ANNEX 2:

Reviews including results from this thesis

Receptor heteromerization in adenosine A_{2A} receptor signaling

Relevance for striatal function and Parkinson's disease

K. Fuxe, MD, PhD; L.F. Agnati, MD, PhD; K. Jacobsen; J. Hillion, PhD; M. Canals, BSc; M. Torvinen, PhD; B. Tinner-Staines; W. Staines, PhD; D. Rosin, PhD; A. Terasmaa, MSc; P. Popoli, MD; G. Leo, PhD; V. Vergoni, PhD; C. Lluis, PhD; F. Ciruela, PhD; R. Franco, PhD; and S. Ferré, MD, PhD

Abstract—Recently evidence has been presented that adenosine A_{2A} and dopamine D_2 receptors form functional heteromeric receptor complexes as demonstrated in human neuroblastoma cells and mouse fibroblast Ltk$^-$ cells. These A_{2A}/D_2 heteromeric receptor complexes undergo coaggregation, cointernalization, and codesensitization on D_2 or A_{2A} receptor agonist treatments and especially after combined agonist treatment. It is hypothesized that the A_{2A}/D_2 receptor heteromer represents the molecular basis for the antagonistic A_{2A}/D_2 receptor interactions demonstrated at the biochemical and behavioral levels. Functional heteromeric complexes between A_{2A} and metabotropic glutamate 5 receptors (mGluR5) have also recently been demonstrated in HEK-293 cells and rat striatal membrane preparations. The A_{2A}/mGluR5 receptor heteromer may account for the synergism found after combined agonist treatments demonstrated in different in vitro and in vivo models. D_2, A_{2A}, and mGluR5 receptors are found together in the dendritic spines of the striatopallidal GABA neurons. Therefore, possible D_2/A_{2A}/mGluR5 multimeric receptor complexes and the receptor interactions within them may have a major role in controlling the dorsal and ventral striatopallidal GABA neurons involved in Parkinson's disease and in schizophrenia and drug addiction, respectively.

NEUROLOGY 2003;61(Suppl 6):S19–S23

The A_{2A}/D_2 heteromeric receptor complex. Evidence has accumulated that intramembrane antagonistic receptor–receptor interactions between adenosine A_{2A} and dopamine D_2 receptors exist in dorsal and especially in the ventral striatum as studied in biochemical and receptor autoradiographic experiments.[1-4] Both receptors are located on the dendritic spines of the striatopallidal GABA neurons.[5,6] In 2002, it became possible to demonstrate the existence of A_{2A}/D_2 heteromeric receptor complexes in membrane preparations from human D_2 receptor (long-form) stably transfected SH-SY5Y neuroblastoma cells and from mouse fibroblast Ltk$^-$ cells stably transfected with human D_2 (long-form) receptors and transiently cotransfected with the A_{2A} receptor double tagged with hemagglutinin.[7] It was observed that the A_{2A}/D_2 heteromeric receptor complexes existed in the absence of exogenous A_{2A} and D_2 receptor agonists and therefore represented constitutive heteromers. Experiments using bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET) techniques indicate that the A_{2A}/D_2 heteromeric receptor complex represents a heterodimer (Canals et al., unpublished observations).

Based on these observations, it is likely that the A_{2A} receptor agonist-induced reduction of D_2 receptor affinity, mainly involving the high-affinity state, is caused by an activation of the A_{2A} receptor in the heteromeric receptor complex causing a conformational change in the binding pocket of the D_2 receptor.[8] A_{2A} receptor activation also leads to a reduction of the G-protein coupling of the D_2 receptor as seen from an antagonism of the GTP-induced cross-regulation of the D_2 receptor with a disappearance of the high-affinity state.[9] These events result in a reduction of D_2 receptor signaling as inferred from the ability of A_{2A} receptor agonists to counteract the reduction of adenylyl cyclase activity[7] and the changes in intracellular Ca^{2+} levels[8,10] induced by D_2 receptors. Conversely, D_2 receptor activation antagonizes A_{2A} receptor signaling by a G_i-mediated inhibition of the A_{2A}/G_{olf}-activated adenylyl cyclase, which seems to be particularly pronounced in the dorsal striatum.[11] The existence of the A_{2A}/D_2 heteromeric receptor complex is probably also the molecular

From the Department of Neuroscience (Drs. Fuxe, Torvinen, and Terasmaa), Karolinska Institute, Stockholm, Sweden; Biomedical Sciences (Drs. Agnati, Leo, and Vergoni), University of Modena, Italy; Department of Anatomy (Dr. Staines, K. Jacobsen and B. Tinner-Staines), University of Ottawa, Canada; Stroke Branch (Dr. Hillion), NINDS, NIH, Bethesda, MD; Department of Biochemistry and Molecular Biology (Drs. Lluis, Ciruela, and Franco, M. Canals), University of Barcelona, Spain; Department of Pharmacology (Dr. Rosin), University of Virginia Health Sciences Center, Charlottesville, VA; Department of Pharmacology (Dr. Popoli), Istituto Superiore di Sanita, Rome, Italy; and Pre-clinical Pharmacology Section (Dr. Ferré), Behavioral Neuroscience Branch, NIDA, NIH, IRP, Baltimore, MD.

Supported by EU QLG3-CT-2001–01056, Swedish Research Council 14X-00715, Spanish Ministry of Science and Technology BIO1999–0601-C02–02, SAF2002–03293, and SAF2001–3474, Intramural Research Program 1089 RI, Italian Ministry of Health (ISS, Italy), Scottish Rite Schizophrenia Research Council, and NIH HL37942 and HL28785.

Address correspondence and reprint requests to Dr. Kjell Fuxe, Department of Neuroscience, Karolinska Institutet, Retzius väg 8, A2:4, S17177, Stockholm, Sweden; e-mail: kjell.fuxe@neuro.ki.se

mechanism underlying the demonstrated coaggregation, cointernalization, and codesensitization of A_{2A} and D_2 receptors on A_{2A} or D_2 receptor agonist treatments.[7] It is of substantial interest that combined D_2 and A_{2A} receptor agonist treatment markedly enhances the cointernalization and codesensitization of the A_{2A} and D_2 receptors. Therefore, the A_{2A}/D_2 heteromeric receptor complex may make A_{2A}/D_2 receptor cotrafficking possible.

Relevance of the A_{2A}/D_2 heteromeric receptor complex for PD and its management. Based on the above, it seems likely that the recently demonstrated antiparkinsonian actions of A_{2A} receptor antagonists in humans (see Chase, page S107) are to a substantial degree caused by blocking the action of endogenous adenosine on A_{2A} receptors of the A_{2A}/D_2 receptor heteromer, leading to enhancement of D_2 receptor signaling.[1,3] This may permit the reduction of the L-dopa dose and thus reduces the development of the L-dopa–induced dyskinesias related to a change in the phenotypic character of striatal GABAergic neurons with overexpression of prodynorphin and glutamic acid decarboxylase (GAD) mRNA levels[12] (see Chen et al., page S74).

Carta et al.[13] (see Carta et al., page S39) have shown that combined treatment with an A_{2A} receptor antagonist and a low dose of L-dopa did not produce the possibly deleterious long-term increases in GAD, dynorphin, and enkephalin mRNA levels. By contrast, repeated treatment with a higher dose of L-dopa alone (which produced the same acute motor stimulant effect as did the combination of L-dopa plus A_{2A} antagonist) led to a significant increase in striatal GAD, dynorphin, and enkephalin expression. This absence of striatal gene inductions with repeated L-dopa plus A_{2A} antagonist was correlated with a stable turning response, in contrast to the sensitized turning response that developed after repeated treatment with L-dopa alone in this hemiparkinsonian model in rats.[13] It is of note that A_{2A} receptor antagonists alone produce antiparkinsonian effects without dyskinesias in parkinsonian monkeys.[14-16] It has also been indicated that long-term L-dopa therapy requires A_{2A} receptors for persistent behavioral sensitization as studied in A_{2A} receptor knockout mice.[17]

The loss of inhibition of A_{2A} receptor signaling by the reduced D_2 receptor signaling in patients with Parkinson's disease (PD) adds to the parkinsonian symptoms, and thus antiparkinsonian actions of A_{2A} receptor antagonists can be related not only to an enhancement of D_2 receptor signaling but also to the blockade of increased A_{2A} receptor signaling of the hypodopaminergic state.[11,18] A_{2A} receptor antagonists can counteract motor inhibitory and cataleptic effects after genetic and pharmacologic disruptions of D_2 receptor-mediated transmission.[19,20]

Data on A_{2A}/D_2 receptor cotrafficking[7] suggest that increased A_{2A}/D_2 receptor cointernalization in response to long-term L-dopa therapy, in combination with increased striatal adenosine tone,[7] may contribute to the deterioration of the therapeutic action of L-dopa. Simply stated, the desensitization may result from a decreased membrane presence of the D_2 receptor.

There is also the possibility that A_{2A} receptor antagonists can show neuroprotective activity because a higher coffee and caffeine intake was associated with reduced risk for PD.[21,22] A_{2A} receptor antagonists may reduce excitotoxicity[23] because, for example, the stimulation of striatal glutamate release by metabotropic glutamate 5 receptor (mGluR5) agonists involves A_{2A} receptors.[24]

In our opinion, an important aspect to be further investigated to reach a better understanding of the sensitization/desensitization process of the D_2 receptors may reside in the stoichiometry of the A_{2A}/D_2 receptor heterodimers vs the A_{2A} and D_2 receptor monomers and homodimers present at the plasma membrane level. In particular, long-term L-dopa therapy may induce internalization of A_{2A} and D_2 receptors when associated as heterodimers, whereas it may not affect A_{2A} receptors existing as monomers or homodimers.[7] Therefore, the relative amount of D_2 receptors in the two forms may be of importance in controlling the manifold aspects (efficacy and potency of D_2 signaling trafficking, sensitization, and desensitization) of the neuronal response to long-term L-dopa therapy.

The A_{2A}/mGluR5 heteromeric receptor complex. Our interest in A_{2A}/mGluR5 receptor interactions started with the demonstration that A_{2A} and group I mGluR receptor agonists could synergistically reduce affinity of D_2 receptor in striatal membranes.[25] Recently it has been possible to show the existence of heteromeric receptor complexes between A_{2A} receptors and the group I mGluR receptor subtype mGluR5 in coimmunoprecipitation experiments in HEK-293 cells cotransfected with Flag-A_{2A} and hemagglutinin mGluR5 receptors and in rat striatal membrane preparations involving native A_{2A} and mGluR5 receptors.[26] In contrast, there was a lack of coimmunoprecipitation between mGluR5 and mGluR1β (an isoform of another group I mGluR receptor) receptors. In agreement, it was found that the A_{2A} and mGluR5 receptors were colocalized on the membrane surface of cotransfected HEK-293 cells as shown with confocal laser microscopy after transient cotransfections. Furthermore, in preliminary fluorescence microscopy experiments with optical sectioning techniques (using the exhaustive photon reassignment process) we have found evidence for a strong A_{2A}/mGluR5 receptor colocalization in rat striatal primary cultures (figure). The detailed quantitative analysis of these results is in preparation (only the images corresponding to the pictures in the figure have been analyzed). It is presently unknown if adapter proteins, such as Homer proteins or the Shank family of scaffold proteins, link mGluR5 and A_{2A} receptors together in the het-

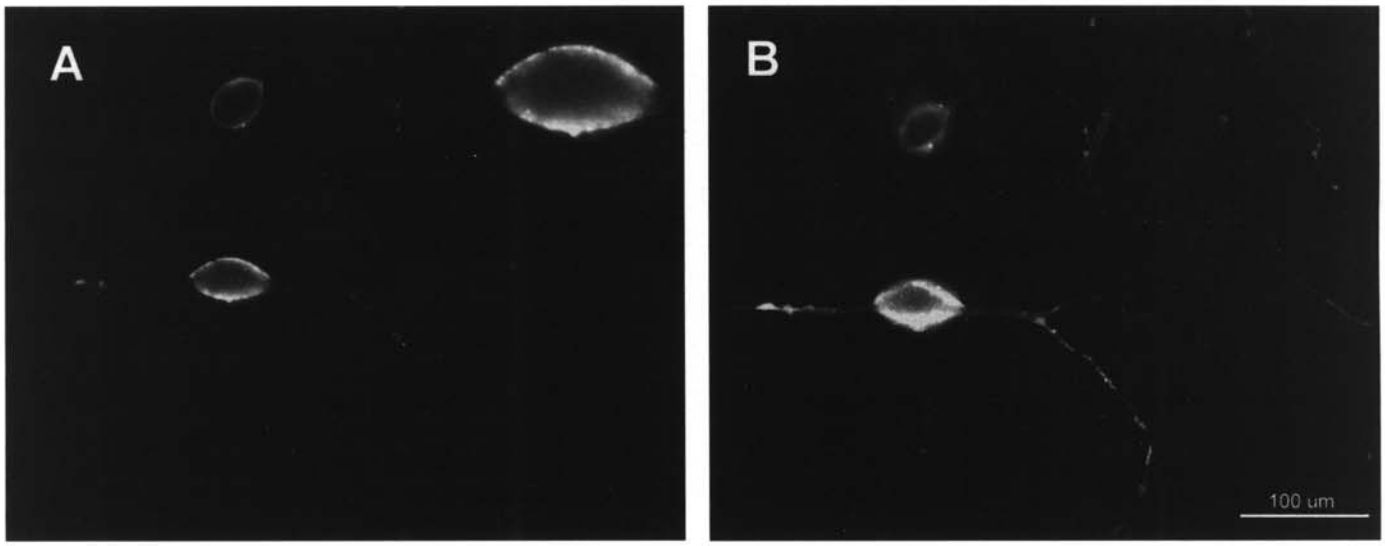

Figure. Colocalization of A_{2A} and metabotropic glutamate 5 (mGluR5) receptor immunoreactivities in the soma (A) and soma and dendrites (B) of striatal neurons in primary cultures (day 15 in vitro). Cryopreserved primary striatal neurons were obtained from QBM Cell Science (R-Cp-502; Ottawa, Canada). Cells were stored in liquid nitrogen until use. Cells were immunostained with rabbit anti-mGluR5 receptor (1:100; Upstate Biotechnology, Lake Placid, NY) and mouse anti-A_{2A} receptor (1:1000) antibodies.[40] Goat antirabbit Alexa-Fluor 488 (1:400; Molecular Probes, Eugene, OR) and goat anti-mouse CY3 (1:400; Sigma Chemical Co., St. Louis, MO) conjugated antibodies were used as secondary antibodies. The immunostaining procedure is the same as described in detail elsewhere for permeabilized cells[41] (the A_{2A} receptor antibody is directed against an intracellular epitope[40]) with some modifications. Cells were analyzed by double immunofluorescence with confocal-like microscopy. Superimposition of the red (CY3) and green (Alexa-Fluor 488) images reveals the A_{2A}/mGluR5 receptor colocalization in yellow (magnification bar, 100 μm). The "Boolean colocalization" (overlap of the field areas between A_{2A} and mGluR5 receptors) at soma level was equal to approximately 33% of the entire soma field area, whereas the "yellow colocalization" (overlap of the field areas between A_{2A} and mGluR5 receptors where red and green emission showed similar intensity of emission) at soma level was equal to approximately 4.5% of the Boolean colocalization. This yellow colocalization most probably represents the area with the highest ratio of A_{2A}/mGluR5 receptor heteromeric complexes vs A_{2A} and mGluR5 homomeric (or monomeric) receptor complexes (for detailed description of the method, see elsewhere[42]).

eromeric receptor complex. The Homer proteins can bind to the C-terminal part of mGluR5 receptor and produce their clustering, and the Shank proteins link together the mGluR5 with the NMDA receptors. The A_{2A}/mGluR5 receptor heteromer was also present in the absence of exogenous agonists and appeared to be preformed, like the A_{2A}/D_2 receptor heteromer.

In the HEK-293 cells, it was possible to give a functional correlate to the A_{2A}/mGluR5 heteromeric receptor complex.[26] Therefore, A_{2A}/mGluR5 receptor coactivation produced a synergistic interaction at the level of extracellular signal-regulated kinase 1/2 (ERK) phosphorylation and c-fos expression. These and other results suggested that the A_{2A}/mGluR5 heteromeric receptor complex is involved in striatal neuron plasticity, including long-term potentiation and depression.[26] This synergism may be brought about by independent signals interacting at the level of the mitogen-activated protein kinase (MAPK) cascade.[27] There is also the possibility that on coactivation of the heteromeric receptor complex, a multireceptor complex may be assembled with receptor tyrosine kinases or nonreceptor tyrosine kinase Src, leading to ERK activation.[28-32] Another possible mechanism involved in the synergism between A_{2A} and mGluR5 receptors could be the modulation of mGluR5 receptor desensitization, which has been demonstrated for NMDA/mGluR5 and group II mGluR/mGluR5 receptor interactions.[33,34] In these cases, the modulation seems to depend on the activation of phosphatase 2B, which reverses the agonist-induced protein kinase C-mediated desensitization of mGluR5 receptors.[33,34] However, this mechanism would imply synergistic interactions at the second-messenger level (Ca^{2+} mobilization),[33,34] which could not be demonstrated in A_{2A}/mGluR5 receptor-cotransfected HEK-293 cells.[26]

A similar A_{2A}/mGluR5 receptor synergism could be demonstrated in the rat striatum for c-fos expression correlated with a synergistic A_{2A}/mGluR5-mediated counteraction of phencyclidine-induced motor activity.[26] It is well known that this motor activity depends on D_2 receptor activity and has so far been blocked only by a high degree of D_2 receptor blockade. Therefore, the results suggest that A_{2A}/mGluR5 receptor costimulation can override a strong D_2 receptor-mediated transmission at the behavioral level. Long-term, but not short-term, treatment with an mGluR5 receptor antagonist can reverse the akinetic deficit in a model of PD.[35] It seems possible that this can in part be caused by an internalization and downregulation of the A_{2A}/mGluR5 heteromeric re-

ceptor complex removing the D_2 receptor from inhibition of its signaling. In addition, there may also exist an acute mGluR5 receptor antagonist-induced counteraction of D_2 receptor blockade with haloperidol as seen from reduced rigidity and catalepsy[36] that may in part be exerted at the network level (e.g., at the level of the subthalamic nigral glutamate system).

The possible existence of A_{2A}/D_2/mGluR5 multimeric receptor complexes. The A_{2A}/mGluR5 heteromeric receptor complexes appear to be preferentially located at dendritic spines of striatopallidal GABAergic neurons[20,37] like the A_{2A}/D_2 heteromeric receptor complexes. Based on the aforementioned observations, it seems a reasonable hypothesis that there exist A_{2A}/D_2/mGluR5 multimeric receptor complexes in the dendritic spines of the striatopallidal GABA neurons.

Observations supporting this hypothesis:

1. A_{2A} and group I mGluR synergistically reduced the affinity of the high-affinity state of the striatal D_2 receptors in membrane preparations.[25]
2. Group I mGluR activation synergistically potentiated the ability of the A_{2A} receptor agonist CGS21680 to counteract D_2 receptor agonist (quinpirole)-induced contralateral rotational behavior.[25]
3. The mGluR5 receptor agonist CHPG reduced the affinity of the high-affinity state of the D_2 receptor, an action potentiated by CGS21680.[38]
4. CHPG inhibited contralateral rotational behaviors induced by quinpirole, an effect potentiated by CGS21680.[38]
5. The mGluR5 receptor agonist CHPG in the nucleus accumbens increased GABA release in the ventral pallidum, an action strongly potentiated by coperfusion with CGS21680.[39]
6. Coperfusion with quinpirole counteracted the increases in ventral pallidal GABA levels by CGS21680 and CHPG.[39]

These results are compatible with the existence of A_{2A}/D_2/mGluR5 multimeric complexes that are important in regulation of the dorsal and ventral striatopallidal GABA neurons and are of high relevance for management of PD (dorsal system) and schizophrenia and drug dependence (ventral system).

References

1. Ferré S, von Euler G, Johansson B, Fredholm BB, Fuxe K. Stimulation of high affinity adenosine A-2 receptors decreases the affinity of dopamine D-2 receptors in rat striatal membranes. Proc Natl Acad Sci USA 1991;88:7238–7241.
2. Ferré S, Fredholm BB, Morelli M, Popoli P, Fuxe K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 1997;20:482–487.
3. Ferré S, Fuxe K. Dopamine denervation leads to an increase in the membrane interaction between adenosine A2 and dopamine D_2 receptors in the neostriatum. Brain Res 1992;594:124–130.
4. Díaz-Cabiale Z, Hurd Y, Guidolin D, et al. Adenosine A_{2A} agonist CGS 21680 decreases the affinity of dopamine D_2 receptors for dopamine in human striatum. Neuroreport 2001;12:1831–1834.
5. Yung KK, Bolam JP, Smith AD, Hersch SM, Ciliax BJ, Levey AI. Immunocytochemical localization of D_1 and D_2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. Neuroscience 1995;65:709–730.
6. Hettinger BD, Lee A, Linden J, Rosin DL. Ultrastructural localization of adenosine A_{2A} receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. J Comp Neurol 2001;431:331–346.
7. Hillion J, Canals M, Torvinen M, et al. Coaggregation, cointernalization and codesensitization of adenosine A_{2A} receptors and dopamine D_2 receptors. J Biol Chem 2002;277:18091–18097.
8. Salim H, Ferré S, Dalal. A et al. Activation of adenosine A_1 and A_{2A} receptors modulates dopamine D_2 receptor-induced responses in stably transfected human neuroblastoma cells. J Neurochem 2000;74:432–439.
9. Ferré S, Snaprud P, Fuxe K. Opposing actions of an adenosine A_2 receptor agonist and a GTP analogue on the regulation of dopamine D_2 receptors in rat neostriatal membranes. Eur J Pharmacol 1993;244: 311–315.
10. Yang S-N, Dasgupta S, Lledo P-M, Vincent J-D, Fuxe K. Reduction of dopamine D_2 receptor transduction by activation of adenosine A_{2A} receptors in stably A_{2A}/D_2 (long-form) receptor co-transfected mouse fibroblast cell lines: studies on intracellular calcium levels. Neuroscience 1995;68:729–736.
11. Fuxe K, Strömberg I, Popoli P, et al. Adenosine receptors and Parkinson's disease. Relevance of antagonistic adenosine and dopamine receptor interactions in the striatum. Adv Neurol 2001;86:345–353.
12. Cenci MA, Lee CS, Bjorklund A. L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. Eur J Neurosci 1998;8:2694–2706.
13. Carta AR, Pinna A, Cauli O, Morelli M. Differential regulation of GAD67, enkephalin and dynorphin mRNAs by chronic-intermittent L-dopa and A_{2A} receptor blockade plus L-dopa in dopamine-denervated rats. Synapse 2002;44:166–174.
14. Grondin R, Bedard PJ, Hadj Tahar A, Gregoire L, Mori A, Kase H. Antiparkinsonian effect of a new selective adenosine A_{2A} receptor antagonist in MPTP-treated monkeys. Neurology 1999;52:1673–1677.
15. Kanda T, Jackson MJ, Smith LA, et al. Adenosine A_{2A} antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 1998;43:507–513.
16. Kanda T, Jackson MJ, Smith LA, et al. Combined use of the adenosine $A_{(2A)}$ antagonist KW-6002 with L-DOPA or with selective D_1 or D_2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp Neurol 2000;162:321–327.
17. Fredduzzi S, Moratalla R, Monopoli A, et al. Persistent behavioral sensitization to chronic L-DOPA requires A_{2A} adenosine receptors. J Neurosci 2002;22:1054–1062.
18. Morelli M, Wardas J. Adenosine A_{2A} receptor antagonists: potential therapeutic and neuroprotective effects in Parkinson's disease. Neurotox Res 2001;3:545–556.
19. Chen JF, Moratalla R, Impagnatiello F, et al. The role of the D(2) dopamine receptor (D(2)R) in $A_{(2A)}$ adenosine receptor ($A_{(2A)}$R)-mediated behavioral and cellular responses as revealed by $A_{(2A)}$ and D(2) receptor knockout mice. Proc Natl Acad Sci USA 2001;98:1970–1975.
20. Aoyama S, Kase H, Borrelli E. Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. J Neuroscience 2000;20:5848–5852.
21. Ross GW, Abbott RD, Petrovitch H, et al. Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA 2000;283: 2674–2679.
22. Schwarzschild MA, Chen JF, Ascherio A. Caffeinated clues and the promise of adenosine $A_{(2A)}$ antagonists in PD. Neurology 2002;58: 1154–1160.
23. Morelli M, Pinna A. Interaction between dopamine and adenosine A_{2A} receptors as a basis for the treatment of Parkinson's disease. Neurol Sci 2001;22:71–72.
24. Pintor A, Pezzola A, Reggio R, Quarta D, Popoli P. The mGlu5 receptor agonist CHPG stimulates striatal glutamate release: possible involvement of A_{2A} receptors. Neuroreport 2000;11:3611–3614.
25. Ferré S, Popoli P, Rimondini R, Reggio R, Kehr J, Fuxe K. Adenosine A_{2A} and group I metabotropic glutamate receptors synergistically modulate the binding characteristics of dopamine D_2 receptors in the rat striatum. Neuropharmacology 1999;38:129–140.
26. Ferré S, Karcz-Kubicha M, Hope BT, et al. Synergistic interaction between adenosine A_{2A} and glutamate mGluR5 receptors: Implications for striatal neuronal function. Proc Natl Acad Sci USA 2002;99:11940–11945.
27. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 2001;76:1–10.
28. Maudsley S, Pierce KL, Zamah M, et al. The β2-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. J Biol Chem 2000;275:9572–9580.
29. Luttrell LM, Roudabush FL, Choy EW, et al. Activation and targeting of extracellular signal-regulated kinases by β-arrestin scaffolds. Proc Natl Acad Sci USA 2001;98:2449–2454.
30. Schulte G, Fredholm BB. Human adenosine A(1), $A_{(2A)}$, A(2B), and A(3) receptors expressed in Chinese hamster ovary cells mediate the phos-

phorylation of extracellular-regulated kinase. Mol Pharmacol 2000;58: 477–482.
31. Marinissen MJ, Gurkind JS. G-protein-coupled receptors and signaling networks: emerging paradigms. Trends Pharmacol Sci 2001;22:368–376.
32. Hall RA, Premont RT, Lefkowitz RJ. Heptahelical receptor signaling: beyond the G protein paradigm. J Cell Biol 1999;145:927–932.
33. Cho K, Bashir ZI. Cooperation between mglu receptors: a depressing mechanism? Trends Neurosci 2002:25:405–411.
34. Alagarsamy S, Marino MJ, Rouse ST, Gereau RW 4th, Heinemann SF, Conn PJ. Activation of NMDA receptors reverses desensitization of mGluR5 in native and recombinant systems. Nat Neurosci 1999;2:234–240.
35. Breysse N, Baunez C, Spooren W, Gasparini F, Amalric M. Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism. J Neurosci 2002;22:5669–5678.
36. Ossowska K, Konieczny J, Wolfarth S, Wieronska J, Pilc A. Blockade of the metabotropic glutamate receptor subtype 5 (mGluR5) produces antiparkinsonian-like effects in rats. Neuropharmacology 2001;41:413–420.
37. Smith Y, Charara A, Hanson JE, Paquet M, Levey AI. GABA(B) and group I metabotropic glutamate receptors in the striopallidal complex in primates. J Anat 2000;196:555–576.
38. Popoli P, Pezzola A, Torvinen M, et al. The selective mGlu(5) receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D(2) receptors in the rat striatum: interactions with adenosine $A_{(2A)}$ receptors. Neuropsychopharmacology 2001;25:505–513.
39. Díaz-Cabiale Z, Vivo M, Del Arco A, et al. Metabotropic glutamate mGlu5 receptor-mediated modulation of the ventral striopallidal GABA pathway. Interactions with adenosine A_{2A} and dopamine D_2 receptors. Neurosci Lett 2002;324:154–158.
40. Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J. Immunohistochemical localization of adenosine A_{2A} receptors in the rat central nervous system. J Comp Neurol 1998;401:163–186.
41. Gines S, Hillion J, Torvinen M, et al. Dopamine D_1 and adenosine A_1 receptors form functionally interacting heteromeric complexes. Proc Natl Acad Sci USA 2000;97:8606–8611.
42. Torvinen M, Ginés S, Hillion J, et al. Interactions among adenosine deaminase, adenosine A_1 receptors and dopamine D_1 receptors in stably cotransfected fibroblast cells and neurons. Neuroscience 2002;3:709–719.

Glutamate $mGlu_5$-Adenosine A_{2A}-Dopamine D_2 Receptor Interactions in the Striatum. Implications for Drug Therapy in Neuro-psychiatric Disorders and Drug Abuse

Sergi Ferré[1]*, Francisco Ciruela[2], Amina S. Woods[3], Meritxell Canals[2], Javier Burgueno[2], Daniel Marcellino[2], Marzena Karcz-Kubicha[1], Bruce T. Hope[4], Marisela Morales[5], Patrizia Popoli[6], Steven R. Goldberg[1], Kjell Fuxe[7], Carme Lluis[2], Rafael Franco[2] and Luigi Agnati[8]

[1]Preclinical Pharmacology Section, [3]Chemistry and Drug Metabolim Section, [4]Behavioral Neuroscience Section and [5]Cellular Neurobiology Section, Department of Health and Human Services, NIH, NIDA, IRP, Baltimore MD 21224, USA, [2]Department of Biochemistry and Molecular Biology, University of Barcelona, 08028 Barcelona, Spain, [6]Department of Pharmacology, Istituto Superiore di Sanita, 00161 Rome, Italy, [7]Department of Neuroscience, Karolinska Institute, 17177 Stockholm, Sweden, [8]Department of Biomedical Sciences, University of Modena, 41100 Modena, Italy

Abstract: There is growing evidence for the existence of not only homomeric, but also functional heteromeric receptor complexes, particularly involving G protein coupled receptors (GPCRs). These include adenosine A_{2A}-dopamine D_2 and adenosine A_{2A}-glutamate $mGlu_5$ receptor complexes. The role of these receptor complexes in receptor function seems to be multiple, involving hetero-modulation of ligand recognition, signalling and trafficking. The preferential localization of A_{2A}-D_2 and A_{2A}-$mGlu_5$ receptor complexes is in the dendritic spines of striatopallidal GABAergic neurons. Results obtained from behavioral and *in vivo* microdialysis experiments have shown an important role of $mGlu_5$-A_{2A}-D_2 receptor interactions in the modulation of the function of the striatopallidal GABAergic neurons. The striatopallidal GABAergic neurons play a key role in the pathophysiology of basal ganglia disorders, like Parkinson's disease, and it is a common pathway for the rewarding effects of opiates and psychostimulants and for the antipsychotic effects of neuroleptics. The formation of receptor complexes modifies the single receptor transducing characteristics and leads to the appearance of "emergent properties". Thus, the study of $mGlu_5$-A_{2A}-D_2 receptor interactions in the striatum reveals new properties of these GPCRs and gives indications for a new rational approach for drug therapy in neuro-psychiatric disorders and drug addiction.

Keywords: Dopamine D_2 receptor; adenosine A_{2A} receptor; glutamate $mGlu_5$ receptor; striatum; heteromeric receptor complexes; Parkinson's disease; schizophrenia; drug addiction.

INTRODUCTION

The basic tenet of the present paper is that the G protein coupled receptors (GPCRs or heptahelical receptors) should be considered not only as single units, but also, frequently, as multimolecular aggregates (see the "receptor mosaic hypothesis", refs. [1,2]). In other words, in most instances GPCRs are functionally connected with other molecules either intrinsic to the plasma membrane or associated with the extracellular or intracellular surface of the membrane. The function of each GPCR can be markedly affected by its interactions with different molecules (especially proteins). "Receptor-receptor interactions" represent a special type of these interactions [1-4]. In the present paper three GPCRs (glutamate $mGlu_5$, adenosine A_{2A} and dopamine D_2 receptors) will be examined. In a first instance, each receptor will be examined as single decoding unit, thereafter the emergent properties of the receptor complexes due to $mGlu_5$-A_{2A}-D_2 receptor interactions will be discussed. The main focus will be on the receptors in the striatopallidal neuron, where there is clear-cut evidence that $mGlu_5$, A_{2A} and D_2 receptors interact. Molecular aspects of these interactions have been evaluated, as well as some of the physiopathological counterparts of these molecular mechanisms have been described [2,5,6]. It is also obvious that on the basis of the experimental evidence presented it is possible to surmise strategies to develop new drugs that may be capable of acting with more selectivity on GPCRs and, thus, to avoid some side-effects of drugs currently in use. For the sake of clarity, some basic aspects of GPCRs will be briefly presented. These aspects should also allow a better understanding of our view and of our hypothesis.

*Address correspondence to this author at the Preclinical Pharmacology Section, Department of Health and Human Services, NIH, NIDA, IRP, 5500 Nathan Shock Drive, Baltimore MD 21224, USA; Tel: 410-550-1586; Fax: 410-550-1648; E-mail: sferre@intra.nida.nih.gov

Some Molecular and Functional Features of GPCRs

It should be noticed that GPCRs span three environments: the extracellular, the intramembrane and the intracellular. Each of these environments has peculiar characteristics (lipophilicity versus hydrophilicity, ion activity, electrostatic fields,…) that obviously affect the steric conformation of the strand of amino acids crossing through it. Four main aspects should be considered:

1568-0150/03 $41.00+.00

The relative topologies of the amino acid domains of the receptors with respect to the other domains, since this aspect leads to the particular steric structure (folding) of the receptor.

The domains of the GPCR that allow the interactions with other molecules in one of the three environments spanned by the receptor, especially the so-called binding pocket for the transmitter and the binding domain for G proteins and also for some scaffold proteins as well as for other receptors.

The trafficking of the receptor with its appearance at cell membrane level, where it can act as a molecular transducer, i.e., as a molecular mediator that links the extracellular signal with the molecular intracellular effector. This aspect will be examined only by considering the receptor internalization phenomenon.

The functional state of the receptor that allows its action as transducer and, therefore, the effectiveness according to which the receptor transduces the signal. This aspect will be examined only by considering receptor sensitization and desensitization phenomena.

GPCR Families

GPCRs comprise the largest family of membrane-bound receptors, representing more than 1% of the genome in vertebrates [7,8]. All GPCRs have in common a central core domain constituted by seven transmembrane (7TM) domains that are connected by three intracellular and three extracellular loops, an extracellular N-terminal segment and an intracellular C-terminal segment. Each of the 7TM domains is composed of 20-27 amino acids. On the other hand, N-terminal and C-terminal segments and the intra- and extracellular loops can vary very much in size. Most GPCRs can be grouped in three major families, according to sequence differences. Family I (or Family A) contains most GPCRs, including receptors for rhodopsin, catecholamines and adenosine (subgroup 1a), the binding site of which is made by a cavity formed in the 7TM region. Rhodopsin is a particular case, where the target of photons, retinal, is covalently linked in this cavity and converted into an active ligand upon light exposure. Other members of Family I (subgroups 1b and 1c) are activated by short peptides or glycoproteins that also bind to the N-terminal segment of the receptor (large in case of subgroup 1c). Family II (or Family B) comprises GPCRs that are activated by large peptides, like glucagon, calcitonin or VIP (vasoactive intestinal peptide), which bind to a relatively large N-terminal domain. Finally, family III (or Family C) includes metabotropic glutamate (mGlu) receptors, a receptor activated by extracellular Ca^{2+} (calcium sensing receptor) and $GABA_B$ receptors [7,8]. Family III receptors contain a very large N-terminal segment that forms two distinct lobes facing each other and separated by a cavity where the ligand binds. The results of a series of studies, including the determination of the crystal structure of the N-terminal domain of $mGlu_1$ receptors suggest that the two lobes close like a "Venus flytrap" upon binding of the ligand [9,10]. Including Family III, irrespective of the localization of the ligand binding domain, a change in conformation of the 7TM core region is the most probable mechanism responsible for receptor activation in all GPCRs [7,8,10].

The change in conformation of the core domain affects the conformation of the intracellular loops, which determines the binding and activation of G proteins. Activation of G proteins is then responsible for activation of different intracellular signaling pathways [8,11]. However, different classes of proteins, other than G proteins, are being recognized to interact with GPCRs. The binding of GPCRs to some of these proteins will determine interactions with different intramembrane and intracellular elements, which will result not only in changes in receptor localization and function but also in G protein-independent signalling [9,11-14]. These proteins include serine-threonine or tyrosine protein kinases, β-arrestins, Homer proteins and other proteins localized in the postsynaptic density (see below). However, they also include GPCRs themselves, which gives the possibility of the formation of GPCR homo- and heteromeric complexes (see below).

Internalization of GPCRs

Receptor desensitization, the decrease of receptor responsiveness to agonist, represents an adaptive mechanism that protects the receptor against acute and chronic over-stimulation. However, GPCR desensitization can also limit the therapeutic usefulness of receptor agonists. GPCR desensitization is the consequence of different mechanisms that in many cases include the phosphorylation-induced uncoupling of the receptor from G proteins, the internalization of cell surface receptors to intracellular compartments, and the down-regulation of the total amount of receptors as a result of receptor degradation and/or decreased synthesis. These mechanisms have different time frames, which range from seconds (phosphorylation) to minutes (internalization) or hours (down-regulation) [12,14,15]. Phosphorylation of serine and threonine residues within the intracellular loops and C-terminal segment of GPCRs can be induced by either GPCR kinases (GRKs) or by second messenger-dependent protein kinases (such as protein kinase A [PKA] and protein kinase C [PKC]). GRKs are made of seven family members that can be classified into three groups according to sequence and function homology: GRK1 and GRK7; GRK2 and GRK 3; GRK4, GRK5 and GRK6) [12]. GRKs selectively phosphorylate activated receptors and, therefore, induce acute homologous desensitization. On the other hand, second messenger-dependent protein kinases induce both acute homologous and heterologous sensitization, since they can indiscriminately phosphorylate agonist-activated and non-activated receptors [12].

Acute homologous desensitization induced by GRK is the first step in the process of receptor internalization dependent on arrestins. Arrestin proteins bind preferentially to agonist-activated and GRK-phosphorylated GPCRs, as opposed to second messenger protein kinase-phosphorylated or non-phosphorylated receptors. When bound to the receptor, arrestins physically prevent the receptor-G protein association, "arresting" the receptor in the desensitized G

protein uncoupled state initially induced by GRK-mediated phosphorylation. Furthermore, arrestins will bind several molecules involved in receptor internalization, such as clathrin, AP-2 and *N*-ethylmaleimide-sensitive fusion protein (NSF) [12,14]. In this way, arrestins serve as adapters that target GPCRs for internalization through clathrin-coated pits. Four arrestin family members have been identified and classified into two groups based on sequence homology and tissue distribution: visual arrestins (visual arrestin and cone arrestin) and β-arrestins [12,14]. The two identified β-arrestins, β-arrestin 1 and β-arrestin 2, have been shown to have different properties [16,17]. Two different classes of GPCRs (which have no relation with the above mentioned GPCR families) have been proposed according to their differential interaction with β-arrestin 1 and β-arrestin 2 [16]. Thus, class A GPCRs, that include β_2 and α_{1b} adrenergic receptors, μ-opioid receptors, endothelin type 1A receptor and some dopamine receptor subtypes, interact with β-arrestin 2 with higher affinity than with β-arrestin 1 [16,18]. On the other hand, class B GPCRs, that include angiotensin type 1 receptor, vasopressin type 2, neurotensin receptor, neurokinin type 1 receptor and thyrotropin-releasing hormone receptor show high affinity for both proteins [16].

Although much has been learned about the desensitization mechanisms that take place during the first seconds or minutes after agonist exposure, little is known about the mechanisms that are involved after longer time of exposure. These, in fact, are the most relevant when considering the therapeutic and secondary effects of exogenous agonists. Although there is a rapid reversibility of the initial processes of desensitization (phosphorylation and initial internalization), down-regulation is characterized by slow or incomplete reversibility following removal of the agonist. The most accepted general mechanism is that once the receptors are internalized they are targeted to "early" endosomes, where they can undergo dephosphorylation and recycling to the plasma membrane (resensitization) or they are further targeted to "late" endosomes or lysosomes for degradation [15]. This model explains the usual dependence of internalization for GPCR resensitization. The "late" endosomes step appears to play an important role in promoting down-regulation of certain GPCRs [15]. However, receptor-specific and cell-specific mechanisms of GPCR endocytosis other than the GRK/β-arrestins/clathrin-coated pits seem to exist. For example, some GPCRs have also been shown to internalize in a β-arrestin-independent manner, by means of small invaginations of the plasma membrane called caveolae (see below). Differential rates of internalization, resensitization and down-regulation together with the rate of receptor synthesis determine the characteristic turnover and, therefore, surface expression of receptors. In some cases, even agonist-induced "upregulation" can be observed, as found with some subtypes of dopamine receptors (D_{2L} and D_3 receptors; see below).

Although initially they were thought to be exclusively involved in receptor desensitization and internalization, β-arrestins have been recently shown to play a key role in G protein independent signaling of GPCRs and, in particular, in GPCR-mediated mitogen-activated protein kinases (MAPKs) activation [19,20]. Thus, in addition to binding different elements involved in receptor internalization, β-arrestins also seem to act as scaffold adapters that coordinate the formation of multiprotein signaling complexes that ultimately lead to activation of MAPKs [19,20]. The assemby of a multiprotein complex at the plasma membrane, which includes receptor and non-receptor tyrosine kinases, allows signal transduction from activated GPCRs to the cytosol located MAPKs, such as extracellular signal-regulated kinases 1 and 2 (ERK1 and 2; in this review, the term MAPK makes reference to ERK1 and 2). This multiprotein complex leads to activation of the small G-protein Ras, which leads to the sequential phosphorylation and activation of the MAPKKK Raf-1, the MAPKK MEK and, finally, MAPK. The coordinating role of β-arrestins on the formation of the multiprotein complex seems to depend on direct interactions with the non-receptor tyrosine kinase Src and other members of the MAPK activation pathway, such as Raf-1 and ERK1 and 2 [19,20]. Src also plays a key dual role in GPCR function, as activator of MAPK signaling [13] and as a required element in agonist-induced receptor endocytosis. Src-mediated tyrosine phosphorylation of dynamin seems to be essential for the well-known role of this protein in constriction of clathrin-coated pits and fission of vesicles [21]. Finally, Src can be activated, not only by binding to β-arrestins, but also by direct interactions with G proteins (Gi and Go, but not Gq) and GPCRs themselves [22,23]. Consequently, β-arrestin binding to the agonist-activated and GRK-phosphorylated GPCR not only terminates receptor-G protein coupling, and therefore, G protein mediated signal transduction, but also initiates a second signal transduction leading to MAPK activation. Thus, by targeting the appropriate multiprotein complexes to clathrin-coated pits, β-arrestins establish a link between MAPK signaling and receptor internalization. In fact, in some systems internalization of signaling protein complexes that include the activated receptor seems to be an additional requirement for MAPK activation, sometimes associated with transactivation of receptor tyrosine kinases [19,20,24,25]. Their association with endocytic vesicles may provide a mechanism for targeting signaling protein complexes to appropriate subcellular localizations, determining if MAPK phosphorylate membrane, cytosolic or nuclear targets [20]. In fact, MAPK plays a predominant role in the mailing of signals from the synapse to the nucleus, since downstream direct or indirect effectors of MAPK are nuclear constitutive transcription factors such as CREB and Elk-1 (see below).

Caveolae were initially identified as flask-shaped plasma membrane invaginations. However, the cloning and characterization of a biochemical marker for caveolae, caveolin, demonstrated the existence of caveolin-rich membrane microdomains that could not be distinguished morphologically from the rest of the plasma membrane [26-29]. The caveolin family comprises a group of proteins (caveolin-1, caveolin-2, with two and three isoforms, respectively, and caveolin-3) encoded by three genes [27,28]. Caveolins are the major component of caveolae and their oligomerization seems to be essential for the formation of the caveolar coat. However, a particular lipid environment, enriched in cholesterol and sphingolipids, is also required for the formation of caveolae [27,28]. Clustering of these lipids constitute specialized detergent

insoluble membrane domains initially called lipid rafts. The "raft hypothesis", as proposed by Simons *et al.* [30,31], postulates the existence of lateral assemblies (rafts) of sphingolipids and cholesterol, which associate with specific proteins while excluding others. Rafts would concentrate signaling and associated molecules within the same plasma cellular domain. Caveolae therefore, are considered to be a specialized form of raft that contains caveolin proteins [26,29]. Caveolin-reach rafts have been shown to be enriched in a variety of signaling molecules, which includes G protein subunits, nitric-oxide synthase and a variety of receptor and non receptor tyrosine kinases (epidermal growth factor [EGF] receptor, platelet-derived growth factor [PDGF] receptor, insulin receptor and Src-related kinases), serine-threonine kinases (GRK, PKA, PKC), and tyrosine-serine-threonine kinases (MAPK). Most of these signaling molecules, in fact, interact directly with caveolin, particularly with a short caveolin cytosolic domain (caveolin-scaffolding domain, or CSD) [27,28]. Besides receptor tyrosine kinases, a fast increasing number of GPCRs are being shown to aggregate, sometimes upon agonist binding, in caveolae. Among others, this includes adenosine A_1 receptors [32] and, so far, two receptors of family III GPCRs: $mGlu_1$ receptors [33] and calcium sensing receptor [34]. Furthermore, we have recently found evidence for co-localization of adenosine A_{2A} and dopamine D_2 receptors with caveolin in CHO cells stably transfected with both receptors (Agnati *et al*. in preparation). Their capacity to concentrate an important number of signaling molecules in a discrete cellular location supports the point of view that caveolae are important functional structures involved in the control of cell signaling [27,28].

As mentioned above, caveolae have also been implicated in GPCR internalization, which may therefore lead to desensitization and to internalization-related signaling. Furthermore, they have been involved in homologous and heterologous GPCR desensitization by a mechanism involving a transient sequestration of receptor by direct binding of activated G protein subunits to caveolins [35]. Co-sequestration of different receptors might lead to heterologous desensitization, a phenomenon recently demonstrated for EGF and PDGF receptors [36] and for adenosine A_{2A} and dopamine D_2 receptors [37]. The involvement of clathrin and/or caveolin-coated vesicles in this A_{2A}-D_2 receptor co-internalization still needs to be determined, although, as indicated above, we have found evidence for co-localization of adenosine A_{2A} and dopamine D_2 receptors with caveolin in a CHO cell line (Agnati *et al.* in preparation). However, caveolins seem to be expressed in low levels in the brain, where they predominate in non-neuronal cells (endothelial cells and astrocytes) [38,39]. Nevertheless, evidence is accumulating which suggests that non-caveolin lipid rafts are abundant in the brain and play very similar roles as caveolae play. Furthermore, proteins functionally similar to caveolins, such as flotillins, are being found in non-caveolin lipid rafts [40]. Specially relevant for the present review is the recent work by Suzuki and coworkers [41,42]. Their results suggest a predominant localization of flotillin lipid rafts in dendritic spines (dendritic rafts) with a postsynaptic and perisynaptic distribution and with direct interactions with the postsynaptic density (see below). Finally, their results also showed the existence of AMPA glutamate receptors in the dendritic rafts, suggesting their involvement in the trafficking of AMPA receptors and, therefore, in synaptic plasticity [41,42].

PHYSIOLOGY OF GLUTAMATE $mGLU_5$ RECEPTORS

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system. Dendritic spines are the preferential site of excitatory synaptic connections and nearly all presynaptic terminals that make synapses on dendritic spines release glutamate. Rapid excitatory synaptic transmission is mediated primarily by the actions of glutamate on two types of ionotropic receptors: AMPA and NMDA. These receptors are mostly localized on the postsynaptic density, an ultrastructurally defined electron-dense thickening of the postsynaptic membrane of the dendritic spines [43,44-46]. In addition to ionotropic glutamate receptors, the postsynaptic density is made of a protein lattice that includes many different organized assemblies of specific proteins. Postsynaptic density proteins are not only involved in signaling, but in the dynamic regulation of different elements of the dendritic spine (including the postsynaptic density itself), which causes both functional and morphological changes that might underlie synaptic plasticity [43-47]. Those changes include local dendritic translation of mRNA [48], phosphorylation and redistribution (exocytosis-endocytosis) of ionotropic receptors [49-52] and changes in dendritic spine cytoskeletal structures [47,53-55].

In addition to its ionotropic AMPA, NMDA and kainate receptors, which mediate fast excitatory glutamate responses, glutamate has metabotropic receptors (mGlu receptors, prototype of Family III GPCRs) that mediate slower, modulatory, responses by coupling with multiple intracellular signalling cascades. Based on sequence similarities, preferred signal transduction mechanisms and pharmacological properties, the 8 subtypes of mGlu receptors so far identified are subdivided into three groups. Group I mGlu receptors includes $mGlu_1$ receptors, with four isoforms ($mGlu_{1a}$ and the short isoforms $mGlu_{1b}$, $mGlu_{1c}$ and $mGlu_{1d}$ receptors), and $mGlu_5$ receptors, with two isoforms [10,56]. In the same way as ionotropic glutamate receptors, Group I mGlu receptors also show a predominant and characteristic postsynaptic localization at glutamatergic synapses. Thus, Group I mGlu receptors are localized in a perisynaptic ring adjacent to the postsynaptic density [57,58]. This localization seems to depend mainly on the existence of specific protein-protein interactions that involve postsynaptic density proteins, such as PSD-95 and Shank [43-46]. PSD-95 is a scaffold protein that binds directly with the C-terminal portion of NR2 subunit of the NMDA receptor; thus, it may be important for clustering of NMDA receptors at synapses [59]. PSD-95 is included on a family of synaptic membrane-associated proteins that are collectively termed membrane-associated guanylyl kinase (MAGUK) proteins [44]. Shank comprises a family of scaffold proteins that contains multiple sites for protein-protein interactions and which is concentrated in a deeper part of the postsynaptic density than is PSD-95 [60]. The connection

between Shank and PSD-95 is made by guanylate kinase-associated protein (GKAP), which constitutes another abundant family of postsynaptic density proteins [60]. Finally, Shank binds to Homer proteins, which selectively binds Group I mGlu receptors [61]. Through these interactions NMDA and Group I mGlu receptors are physically connected. In fact, there is evidence for synergistic NMDA receptor interactions with Group I mGlu receptor interactions, the most common being a potentiation of NMDA-mediated responses by $mGlu_5$ receptor stimulation (see below and refs. [62-64]). In addition, NMDA receptor activation seems to be required for some effects of $mGlu_5$ receptor activation (see below). As discussed below, there is also evidence for an NMDA receptor-mediated potentiation of $mGlu_5$ receptor function, by reversal of basal PKC-induced $mGlu_5$ receptor desensitization [65].

Three main genes encoding Homer proteins have been identified; they generate diverse isoforms by alternative splicing [66]. Homer proteins are characterized by a highly conserved N-terminal EVH1 domain. This EVH1 domain binds to a distal, proline-rich region of the C-terminal portion of $mGlu_5$ receptor (both isoforms) and the long isoform of $mGlu_1$ receptor [66,67]. On the other hand, Homer proteins do not bind to Group II or III mGlu receptors [66,67]. The same EVH1 domain is responsible for the Homer-Shank interaction [61,66] and for the direct interaction with inositol triphosphate (IP3) and ryanodine receptors, which are localized in the endoplasmic reticulum [61,66]. In addition to the EVH1 domain most Homer proteins contain a coiled-coil C-terminal domain (CC domain), which mediates both homomeric and heteromeric association among the different isoforms [66]. The CC domain, therefore, confers the functional capacity of cross-linking Group I mGlu receptors and related Homer interacting proteins, like Shank and IP3 and ryanodine receptors. In addition to the constitutively expressed Homer 1b and Homer 1c, the Homer 1 gene can also generate two short isoforms which lack the CC domain. These short-form isoforms, called Homer 1a and Ania 3, are immediate-early gene (IEG) products the transcription of which is increased with neuronal activation [67,68]. Glutamate receptor (specially NMDA receptor) activation has also been shown to induce Homer 1a expression [69,70], involving MAPK signaling [70]. The Homer 1 gene contains 10 exons for the alternatively spliced constitutive forms, Homer 1b/c, while Homer 1a and Ania 3 end within the 5 intron, that separates the exons encoding the N-terminal EVH1 domain (exons 1-5) from those encoding the CC domain (exons 6-10) [68]. Bottai *et al.* have recently shown that there is a switch from the constitutive expression of Homer 1b/c to the activity-dependent expression of Homer 1a and Ania 3, which entails intronic to exonic sequence conversion and transcript termination within the central intron of the Homer 1 gene [68].

By counteracting CC-oligomerization of long isoforms, the Homer IEG products are natural competitive antagonists of the constitutive long-form isoforms and their expression disrupts the coupling of Group I mGlu receptors to other Homer interacting proteins [69,71]. This implies modifications of mGlu receptor signalling and trafficking. For both $mGlu_1$ and $mGlu_5$ receptors an increased Homer 1a expression unmasks an agonist-independent, constitutive activity of the receptor [69,72]. Homer 1a acts as an indirect Group I mGlu receptor agonist. The effects on trafficking, however, depend on the mGlu receptor subtype. Recent experimental findings strongly suggest that Homer 1b and Homer 1c cause a retention of $mGlu_5$ receptors in the endoplasmic reticulum (ER), and Homer 1a triggers the trafficking of $mGlu_5$ receptors to the plasma membrane [72-74]. ER retention induced by the long Homer isoforms could be just related to their ability to cross-link $mGlu_5$ receptors to the ER proteins IP3 and ryanodine receptors. For different GPCRs homo- and heteromerization seems to be a prerequisite for exiting the ER. With the formation of the GPCR dimer the amino acid sequences involved in ER retention (directly or through additional proteins, such as the long Homer isoforms) would be hidden. In fact, Coutinho *et al.* showed that Homer 1c prevents $mGlu_5$ receptor homodimerization [74]. In contrast to $mGlu_5$ receptors, both Homer 1a and Homer 1c have been reported to increase cell surface clustering of $mGlu_{1a}$ receptors [75,76]. Thus, a differential Homer-mediated regulation between $mGlu_5$ and $mGlu_1$ receptor trafficking seems to exist. In this way the levels of constitutive and inducible Homer proteins might determine the predominant mGlu receptor subtype expressed at the surface of cells containing both receptors.

Group I mGlu receptors couple preferentially to phospholipase C (PLC) through coupling to Gq proteins (see Fig. (**1**)). Activation of PLC induces a release of inositol (1,4,5)triphosphate (IP3) and diacylglycerol (DAG), which cause the release of intracellular Ca^{2+} and the activation of protein kinase C (PKC), respectively [10,77]. In addition to the preferential Gq-PLC pathway, Group I mGlu receptors have also been shown to use other signaling pathways, such as adenylyl cyclase [78,79] and MAPK [80,81] pathways. Work by Francesconi and Duvoisin [78,79] suggests that Group I mGlu receptors stimulate adenylyl cyclase by their ability to couple to Gs proteins. Depending on the cellular system used, Group I mGlu receptor-mediated MAPK activation can be dependent on or independent of PKC activation and, therefore, dependent on or independent of the initial activation of PLC pathway. In either case, activation of non-receptor tyrosine kinases seems to be required. Among the different major gene families of non-receptor tyrosine kinases so far identified the protein products of the *c-src* family, such as Src and Fyn, have been the most extensively studied. However, other non-receptor tyrosine kinases, such as the cell-adhesion kinase β (CAKβ; also called Pyk2, FAK-2, CADTK or RAFTK), which belongs to the focal adhesion (FAK) family, seem to play an important role in glutamatergic synapses [82]. There is evidence showing that Src, Fyn and CAKβ are specially concentrated in the postsynaptic density and that PKC or an increased extracellular Ca^{2+} lead to Src activation secondarily to CAKβ phosphorylation [82,83]. This cascade of events have been implicated in the above-mentioned Group I mGlu receptor-mediated potentiation of NMDA receptor function [84-86]. In fact, the NMDA receptor subunit 2B (NR2B) is the major tyrosine phosphorylated protein of the postsynaptic density fraction [87]. Furthermore, a recent study by Choe and Wang suggests that NMDA receptors are involved in the activation

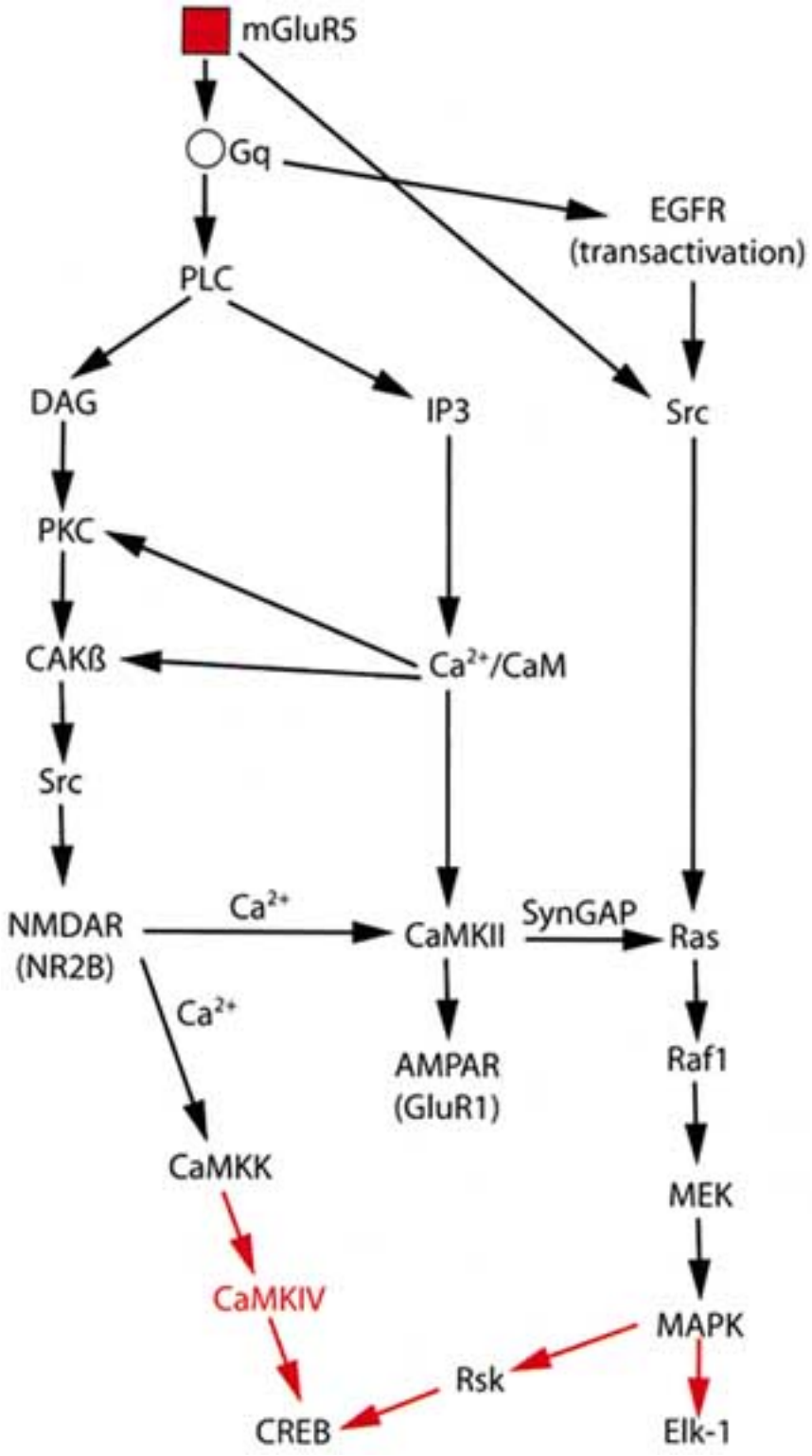

Fig. (1). Signal transduction mechanisms of the glutamate metabotropic $mGlu_5$ receptor. Solid and broken arrows: stimulatory and inhibitory effects, respectively (see text). Red arrows: signaling into the nucleus. AMPAR: AMPA receptor; CAKβ: cell-adhesion kinase β; CaM: calmodulin; CaMK: calmodulin kinases; CREB: cAMP response element binding protein; DAG: diacylglycerol; EGFR: epidermal growth factor receptor; Elk-1: member of the ternary complex factor family of transcription factors; IP3: inositol (1,4,5)triphosphate; MAPK: mitogen-activated protein kinases; MEK: MAPK kinase; NMDAR: NMDA receptor; PKC: protein kinase C; PLC: phospholipase C; Raf-1: member of the Raf family of MAPK kinase kinases; Ras: member of the small GTPase protein superfamily; Rsk: Ribosomal S6 kinases; Src: member of the Src family of non-receptor tyrosine kinases; SynGAP: synaptic Ras activating protein.

of MAPK and the constitutive transcription factors CREB (cAMP response element binding protein) and Elk-1 induced by the stimulation of Group I mGlu receptors in the striatum [88]. Both PKC and Ca^{2+}/calmodulin-dependent protein kinases (CAMK) were also involved [88] Fig. (**1**). However, Peavy *et al*. have shown that $mGlu_5$ receptor stimulation produces MAPK activation in astrocytes which is PLC, Ca^{2+} and PKC-independent but dependent on Gq protein activation, transactivation of a receptor tyrosine kinase (EGF receptor) and activation of Src [81]. Their results, which included $mGlu_5$ receptor agonist-induced co-immunoprecipitation of EGF and $mGlu_5$ receptors, are consistent with the formation of multiprotein signaling complexes leading to MAPK activation (see above and refs. [19,20]). Finally, a G protein-independent and Src-dependent signaling mediated by Group I mGlu receptors has also been reported [89]. This might be related to a β-arrestin-Src interaction (see above), since Group I mGlu receptors have been shown to interact with β-arrestins and to undergo agonist-induced GRK/β-arrestin-dependent internalization [90,91] (Fig. (**1**)).

CAMK comprises a group of kinases that have been shown to be involved in the coupling of synaptic activity (Ca^{2+} influx through NMDA receptors) to synaptic plasticity (long-term potentiation [LTP] and long-term depression [LTD]) at glutamatergic synapses [46,50,92-94]. Stimulation of NMDA receptors, by increasing Ca^{2+} entry and binding to calmodulin, induces activation of CaMKII, which causes its translocation to the postsynaptic density and its binding to NMDA receptors (preferentially to NR2B). Once the interaction with NMDA receptors is established, Ca^{2+} and calmodulin are no longer required for CaMKII to be activated and NR2B-bound CaMKII remains partially active even after calmodulin dissociation, which might serve as a form of "molecular memory" of a previous brief stimulation [94,95]. The postsynaptic density protein SynGap, a synaptic Ras-GTPase activating protein that binds to PSD-95, is one of the main substrates of CaMKII. CaMKII-induced SynGap phosphorylation activates MAPK signaling by stopping inactivation of Ras [96] Fig. (**1**). AMPA receptors (preferentially the GluR1 subunit) are also substrates for CaMKII. CaMKII-mediated GluR1 phosphorylation potentiates AMPA receptor-mediated current and it is involved in the recruitment of AMPA receptors to the synapse from some intracellular pool, a mechanism most probably involved in LTP [49-52]. Although CREB is another potential substrate for CaMKII, the nuclear enriched CaMKIV (which is activated by a Ca^{2+}/calmodulin CaMKK) seems to be more involved in CREB phosphorylation Fig. (**1**). Work by Wu *et al.* suggests that CaMKIV and MAPK represent fast-sensitive and slow-sensitive convergent pathways, respectively, for activity-dependent CREB phosphorylation [93]. Altogether these signaling pathways suggest that $mGlu_5$ receptors can have an important role in the modulation of synaptic plasticity in the dendritic spines, which are believed to act as functional cellular compartments that constitute the main site of neuronal plasticity and long-term memory. In fact, stimulation of Group I mGlu receptors has been shown to induce morphological changes in the dendritic spines as well as to stimulate dendritic protein synthesis, chemical LTD and internalization of AMPA and NMDA receptors, and these processes seem to be interrelated [48,97-103] (Fig. (**1**)).

PKC activation seems to play a more important role in rapid agonist-induced $mGlu_5$ receptor desensitization than the homologous GRK-dependent desensitization [104]. Any manipulation that elevates PKC activity (or decreases the activity of serine/threonine phosphatases) is likely to result in a reduction of $mGlu_5$ receptor function. On the other hand, reductions of PKC activity (or increases in the activity of serine/threonine phosphatases) would result in an increased $mGlu_5$ receptor function [65,104]. PKC-dependent homologous and heterologous desensitization may be important in regulating synaptic transmission and plasticity under normal conditions. It has been suggested that the oscillatory signaling of Group I mGlu receptors, which results in repetitive base-line oscillations in intracellular free Ca^{2+} (with a frequency of 25-40 s for $mGlu_5$ receptors), might be related to repetitive cycles of PKC-dependent phosphorylation and dephosphorylation [91]. Although PKC inhibitors do not counteract Ca^{2+} oscillations [105], an enhancement of $mGlu_5$ receptor function by concomitant stimulation of NMDA receptors has been related to a reversal of a basal PKC-induced $mGlu_5$ receptor desensitization by NMDA receptor-mediated activation of protein phosphatases [65]. More recently, a similar mechanism has been thought to be involved in Group II mGlu receptor-mediated potentiation of the effects of Group I mGlu receptor activation. In this case, however, Group II mGlu receptor stimulation would counteract a PKA-mediated $mGluR_5$ heterologous desensitization [106]. However, a role of PKA in $mGlu_5$ receptor desensitization is controversial, since other studies suggest that PKA activation increases $mGlu_5$ receptor function [79].

PHYSIOLOGY OF ADENOSINE A_{2A} RECEPTORS

Extracellular adenosine depends mostly on the intracellular concentration of adenosine and nucleotides, such as ATP, AMP, cAMP [107]. In some brain areas, like the hippocampus, most of the extracellular adenosine seems to depend mostly on intracellular adenosine, the concentration of which depends on the rate of breakdown and synthesis of ATP [107]. Thus, adenosine is released as a neuromodulator [108] by the effector cells in response to an increased metabolic demand [109]. However, in the striatum, it has recently been suggested that the main source of extracellular adenosine is intracellular cAMP [110], which is metabolized to AMP by means of phosphodiesterases and then to adenosine by the ectoenzyme 5'nucleotidase. Since cAMP can only be generated by the action of the enzyme adenylyl cyclase, striatal extracellular adenosine would mostly reflect an increased activation of receptors positively linked to adenylyl cyclase. The actions of adenosine are mediated by specific G protein receptors. From the four subtypes of adenosine receptors so far identified (A_1, A_{2A}, A_{2B} and A_3) A_{2A} receptors are specially concentrated in the striatum [110-111], where they are mostly localized in the GABAergic striatopallidal neurons, colocalized with dopamine D_2 receptors (see below, and refs. [112,113]. The recent ultrastructural analysis of the localization of A_{2A} receptors in the rat striatum show a very similar localization

to that described for $mGlu_5$ receptors in primates [58]. That is, A_{2A} receptors are mostly localized postsynaptically in the dendrites and dendritic spines of the striatal GABAergic neurons. Importantly, A_{2A} receptor-immunoreactivity is observed primarily at glutamatergic (asymmetric) synapses [114].

The major signal transduction pathway used by A_{2A} receptors depends on the activation of adenylyl cyclase, by means of G_s-G_{olf} coupling [115,116] (see Fig. (**2**). A_{2A} receptor mediated adenylyl cyclase activation generates cAMP, which activates a cAMP-dependent protein kinase (PKA), which in turn regulates the state of phosphorylation of various substrate proteins. One of those proteins, DARPP-32 (dopamine and cyclic adenosine 3', 5'-monophosphate-regulated phophoprotein, 32 kDa) is expressed in very high concentration in the GABAergic efferent neurons [115,116]. PKA-induced phosphorylation at a single site (Thr-34 of the rat sequence) converts DARPP-32 into a potent and selective inhibitor of protein phosphatase-1 (PP-1) [116]. Furthermore, DARPP-32 is, at another site (Thr-75), also phosphorylated under basal conditions, which converts it into an inhibitor of PKA [117]. PKA stimulates protein phosphatase-2A, which is the predominant phosphatase responsible for dephosphorylation of phospho-Thr-75-DARPP-32 [117]. It has been demonstrated that the removal of the inhibitory constraint on PKA by dephosphorylation of phospho-Thr-75-DARPP-32 provides a mechanism of amplification of the PKA signal transduction pathway [117]. Fisone and coworkers have demonstrated that in striatal slices stimulation of A_{2A} receptors produces phosphorylation of DARPP-32 at Thr-34 and dephosphorylation of DARPP-32 at Thr-75 [118-121] (Fig. (**2**)). CREB is another protein phosphorylated by PKA. Induction of cAMP liberates the catalytic subunits of PKA, which diffuse into the nucleus and induce cellular gene expression by phosphorylating CREB at a serine residue (Ser133) [122]. CREB activity declines after a couple of hours of continuous stimulation, due to dephosphorylation at Ser133 by PP-1 [122]. Thus, through PKA activation A_{2A}R stimulation in the GABAergic striatopallidal neurons can potentially produce a sustained increase in the transcription of some CREB modulated genes by a mechanism involving increased CREB phosphorylation and decreased CREB dephosphorylation (through phospho-Thr-34-DARPP-32 mediated inhibition of PP-1 activity) Fig. (**2**). The immediate-early gene *c-fos* and the preproenkephalin and neurotensin genes are very well studied target genes the promoters of which contain consensus sites for CREB-P binding [123-127]. Recent studies have shown that A_{2A} receptor stimulation can, under certain conditions, increase the expression of *c-fos* (and other immediate-early genes), preproenkephalin and neurotensin genes (see below).

In addition to the preferential Gs(olf)-adenylyl cyclase pathway, A_{2A} receptors have also been shown to signal through PLC [128], phosphatidylinositol 3-kinase (PI3K)-Akt [129] and MAPK [130-135] pathways. Depending on the cellular system used A_{2A}-receptor mediated MAPK activation can be dependent on or independent of cAMP accumulation and PKA activation and, therefore, on the initial activation of the adenylyl cyclase pathway. Accumulation of cAMP and activation of MAPK have been shown to produce conflicting cellular responses, such as proliferation, growth arrest, apoptosis and differentiation, depending on the duration, strength of the external stimulus and cell type. These conflicting results seem to depend largely on the differential cross-talk between MAPK and cAMP/PKA and between MAPK and PI-3K/Akt signaling pathways in different situations [136,137]. Instead of the Ras-Raf-1-MEK-ERK1-2 cascade, cAMP accumulation produces activation of another GTPase of the Ras family, Rap1, which leads to the sequential phosphorylation of the Raf isoform B-Raf, MEK and ERK1-2 [138]. Activation of Rap1 by cAMP might depend on both PKA-dependent and -independent mechanisms. PKA-induced phosphorylation of Src is required for PKA to activate Rap1 [139] and a family of cAMP-binding proteins termed cAMP-GEFs or Epacs seems to mediate PKA-independent activation of Rap1 [140,141]. Although Rap1 can bind and activate B-Raf, it can also bind but inactivate Raf-1 (by blocking Ras binding to Raf-1 [138]). Thus, the predominant Raf isoform seems to be the main determinant of the final effect of cAMP on MAPK activation. A predominance of B-Raf or Raf-1 will produce a cAMP-induced increase or decrease of MAPK activation, respectively [142]. Morice *et al.* found a predominant expression of B-Raf versus Raf-1 in the striatum, which was mostly localized in dendrites, which suggests that A_{2A} receptor-induced striatal stimulation of adenylyl cyclase might predominantly lead to MAPK activation [143] (Fig. (**2**)). Nevertheless, A_{2A} receptor stimulation has been shown to produce MAPK activation by a Ras-dependent pathway in cells that did not express B-Raf (HEK 293 cells transfected with A_{2A} receptors; [131]). As demonstrated in the same and other cell lines with the activation of transfected β_2-adrenergic receptors, the mechanism for this Ras-dependent activation of MAPK seems to require $G\beta\gamma$ subunits and activation of Src (see above, and ref. [142]). The differential activation of Src seems to determine the ability of of this non-receptor tyrosine kinase to activate MAPK through either Rap-1 (PKA-dependent phosphorylation) or Ras (PKA-non-dependent phosphorylation) [142]. There is cross-talk between the MAPK and Akt signaling pathways, mostly due to inhibition of the MAPK signaling by Akt-induced phosphorylation of Raf-1 [136,144] and the stimulation of PI3K by Ras [145]. The balance between Akt and MAPK signaling seems to depend mostly on the kind of agonist and the cellular background or stage of differentiation [136]. Finally, Lee *et al.* have shown that A_{2A} receptor stimulation causes PI3K activation by transactivation of Trk neurotrophin receptors by a Ca^{2+}- and Src-dependent mechanism [129] (Fig. (**2**)).

A rapid agonist-induced desensitization of A_{2A} receptors has been described in different cellular systems [146-153]. This rapid desensitization involves A_{2A} receptor phosphorylation and, unlike $mGlu_5$ receptor desensitization (which mostly depends on PKC activation; see above), it is mostly dependent on GRK (homologous desensitization) (Palmer *et al.* 1994; Palmer and Stiles, 1997; 150,152-154). Palmer and Stiles have identified the domain of the A_{2A} receptor specifically responsible for this desensitization in the proximal portion of the C-terminal segment (Thr-298) [154]. An unexpected additional finding of this work was an apparent functional redundancy of the long C-terminal

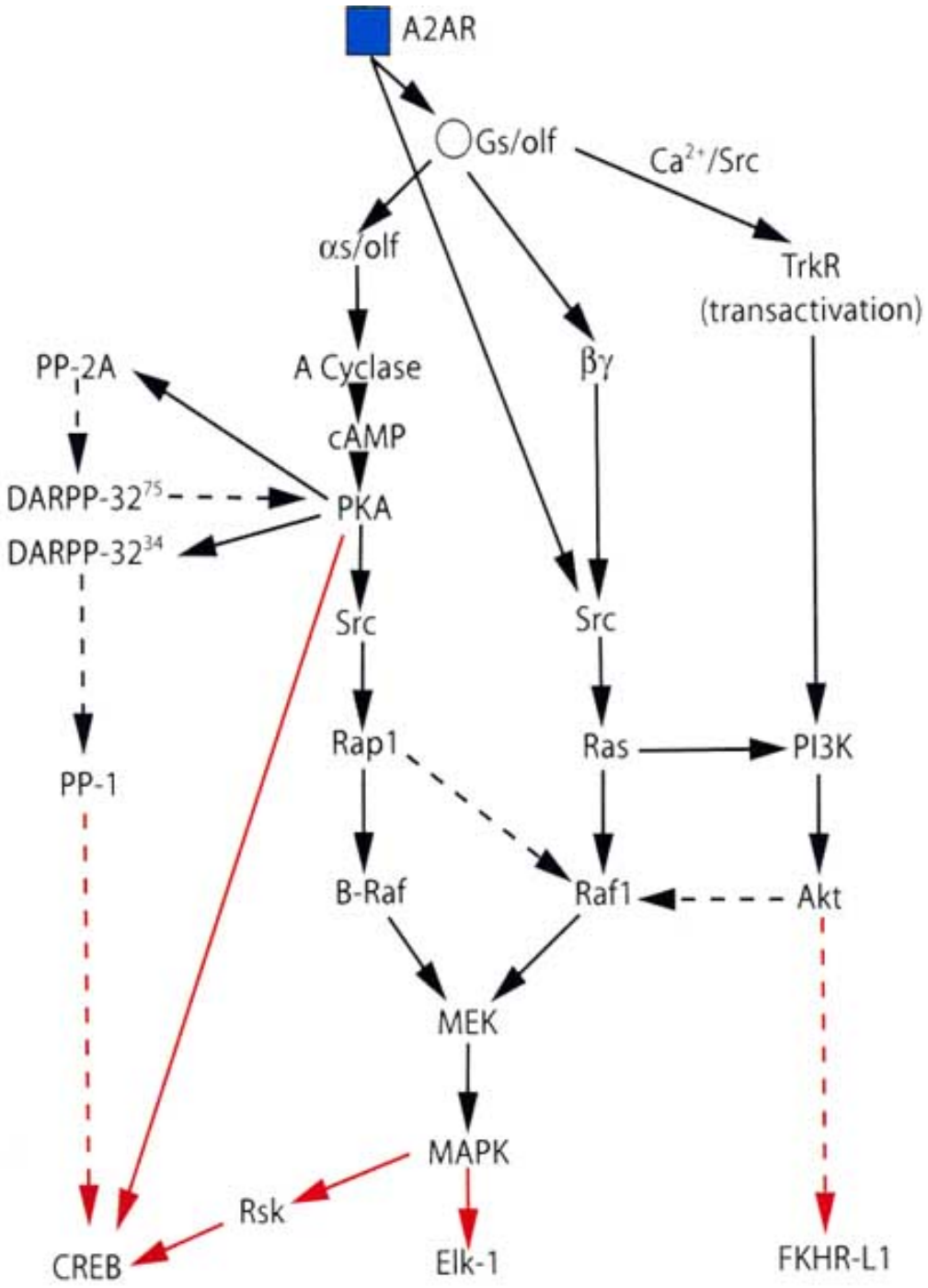

Fig. (2). Signal transduction mechanisms of the adenosine A_{2A} receptor. Solid and broken arrows: stimulatory and inhibitory effects, respectively (see text). Red arrows: signaling into the nucleus. A Cyclase: adenylyl cyclase; Akt: serine-threonine kinase also known as protein kinase B; CREB: cAMP response element binding protein; DARPP-32 dopamine and cAMP-regulated phophoprotein, 32 kDa; Elk-1: member of the ternary complex factor family of transcription factors; FKHR-L1: transcription factor of the Forkhead family; MAPK: mitogen-activated protein kinases; MEK: MAPK kinase; PI3K: phosphatidylinositol 3-kinase; PKA: protein kinase A; PP-1 and PP-2A: protein phosphatases 1 and 2A, respectively; Raf-1 and B-Raf: members of the Raf family of MAPK kinase kinases; Ras and Rap1: members of the small GTPase protein superfamily; Rsk: Ribosomal S6 kinases; Src: member of the Src family of non-receptor tyrosine kinases; TrkR: neurotrophin receptor.

segment of the A_{2A} receptor, distal to the mentioned domain. Removal of this region (95 amino acids) did not affect G protein coupling or the ability of the receptor to desensitize [154]. Selective activation of G proteins by stimulation of the A_{2A} receptor is predominantly dictated by its 3IL (in its N-terminal portion) [155]. The C-terminal segment seems to

be required for the transition of the A_{2A} receptor to the activated state, since its truncation blunts constitutive activity [135]. Finally, the C-teminal segment of the A_{2A} receptor seems to be involved in the formation of A_{2A}-D_2 heteromeric complexes and to the interaction of A_{2A} receptor with the actin cytoskeleton (see below). A longer agonist exposure induces receptor internalization, which has been shown to be a necessary step for either resensitization or down-regulation of A_{2A} receptor through clathrin-coated vesicles [150,152].

PHYSIOLOGY OF DOPAMINE D_2 RECEPTORS

The striatum receives the most dense dopamine innervation and contains the highest concentration of dopamine receptors in the brain [156]. On the basis of morphological and pharmacological characteristics, dopamine receptors are classified as D_1-like, which include D_1 and D_5 receptors, or as D_2-like receptors, which include the long and short isoforms of the D_2 receptor (termed D_{2L} and D_{2S}, respectively), the D_3 and the D_4 receptors [157]. D_2 receptors have a short C-terminal segment and a very long 3IL. Both the N-terminal and C-terminal portions of the 3IL seem to be involved in the G protein coupling of D_2 receptors [158-161]. The two isoforms of D_2 receptors are generated by alternative splicing and D_{2L} differs from D_{2S} by the presence of an additional 29 amino acids within the middle portion of the 3IL [157]. Recent studies suggest that the N-terminal portion of the 3IL of the D_2 receptor is involved in interactions with actin cytoskeleton-binding proteins (see below). Finally, our results suggest that two strings of basic residues of the 3IL of the D_2 receptor establish coulombic interactions with some acidic residues of the distal part of the C-terminal segment of the A_{2A} receptor (in preparation). One string of basic amino acids of the D_2 receptor is also located in the N-terminal portion of the 3IL, while the other string is located in the middle portion of the 3IL of only the D_{2L} (amino acids missing in the D_{2S}). These interactions are most probably involved in the formation of A_{2A}-D_2 heteromeric receptor complexes (see below). Although these two isoforms of D_2 receptors may be differentially phosphorylated and can display differential G protein interactions [162-164], no clear functional differences have been found on signaling. More clear-cut differences have been found, however, on intracellular trafficking. First, the processing to the mature receptor differs between the two isoforms. The D_{2S} receptor is processed faster than the D_{2L} receptor and a significant portion of D_{2L} receptor remains in its partially processed form in an intracellular compartment and does not reach the plasma membrane [165]. More relevant from a therapeutic and patho-physiological point of view is the differential response of D_{2L} and D_{2S} to prolonged exposure to agonists (see below and ref. [166]). In some cell systems both isoforms have been shown to internalize upon agonist exposure by a GRK-β-arrestin dependent mechanism [18,167]. However, the degree of internalization of D_{2S} was greater than for D_{2L} receptors [167]. In fact, in other cell systems, D_{2L} receptors have been found to even up-regulate in response to agonist pretreatment [168-171], which seemed to be related to agonist-induced translocation to the membrane of existing intracellular receptors and to *de novo* receptor synthesis [171]. According to Ng *et al.* [171] this up-regulation might underlie the reported resistance (or slow onset) of D_{2L} receptors to desensitization [37,168-171]. Finally an even more striking difference between D_{2L} and D_{2S} receptors is their differential cellular localization in the brain. Thus, in the striatum, D_{2L} receptors are the postsynaptic D_2 receptors, localized mainly in the GABAergic striatopallidal neurons and, therefore, colocalized with A_{2A} receptors. On the other hand, D_{2S} receptors are the presynaptic D_2 receptors, localized mainly in the dopaminergic cells [172-174].

The dopamine D_2 receptor (both D_{2L} and D_{2S}) has been shown to signal through multiple pathways when studied in different cell systems [157] (Fig. (**3**)). It becomes a challenge, therefore, to determine the main signaling pathways utilized by D_2 receptors in the central nervous system. It was initially thought that inhibition of adenylyl cyclase was the main functional consequence upon agonist-induced activation of D_2 receptors (reviewed in ref. [157]). In fact, this seems to be the predominant D_2 receptor signaling in the striatum. Strong impairment of D_2 receptor function is observed after the genetic inactivation of either PKA or of type 5 adenylyl cyclase (AC5) [175,176], the main adenylyl cyclase isoform found in the striatum [176,177]. In each case there is a loss of the biochemical and behavioral effects of D_2 receptor antagonists [175,176]. D_2 receptor-mediated adenylate cyclase inhibition has also been shown in other cellular systems and seems to be dependent on the coupling of the receptor to Gi proteins [178,179]. Supression of Ca^{2+} currents through L-type voltage dependent Ca^{2+} channels (L-type VDCCs) is one of the best established adenylyl cyclase-independent signaling pathways of D_2 receptors demonstrated in cell lines, lactotroph cells and striatal neurons [179-181]. This pathway seems to involve Go, βγ units, PLC activation, IP3 dependent-intracellular Ca^{2+} mobilization and activation of the Ca^{2+}-dependent serine-threonine phosphatase calcineurin (PP-2A) [180,181] (Fig. (**3**)). Calcineurin has not only been shown to reduce L-type VDCC currents in different cell preparations [181], but to be a main phosphatase involved in dephosphorylation of DARPP-32 (at Thr-34; see above). D_2 receptor activation produces DARPP-32 dephosphorylation both by inhibiting adenylyl cyclase activity and by a Ca^{2+}/calcineurin-dependent and adenylyl cyclase-independent mechanism [182] (Fig. (**3**)).

D_2 receptor stimulation has been shown to activate MAPK in different cell lines [179,183-185], in brain slices [186] and in striatal primary cultures [187]. CaMK, PKC, DARPP-32 and elevated Ca^{2+} seem to be important components of the D_2 receptor-mediated signaling involved in MAPK activation [186]. Interestingly, MAPK activation can only be produced in the dopamine-denervated striatum [188]. Furthermore, MAPK activation might be involved in the sensitized locomotor response to D_2 receptor stimulation in unilateral 6-hydroxydopamine-lesioned rats [188]. However, recent studies have shown that D_2 receptor agonists specifically inhibit the activation of MAPK in the striatopallidal neurons induced by corticostriatal afferent stimulation [189]. According to these studies, the previously reported D_2 receptor agonist-induced MAPK activation in the dopamine-denervated striatum [188] seems to be only

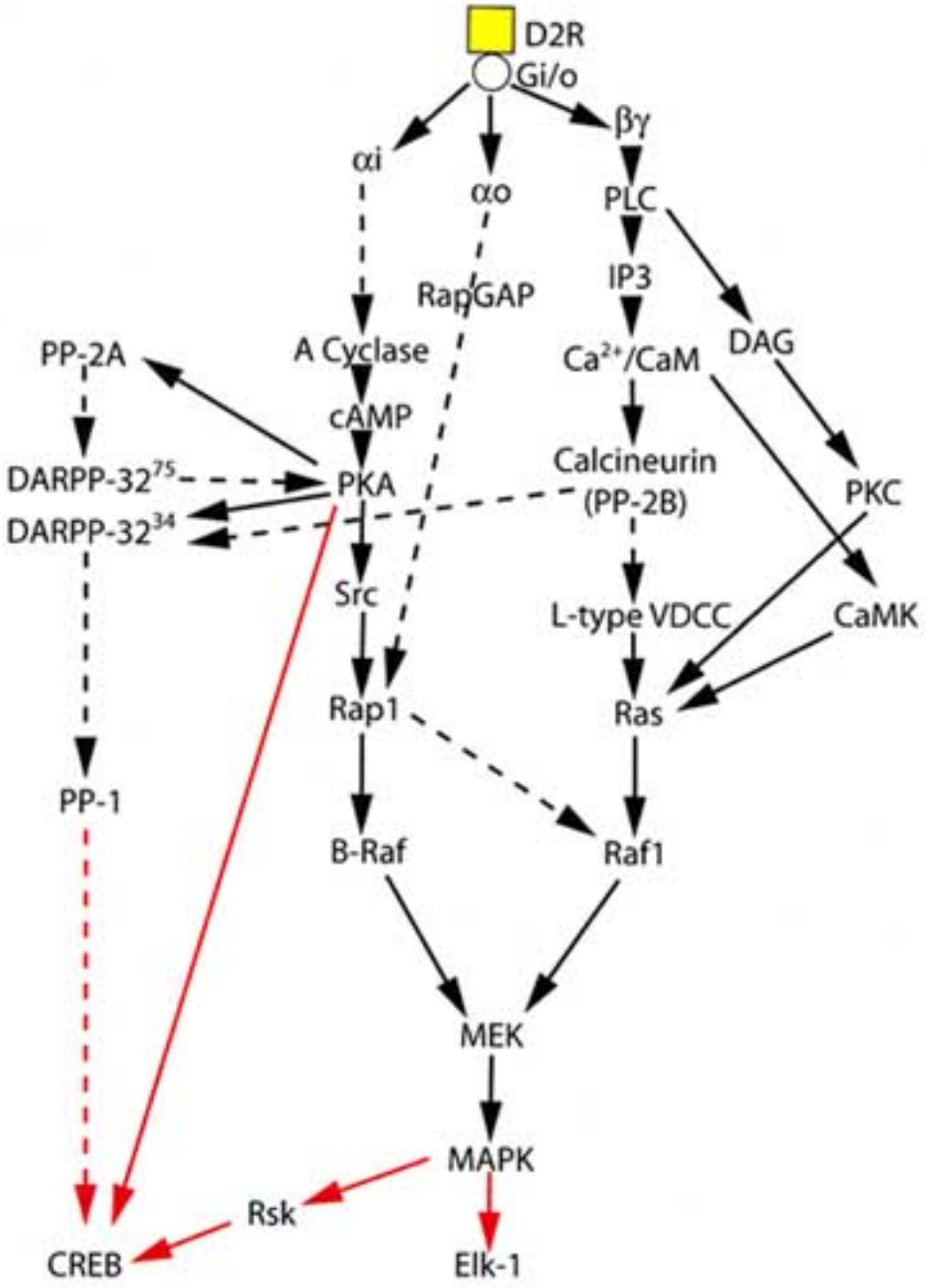

Fig. (3). Signal transduction mechanisms of the dopamine D_2 receptor. Solid and broken arrows: stimulatory and inhibitory effects, respectively (see text). Red arrows: signaling into the nucleus. A Cyclase: adenylyl cyclase; CaM: calmodulin; CaMK: calmodulin kinases; CREB: cAMP response element binding protein; DAG: diacylglycerol; DARPP-32: dopamine and cAMP-regulated phophoprotein, 32 kDa; Elk-1: member of the ternary complex factor family of transcription factors; FKHR-L1: transcription factor of the Forkhead family; IP3: inositol (1,4,5)triphosphate; MAPK: mitogen-activated protein kinases; MEK: MAPK kinase; PKA: protein kinase A; PKC: protein kinase C; PP-1, PP-2A and PP-2B: protein phosphatases 1, 2A and 2B (also called calcineurin), respectively; Raf-1 and B-Raf: members of the Raf family of MAPK kinase kinases; Ras and Rap1: members of the small GTPase protein superfamily; RapGAP: Rap-GTPase activating protein; Rsk: Ribosomal S6 kinases; Src: member of the Src family of non-receptor tyrosine kinases; VDCC: voltage-dependent calcium channel.

induced in striatal interneurons [189]. On the other hand, D_2 receptor antagonist treatment results in a strong and selective MAPK activation in striatopallidal neurons [189]. The same inhibitory effect of D_2 receptor agonists has been observed in pituitary cells and in the prolactin-secreting pituitary cell line GH4ZR7, where the D_2 receptor-mediated inhibitory

modulation of MAPK activation has recently been characterized [180,190]. In these cells, both adenylyl cyclase-dependent and independent mechanisms for the D_2-receptor mediated inhibition of MAPK activation were found and D_2 receptor stimulation not only antagonized stimulatory effects of exogenous hormones (e.g. TRH) on MAPK, but also suppressed basal levels of activated MAPK. Rap 1 inactivation could be an additional mechanism by which D_2 receptor activation counteract an adenylyl cyclase-dependent inhibition of MAPK [180]. This would be related to the demonstrated direct interaction between Go (the Gαo subunit) and a Rap1 GTPase-activating protein (Rap1GAP), which results in the sequestration of Rap1GAP and the consequent increase in activated Rap1. On the other hand, activation of Go would lead to the release of Rap1GAP with the consequent Rap1 inactivation [191] (Fig. (**3**)). Inhibition of Ca^{2+} influx through L-type VDCCs and activation of a phosphotyrosine phosphatase have been proposed as possible mechanisms involved in the adenylyl cyclase-independent D_2 receptor-mediated inhibition of MAPK activation [180,190]. L-type VDCCs play a key role in conveying information from the membrane (neuronal activation) to the nucleus (neuronal plasticity) [192]. Furthermore, this signaling involves the formation of an L-type VDCC/calmodulin complex and the consequent activation of MAPK [192]. Therefore, in the striatopallidal neuron, the D_2 receptor-mediated modulation of L-type VDCC currents [181] might be the main mechanism involved in the adenylyl cyclase-independent D_2 receptor-mediated inhibition of MAPK activation (Fig. (**3**)).

HOMO- AND HETEROMERIZATION OF GLUTAMATE mGLU$_5$, DOPAMINE D$_2$ AND ADENOSINE A$_{2A}$ RECEPTORS

In recent years an increasing number of biochemical (non-denaturing SDS-PAGE, coimmunoprecipitation and biotinylation) and biophysical (FRET, Fluorescence Resonance Energy Transfer, and BRET, Bioluminiscence Resonance Energy Transfer) techniques have revealed the existence of GPCR homo- and heteromerization. Early studies suggested that GPCRs could be present as dimers in the plasma membrane. To date several GPCRs, including the β-adrenergic receptor [193], the δ-opioid receptor [194], the adenosine A_1 receptor [195], the $mGlu_5$ receptor [196,197] and the dopamine D_2 receptor [166,198] have been shown to form dimers in native tissues or when expressed in mammalian cells. Dimerization of the β-adrenergic receptor is important for efficient receptor-G protein coupling [193], whereas for the opioid receptor dimerization seems to have a role in the regulation of receptor internalization [194]. Similarly, different domains of the proteins appear to be responsible for the interaction of the different receptors.

For $mGlu_5$ receptors there is good evidence that the N-terminal domain plus the first transmembrane domain play a key role in the formation of homodimers, suggesting that determinants within these domains are responsible for the interaction of the receptor molecules [196]. Indeed, cysteine residues present within the N-terminal domain are responsible for the disulphide bonding of the dimers [196,197]. Other members of the mGlu receptor family do not form heteromers, even if they belong to the same mGlu receptor group, such as $mGlu_{1a}$ and $mGlu_5$ receptors [196,197]. Furthermore, these receptors do not form heteromers with their different C-terminal tail splice variants, such as $mGlu_{1a}$ and $mGlu_{1b}$ receptors [199]. The existence of dimeric forms of the D_2 receptor was initially shown by means of biochemical techniques. These observations were based on the fact that several homomers are resistant to the denaturation properties of SDS. Thus, it was possible early on by Ng and colleagues to show that the D_2 receptor expressed in cell lines formed molecular species not only corresponding to monomers but also dimers [166,200]. Finally, we have recently found evidence for A_{2A} receptor homodimerization in transiently transfected HEK cells using the classical SDS-PAGE approach. In the same cellular model this homodimerization was corroborated by FRET and BRET experiments (results in preparation). The techniques of FRET and BRET, which require distances as close as 5-10 nM between donor and acceptor for energy transfer, have been very valuable in detecting dimers in living cells without the risk of solubilization artefacts (for review, see ref. [201]). When we isolated the membrane A_{2A} receptor by cell surface protein biotinylation, using a membrane impermeable biotin-ester and streptavidin-agarose affinity precipitation of the membrane proteins, we could demonstrate that the receptor was present as a dimer in the plasma membrane (in preparation).

Gouldson *et al.* hypothesized that domain swapping with involvement of transmembrane regions 5 and 6 is involved in homodimerization of β_2 adrenergic receptors [202]. Peptides corresponding to transmembrane region 6 of the β_2 adrenergic receptor were found to disrupt the dimerization as well as receptor activation, indicating a participation of the hydrophobic forces in the transmembrane region 6 in the dimerization interface. Also, agonist stimulation was found to stabilize the dimeric state of the receptor, while inverse agonists favored the monomeric species; this suggests that interconversion between monomeric and dimeric forms may be important for biological activity [193]. Similarly, peptides corresponding to the D_2 receptor transmembrane regions 6 and 7 dissociated the D_2 receptor dimer. Such peptide actions on the dimer were receptor- and region-specific. Interestingly, ligands did not bind to peptide-dissociated D_2 receptors and D_2 receptor dimers also dissociated as a function of increasing temperature and in the presence of acid environment [200]. Thus, for some GPCRs, such as β_2 adrenergic and D_2 receptors, homodimerization seems to involve specific intermolecular, noncovalent, electrostatic interactions of residues within the transmembrane α−helices. In this model the receptor dimer is rearranged in a much more stable conformation favored by the interaction of the hydrophobic interfaces [203].

The existence of GPCR heteromers was first postulated in 1993 by Zoli *et al.* [204] to provide a molecular explanation to the large amount of evidence obtained on the intramembrane and functional receptor-receptor interactions between GPCRs. Since then, the existence of heteromers of several GPCRs has been reported (see ref. [2]). We have recently demonstrated by means of coimmunoprecipitation experiments that the D_2 and A_{2A} receptors form heteromeric complexes in membrane preparations from D_{2L}-transfected

SH-SY5Y neuroblastoma cells containing native A_{2A} receptor and from mouse fibroblast Ltk$^-$ cells stably transfected with D_{2L} receptors and transiently cotransfected with A_{2A} receptors [37]. By means of double immunofluorescence experiments we showed a high degree of colocalization of these two receptors in the SH-SY5Y human neuroblastoma cells and in cultured striatal neurons [37]. Finally, long term exposure to A_{2A} and D_2 receptor agonists resulted in coaggregation, cointernalization and codesensitization of both receptors [37]. Recently, these results have been corroborated and expanded by means of FRET and BRET techniques in transiently transfected HEK cells with enzymatically/fluorescently tagged A_{2A} and D_2 receptors (results in preparation). We also have preliminary data suggesting a direct interaction between receptors through the C-terminal segment of the A_{2A} receptor and the 3IL of the D_2 receptor. Using peptides corresponding to the 3IL of the D_2 receptor and by means of pull-down experiments we were able to bring down a fusion protein containing the C-terminal tail of the A_{2A} receptor. Similar experiments were performed with the A_{2A} receptor expressed in HEK cells in order to demonstrate that the interaction also takes place with the full-length A_{2A} receptor. Molecular homology modeling of A_{2A} and D_2 receptors, followed by automatic docking simulation, support the involvement of those domains in the formation of A_{2A}-D_2 receptor heteromers (results in preparation). Similarly, we have also demonstrated heteromerization of $mGlu_5$ and A_{2A} receptors by means of Western-blotting of immunoprecipitates of either Flag-A_{2A} or HA-$mGlu_5$ in membrane preparations from co-transfected HEK-293 cells and of native A_{2A} and $mGlu_5$ receptors in rat striatal membrane preparations [133]. Also, by means of double immunofluorescence experiments, overlap in the distribution of the $mGlu_5$ and A_{2A} receptors was found in this cellular model.

It remains to be resolved whether A_{2A} and $mGlu_5$ receptors are physically associated by means of direct protein-protein interactions (true heterodimers) or they are linked via additional cytosolic proteins. Thus, interactions of the receptors' cytoplasmic C-termini or intracellular loops with specific targeting proteins provide an alternative mechanism for GPCR heteromerization. The best example is the coupling of NMDA with Group I mGlu receptors by means of PSD-95-Shank-GKAP protein complexes (see above). Furthemore, through these interactions NMDA and Group I mGlu receptors are connected to the cytoskeleton, since Shank also binds to cortactin, which directly interacts with actin [60]. Also, filamin-A, a member of the α-actinin/spectrin/dystrophin family of actin-binding proteins has recently been identified as a binding partner of $mGlu_5$ receptors. Filamin-A is a ubiquitously expressed dimeric actin-cross-linking phosphoprotein that promotes orthogonal branching of actin filaments, contributing to the formation of the actin meshwork adjacent to the surface cell membrane and linking membrane glycoproteins to the actin cytoskeleton [205]. Several dopamine D_2 receptor-interacting proteins, including filamin-A, have been identified. It is the N-terminal portion of the 3IL of the D_2 receptor that interacts with filamin-A [206]. This interaction, like that for the $mGlu_5$ receptor, may be important for establishing the correct subcellular localization of D_2 receptor. Similarly, it has been demonstrated that the N-terminal portion of the 3IL of the D_2 receptor also interacts with protein 4.1N [207], a recently identified member of the 4.1 family of cytoskeletal-associated proteins that is specifically enriched in neurons. The 4.1 proteins are critical components of the spectrin/actin cytoskeleton and provide attachement between cytoskeleton and the cell membranes. Protein 4.1N has been shown to directly interact with the GluR1 subunit of the AMPA receptor and to colocalize with AMPA receptors at excitatory synapses [208]. It is suggested that protein 4.1N-D_2 receptor interaction is required for localization or stability of dopamine receptors at the neuronal plasma membrane [207]. Spinophilin, a recently characterized F-actin and PP-1-binding protein with a single PDZ domain is also a protein that interacts with the 3IL of D_2 receptor [209]. It has been suggested that spinophilin might also be important for linking D_2 receptors to downstream signaling molecules and the actin cytoskeleton [209]. Currently and by similar approaches we are searching proteins that interact directly with the C-terminal tail of the A_{2A} receptor. An actin binding protein, actinin, is a potential partner of the A_{2A} receptor C-terminal tail. Actinin, like filamin-A, is an actin crosslinking protein that shares similar properties including the formation of antiparallel homodimers (Burgueno *et al.*, in preparation). In conclusion, the actin cytoskeleton may serve as a platform for assembling GPCRs in specific plasma membrane domains and also may participate in stabilizing direct interactions between these receptors necesaries for the formation of homo- and heteromers of GPCRs. It is important to note that the Homer-Shank-actin cytoskeleton interaction involves a linkage of mGlu receptors, and potentially adenosine and dopamine receptors, with the NMDA receptor scaffold. Thus, the Homer-Shank-actin cytoskeleton interaction could be of special importance in processes of synapse formation and plasticity, where the docking of mGlu, adenosine and dopamine receptors to a pre-established NMDA receptor-associated PSD-95 complex could play a critical role (see above). On the other hand, this physical association between NMDA, mGlu, adenosine and dopamine receptors could be important in the fine tuning of glutamate-adenosine-dopamine cross-talk which is described below (see also Fig. (**4**)).

GLUTAMATE $mGLU_5$ -ADENOSINE A_{2A}-DOPAMINE D_2 RECEPTOR INTERACTIONS IN THE STRIATUM. IMPLICATIONS FOR DRUG THERAPY IN NEURO-PSYCHIATRIC DISORDERS

The basic intrastriatal circuitry is quite simple, since GABAergic projection neurons, constitute more that 90% of the striatal neuronal population. The GABAergic projection neurons mainly convey information carried by glutamatergic cells located in cortical and limbic areas, like the amygdala and hippocampus, and by dopaminergic mesencephalic cells, which are located in the substantia nigra and ventral tegmental area, and [210,211]. There are two subtypes of striatal GABAergic efferent neurons, the striatopallidal and strioentopeduncular neurons. As mentioned before, adenosine A_{2A} and dopamine D_2 (D_{2L}) receptors are colocalized in the striatopallidal GABAergic neurons, which also contain the peptide enkephalin. The other subtype of striatal GABAergic efferent neurons, the striatoento-

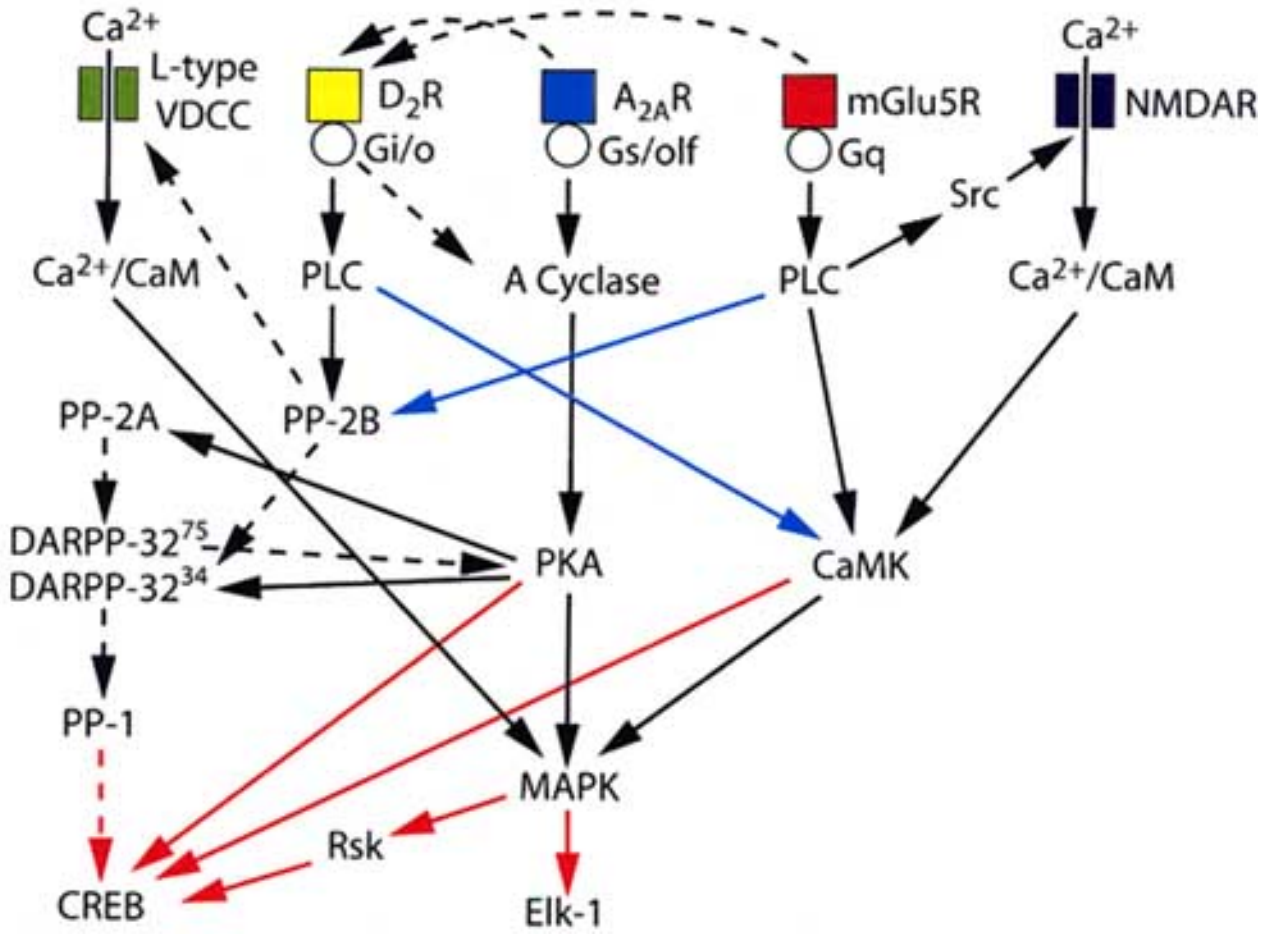

Fig. (4). Possible mechanisms involved in the multiple biochemical interactions between dopamine D_2 receptors, adenosine A_{2A} receptors and $mGlu_5$ receptors (see text). Antagonistic A_{2A}-D_2 receptor and $mGlu_5$-D_2 receptor interactions have been demonstrated at the intramembrane level and a strong antagonistic D_2-A_{2A} receptor interaction has been found at the adenylyl-cyclase level. MAPK seems to be a main coincidental signal involved in synergistic A_{2A}-$mGlu_5$ receptor interactions. D_2 receptors stimulation counteracts A_{2A} and $mGlu_5$ receptor-mediated MAPK activation by acting at different levels (adenylyl cyclase, PKA and through inhibition of L-type VDCCs). Some of the effects of $mGlu_5$ receptors are mediated by modulating NMDA receptor function. Solid and broken arrows: stimulatory and inhibitory effects, respectively. Blue arrows: Ovious but not predominant signaling pathways. Red arrows: signaling into the nucleus. CaM: calmodulin; CaMK: calmodulin kinases; CREB: cAMP response element binding protein; Elk-1: member of the ternary complex factor family of transcription factors; DARPP-32: dopamine and cAMP-regulated phophoprotein, 32 kDa; MAPK: mitogen-activated protein kinases; NMDAR: NMDA receptor; PLC: phospholipase C; PKA: protein kinase A; PKC: protein kinase C; PP-1, PP-2A and PP-2B: protein phosphatases 1, 2A and 2B (also called calcineurin); Rsk: Ribosomal S6 kinases; VDCC: voltage-dependent calcium channel.

peduncular GABAergic neurons, contain the peptides dynorphin and substance P and dopamine receptors predominantly of the D_1 subtype (reviewed in ref. [6]). Striatal $mGlu_5$ receptors are mostly localized in the striatal GABAergic efferent neurons, but without an obvious preference for either the striatopallidal or the striatoentopeduncular subtypes [212- 2134]. In any case, a distinct colocalization of $mGlu_5$, A_{2A} and D_2 receptors exists in the dendritic spines of the striatopallidal neurons (see above and ref. [2]). Stimulation of the striatoentopeduncular GABAergic neurons results in motor activation and stimulation of the striatopallidal neurons produces motor inhibition. Dopamine, or dopamine agonists, will induce motor activation by activating the striatoentopeduncular neurons (acting on stimulatory D_1 receptors) and by depressing the striatopallidal neurons (acting on inhibitory D_2 receptors) [211,215]. In Parkinson's disease a preferential degeneration of the nigrostriatal dopaminergic system produces striatal dopamine depletion with the consequent impairment of the functioning of these circuits, which is associated with hypokinesia. Hyperactivity of the GABAergic striatopallidal neurons due to the release from the strong D_2 receptor mediated tonic inhibitory effects of endogenous dopamine is probably the main pathophysiological mechanism responsible for this hypokinesia [216].

The GABAergic striatopallidal neurons that originate in the ventral part of the striatum, the nucleus accumbens (NAc), play a key role in the conversion of motivation into action *via* transfer of information from the limbic to the motor system (for review, see ref. [5]). In fact, it is a final common pathway for opiate and psychostimulant mediated reward [217] and a common target for antipsychotic drugs [5]. Thus, blockade of D_2 receptors in the ventral GABAergic striatopallidal neurons seems to be involved with the antipsychotic effects of neuroleptics or, at least,

with their therapeutic effect on the positive symptoms of schizophrenia. On the other hand, blockade of D_2 receptors in the dorsal GABAergic striatopallidal neuron is associated with the extrapyramidal side effects of neuroleptics, such as parkinsonism. One of the possible properties of atypical neuroleptics, which have a decreased liability of those side effects, is a preferential D_2 receptor blockade in the ventral striatum (see below).

The formation of receptor complexes modifies the single receptor transducing characteristics and leads to the appearance of "emergent properties" [218]. The study of glutamate $mGlu_5$-adenosine A_{2A}-dopamine D_2 receptor interactions in the striatum reveals new properties of these GPCRs and gives indications for a new rational approach for drug therapy in neuro-psychiatric disorders. As previously discussed, evidence for the existence of physical interactions between A_{2A} and D_2 receptors and between A_{2A} and $mGlu_5$ receptors has been obtained with coimmunprecipitation experiments in cell lines which express, constitutively, or after stable or transient cotransfection, the corresponding receptors [37,133]. A_{2A}-$mGlu_5$ heteromeric receptor complexes have also been demonstrated in rat striatal membrane preparations with coimmunoprecipitation experiments [133]. Different behavioral and biochemical models have demonstrated the existence of functionally significant antagonistic A_{2A}-D_2 receptor and $mGlu_5$-D_2 receptor interactions and synergistic A_{2A}-$mGlu_5$ receptor interactions. At the biochemical level, by using membrane preparations of rat striatum or of cell lines expressing the corresponding receptors, and by using receptor autoradiography in rat and human striatal sections, it has been shown that stimulation of A_{2A} receptors decreases the affinity of D_2 receptors for dopamine or dopamine agonists [115,219-226]. Also, in membrane preparations from rat striatum, non-selective stimulation of Group I mGlu receptors or selective stimulation of $mGlu_5$ receptors decreased the affinity of D_2 receptors for dopamine [221,227,228]. Finally, costimulation of A_{2A} receptors and Group I mGlu receptors or $mGlu_5$ receptors exerts a synergistic effect on the modulation of the binding characteristics of D_2 receptors [221,227,228].

In addition to the intramembrane receptor-receptor interactions, the existence of A_{2A}-D_2 and A_{2A}-$mGlu_5$ heteromeric receptor complexes provides the possibility for close cross talk between A_{2A}, D_2 and $mGlu_5$ receptors (Fig. (**4**)). A strong antagonistic interaction between A_{2A} receptors and D_2 receptors at the adenylyl cyclase level has been demonstrated in different cell lines [37,115]. In CHO cells stably cotransfected with A_{2A} and D_2 receptor cDNAs, stimulation of A_{2A} receptors produced a strong stimulation of cAMP, CREB phosphorylation and increased *c-fos* expression [115]. A selective D_2 receptor agonist dose-dependently counteracts these effects and a complete blockade is attained at low concentrations of the D_2 receptor agonist [115]. Similarly, in striatal slices a D_2 receptor agonist completely counteracts Thr-34 phosphorylation of DARPP-32 by A_{2A} receptor stimulation [120]. Striatal D_2 receptors are tonically stimulated by basal endogenous levels of dopamine, and many experimental results indicate that this tonic D_2 receptor stimulation strongly counteracts the tonic A_{2A} receptor stimulation induced by the basal striatal levels of adenosine. Through this antagonistic D_2-A_{2A} receptor interaction, some products of A_{2A} receptor activation are kept at a low concentration under normal basal conditions. Inactivation of striatal D_2 receptor-mediated neurotransmission (by administration of D_2 receptor antagonists, striatal dopamine depletion or genetic D_2 receptor inactivation) liberates A_{2A} receptor-mediated function from the strong D_2 receptor-mediated tonic inhibition. This results in a very significant increase in the striatal expression of c-Fos, phospho-Thr-34-DARPP-32, enkephalin and neurotensin, all of which are partially counteracted by genetic inactivation or pharmacological blockade of A_{2A} receptors [119,175,229-240]. At the behavioral level A_{2A} receptor antagonists also counteract the motor depressant and cataleptic effects secondary to the genetic inactivation or pharmacological interruption of D_2 receptor mediated neurotransmission (see below). An important part of the biochemical and behavioral effects induced by interruption of D_2 receptor-mediated neurotransmission seems, therefore, to be due to the liberation of A_{2A} receptor signaling. This can have implications for the possible application of A_{2A} receptor antagonists in Parkinson's disease (see below). On the other hand, the biochemical effects related to adenylyl cyclase activation, which are only clearly apparent with the removal of the D_2 receptor-mediated inhibitory tone, do not seem to play a major role in some functional and behavioral effects produced by the administration of A_{2A} receptor agonists and antagonists. For instance, the systemic administration of a low dose of a selective A_{2A} receptor agonist produces a pronounced motor depression (already shown to be centrally mediated; see ref. [241]) and selectively counteracts D_2 receptor agonist mediated behaviours [242,243] without inducing an increased *c-fos* striatal expression [133,230,233]. In fact, AC5 knockout mice (see above) have a loss of A_{2A} and D_2 receptor-mediated adenylyl-cyclase signaling, loss of behavioral effects of D_2 receptor antagonists and preservation of the motor depression induced by A_{2A} receptor agonists [176]. Most probably the adenylyl cyclase-independent behavioral effects of A_{2A} receptor agonists are dependent on adenylyl cyclase-independent signaling pathways (see above) or to the reciprocal antagonistic A_{2A}-D_2 receptor intramembrane interaction, which could be more effective at modulating other D_2 receptor-mediated signaling pathways, such as the decrease in L-type VDCC currents (see above) (Fig. (**4**)).

As mentioned above, the systemic administration of A_{2A} receptor antagonists counteracts most of the biochemical (see above) as well as the motor depressant and cataleptic effects secondary to the genetic inactivation or pharmacological interruption of D_2 receptor mediated neurotransmission. This has been repeatedly demonstrated in a number of experimental models involving rodents pretreated with D_2 receptor antagonists, reserpine or MPTP or after genetic inactivation of D_2 receptors [231,237,238,244-250] or involving MPTP-treated monkeys [251,252]. These experiments suggest that A_{2A} receptor antagonists might be useful in the treatment of Parkinson's disease. However, although it has been claimed that A_{2A} receptor antagonists might be devoid of the secondary dyskinetic effects associated with treatment with dopamine agonists [251,252], it is still a matter of debate as to whether they could be used alone or if they would be more efficacious when combined

with D_2 receptor agonists [248]. In fact, in another classical experimental model of Parkinson's disease, the rat with a unilateral lesion of the nigrostriatal pathway [253], A_{2A} receptor antagonists do not produce the behaviour that predicts antiparkinsonian activity: turning behavior away from the lesioned side [248,254,255]. Nevertheless, in all animal models of Parkinson's disease thus far tested (reserpinized mice, rats with unilateral 6-OH-dopamine lesions, MPTP-treated monkeys) A_{2A} receptor antagonists strongly potentiate the motor activation induced by D_2 receptor agonists [228,248,252,255-259]. Importantly, co-treatment with A_{2A} receptor antagonists and L-DOPA does not increase the non-wanted dyskinetic effects in MPTP-treated monkeys [252,257]. Finally, recent electrophysiological experiments in the rat dopamine denervated striatum showed that the infusion of an A_{2A} receptor antagonist does not produce any effect on its own, but strongly potentiates the D_2 receptor agonist-induced inhibition of striatal neuronal activity [255]. Altogether, these results suggest that some pharmacological and, maybe, therapeutic effects secondary to A_{2A} receptor blockade can only be observed with the concomitant stimulation of D_2 receptors. This is also in agreement with some results obtained with D_2 receptor knockout mice where the motor effects of A_{2A} receptor antagonists were attenuated [238]. These experiments, performed under complete inactivation of D_2 receptors, demonstrate that some A_{2A} receptor functions are dependent on the integrity of D_2 receptors. This was shown even more dramatically in recent studies by Zahniser *et al.* where a very significant functional uncoupling of A_{2A} receptors (lack of A_{2A} receptor agonist induced GABA release in striatal-pallidal slices) was found in D_2 receptor knockout mice [239]. On the other hand, decreased striatal dopamine release and decreased psychostimulant-induced motor activation (functional striatal hypodopaminergic activity) has been found in mice lacking A_{2A} receptors [240,260].

We are beginning to understand the physiological conditions (where there is no interruption of D_2 receptor mediated neurotransmission) that allow A_{2A} receptor stimulation to produce a clear adenylyl cyclase activation, with the corresponding increase in phospho-Thr-34-DARPP-32 and CREB phosphorylation and increases in the expression of *c-fos* and the preproenkephalin and neurotensin genes. One such condition seems to be $mGlu_5$ receptor co-activation [133]. In HEK-293 cells transiently transfected with A_{2A} and $mGlu_5$ receptors stimulation of A_{2A} receptors produced an increase in cAMP levels that was not significantly modified by the concomitant stimulation of $mGlu_5$ receptors [133]. Stimulation of either $mGlu_5$ or A_{2A} receptors induces an increase in $[Ca^{2+}]_i$ but, again, there is no synergistic A_{2A}-$mGlu_5$ receptor interaction [133]. Nevertheless, there is a strong functional synergistic A_{2A}-$mGlu_5$ receptor interaction on MAPK and on *c-fos* expression. Importantly, the effect on c-fos expression was counteracted by incubation with the MEK inhibitor PD98059 [133]. A significant *c-fos* induction is also obtained in the rat striatum, preferentially in the NAc, after the concomitant central administration of A_{2A} and $mGlu_5$ receptor agonists, which is ineffective when they are administered alone [133]. MAPK seems, therefore, to be a main biochemical integration element responsible for the synergistic interactions between A_{2A} and $mGlu_5$ receptors (Fig. (**4**)). Alternatively. There might be levels of interaction upstream from MAPK, such as non-receptor tyrosine kinases or other enzymes recently demonstrated to be activated by GPCRs through G protein-independent signaling (see above). In any case, concomitant stimulation of A_{2A} and $mGlu_5$ receptors seems to allow A_{2A} receptor stimulation to overcome the tonic inhibitory effect of dopamine and induce striatal *c-fos* expression. We favor the idea that this mechanism can take place under conditions of intense glutamatergic neurotransmission, which is known to induce adenosine release, most probably due to the neuronal metabolic demand imposed by the increased excitatory input [107,109]. This is also consistent with previous studies showing that corticostriatal stimulation produces selective MAPK activation and induction of immediate early genes in the striatopallidal neurons [189,261,262]. That MAPK activation is responsible for the striatal induction of immediate early genes after corticostriatal stimulation is supported by the reported counteracting effects of MEK inhibitors [263].

The role of $mGlu_5$ receptors and their interactions with A_{2A} and D_2 receptors in animal models of Parkinson's disease is beginning to be evaluated. In unilaterally 6-OH-dopamine-lesioned rats, the intracerebral administration of a selective $mGlu_5$ receptor agonist selectively inhibits the contralateral turning induced by a D_2 receptor agonist [228]. The effect of the $mGlu_5$ agonist is potentiated by an A_{2A} receptor agonist and attenuated by an A_{2A} receptor antagonist [228]. These results suggest that $mGlu_5$ receptor antagonists, alone or in combination with A_{2A} receptor antagonists or D_2 receptor agonists or both, might provide a new therapeutic approach for basal ganglia disorders, such as Parkinson's disease. In fact, some studies have recently shown antiparkinsonian-like effects of $mGlu_5$ antagonists in rats [264].

Preferential blockade of D_2 receptors in the ventral striatum (with relative sparing of D_2 receptors in the dorsal striatum) is one of the main factors responsible for the atypical profile of an antipsychotic. Typical antipsychotics, like haloperidol, increase *c-fos* expression in the dorsal and ventral striatum, while atypical antipsychotics, like clozapine, selectively elevates *c-fos* expression in the ventral striatum, and specially in the shell of the nucleus accumbens [234,265-267]. A similar differential response to haloperidol and clozapine has also been demonstrated for the striatal expression of neurotensin [267]. It has also been shown that the increased *c-fos* expression in the ventral striatum induced by both haloperidol and clozapine selectively takes place in the ventral GABAergic striatopallidal neuron [268] and that it can be counteracted by the systemic administration of an A_{2A} receptor antagonist [234]. These results raise the possibility that A_{2A} receptors might be involved in the mediation of the antipsychotic effects of neuroleptics. The D_2, A_{2A} and $mGlu_5$ receptor-mediated modulation of GABAergic neurotransmission in the ventral striatopallidal neurons has been studied with the dual-probe *in vivo* microdialysis technique [220,269]. The perfusion of a D_2 receptor antagonist, an A_{2A} receptor agonist or a $mGlu_5$ receptor agonist in the NAc increases GABA levels in the ipsilateral ventral pallidum [220,269]. Co-perfusion of A_{2A} receptor agonist and D_2 receptor antagonist or co-perfusion

of A_{2A} and $mGlu_5$ receptor agonists produces synergistic increases in pallidal GABA [220,269]. Finally, a D_2 receptor agonist completely counteracts the effect of the A_{2A} receptor agonist and partially counteracts the effect of A_{2A} plus $mGlu_5$ receptor agonists [269]. From these results, we have suggested a possible use of A_{2A} receptor agonists as antipsychotic drugs, alone or in combination with D_2 receptor antagonists, which could imply a reduction in the secondary effects of the already established antipsychotic [220]. In fact, the systemic administration of an A_{2A} receptor agonist gives the pharmacological profile of an atypical antipsychotic in several animal models [242,243,270]. Another possible therapetic approach is the use of $mGlu_5$ receptor agonists, alone or in combination with A_{2A} receptor agonists, D_2 receptor antagonists, or the combination of the two.

GLUTAMATE $mGLU_5$-ADENOSINE A_{2A}-DOPAMINE D_2 RECEPTOR INTERACTIONS IN THE STRIATUM. IMPLICATIONS FOR NEURO-ALTERATIONS IN DRUG USE AND ABUSE

An important variable when targeting GPCRs in drug therapy is the possible receptor adaptations due to long exposure to agonists. A clear example is the potential application of A_{2A} receptor agonists in hypertension. A_{2A} receptors are present in the cardiovascular system and their stimulation induces hypotension [271,272]. Continuous administration of an A_{2A} receptor agonist causes rapid and complete tolerance to the drug's hypotensive effects trough downregulation of A2A receptors [273]. Tolerance may limit the potential use of A_{2A} agonists not only in hypertension, but also in schizophrenia (see above and ref. [5]). As summarized above, D_2 receptors are established targets for drug therapy in the treatment of neuro-psychiatric disorders, such as Parkinson's disease and schizoprenia. It was already mentioned that in cell lines the D_{2L} receptor isoform is resistant to desensitization and that long-term agonist exposure is associated with up-regulation of D_{2L} receptors. On the other hand the D_{2S} isoform always down-regulates and shows faster and stronger desensitization (see above and refs. [168-171]. It was also mentioned that striatal D_{2L} receptors are localized postsynaptically, mainly in the GABAergic striatopallidal neurons; therefore, they are the ones colocalized with A_{2A} receptors. D_{2S} receptors are presynaptic D_2 receptors localized mainly in the dopaminergic cells [172-174]. Thus, now it remains to be established which are the adaptations of both D_2 receptor isoforms that take place in the brain with long-term agonist exposure.

There are already experimental data which support that the D_2 autoreceptors (D_{2S} receptors) localized in dopaminergic cells are very sensitive to down-regulation upon exposure to selective D_2 receptor agonists [274-276] or to indirect dopamine agonists, such as the psychostimulants cocaine or amphetamine. Thus, repeated cocaine or amphetamine administration is associated to subsensitivity of both axonal and somatodendritic D_2 autoreceptors (reviewed in ref. [277]). D_2 autoreceptors exert an inhibitory effect on firing, dopamine synthesis and dopamine release of dopaminergic cells [274-276] and they seem to play a crucial role in mediating the autoinhibition observed in conditions of high extracellular dopamine concentration, such as those obtained with the administration of psychostimulants [278]. D_2 autoreceptor subsensitivity seems to be the main mechanism involved in the increase in dopamine release induced by repeated administration of psychostimulants [277] and seems to involve a reduction in Gi and Go levels [279] in addition to receptor down-regulation. Behavioral sensitization to psychostimulants makes reference to the increased motor stimulant effects to these drugs upon subsequent exposure. Core features of this sensitization are its long-lasting quality, with long-lasting or irreversible neurobiological alterations [280], cross-sensitization between different psychostimulants and environmental stressors [281,282] and faster development in a novel environment [283]. Although in behavioral sensitization to psychostimulants a good correlation exists between motor activation and striatal dopamine release, mechanisms other than D_2 autoreceptor subsensitivity seem to be involved, which also vary among different psychostimulants [277]. Nevertheless, all mechanisms involved in behavioral sensitization seem to result from time-dependent neuro-alterations within the circuitry that includes the dopaminergic cells that innervate the nucleus accumbens. In fact, evidence also exists for an increased sensitivity of postsynaptic D_2 receptors to agonists with repeated administration of cocaine and heroin [284]. These effects seem to be especially salient during the early phases of drug withdrawal [284], a period during which releasability of dopamine is often diminished or unaffected [277]. The period of behavioral sensitization of D_2 agonists is also associated to D_2 receptor agonist-mediated relapse to cocaine- and heroin- seeking behaviour [284]. These results suggest that the increased sensitivity of D_2 receptors (most probably D_{2L}) to agonists induced by drugs of abuse may play a permissive role in drug-induced relapse behavior. In fact, stimulation of D_2 receptors (but not D_3 or D_1 receptors) appears to be necessary for induction of relapse to cocaine-seeking behaviour in non-human primates [285].

Psychostimulant-induced psychosis in humans has been suggested to be related to behavioral sensitization to psychostimulants in animals (for review, see ref. [286]). Subjects who experience methamphetamine psychosis when chronic abusers remain vulnerable to methamphetamine-induced psychosis even after years of withdrawal [287]. In these subjects a low dose of methamphetamine or a stressful event can produce a recurrence of a psychotic episode [288]. Furthermore, an "endogenous" sensitization to psycho-stimulants has also been demonstrated in schizophrenic patients (reviewed in ref. [286]) and brain imaging studies have shown evidence for increased amphetamine-induced striatal dopamine release in schizophrenia [289,290]. Therefore, similar patho-physiological mechanisms might take place in psychostimulant-induced psychosis and in schizophrenia, which could share some of the neuro-alterations associated with behavioral sensitization to psychostimulants in animals. Laruelle [286] suggests that a common mechanism leading to these neuro-alterations is an initial period of excess dopaminergic transmission, which leaves long-lasting alterations in the brain. One main alteration is a sensitization of the dopaminergic systems, which might depend on both pre- and post-synaptic changes in D_2 receptors, such as subsensitivity of D_2 autoreceptors

(D_{2S}) and supersensitivity of post-synaptic D_2 receptors (D_{2L}) to agonists (or endogenous dopamine). In schizophrenia, it is suggested that the initial period could be related to a dysregulation of dopaminergic neurons of the ventral tegmental area, most probably involving their glutamatergic afferents from the prefrontal cortex. Thus, these glutamatergic neurons are hypothesized to exert an important role in the induction of sensitization to psychostimulants [291,292]. Furthermore, a neonatal lesion of the medial temporal lobe, a process that mimics some neurodevelopmental changes of the schizophrenic brain [293], disrupts the prefrontal cortical regulation of striatal dopamine [294].

One more pathological state to be considered as a manifestation of sensitization to the effects of dopaminergic agonists is the L-DOPA-induced motor response complications of patients with Parkinson's disease and, in particular L-DOPA-induced dyskinesia. Striatal dopamine denervation and intermittent or pulsatile dopamine-receptor stimulation seem to be the two main conditions necessary for the development of L-DOPA-induced dyskinesia [295,296]. It is very well established that dopamine denervation leads to an increased sensitivity of striatal dopamine receptors to the effects of agonists [253]. Both post-synaptic D_1 and D_2 receptors are involved and D_2 receptor supersensitivity seems to be mediated by both receptor upregulation and enhanced coupling to G proteins [297-300]. Similarly, in untreated parkinsonian patients most studies suggest that there is an upregulation of D_2 receptors (see, for instance, refs. [301,302]). Some experimental findings in the unilateral 6-OH-dopamine-lesioned rat indicate that chronic treatment with high doses of L-DOPA can only produce a partial recovery of the denervation-induced supersensitivity of striatal post-synaptic receptors to agonists [303]. In agreement, in unilateral 6-OH-dopamine-lesioned rats, high doses of L-DOPA do not affect enkephalin levels or preproenkephalin mRNA in striopallidal GABAergic neurons [304,305]. This demonstrates the existence of the previously hypothesized irreversibility of denervation-induced supersensitivity of striatal dopaminergic receptors [303], which might be related to the resistance of post-synaptic D_2 receptors (D_{2L}) to down-regulation. Also in rats with unilateral striatal dopamine denervation, lower doses or intermittent L-DOPA treatment induces behavioral sensitization (turning behavior and abnormal involuntary movements [AIM]), with increased turning behaviour induced by L-DOPA and with cross-sensitization to apormorphine or to the D_2 receptor agonist bromocriptine [306-310]. The analysis of apomorphine-induced *c-fos* expression in the denervated striatum and ipsilateral globus pallidus [309] strongly suggests that L-DOPA pre-treatment increases and decreases the denervation-induced sensitization of D_2 and D_1 receptors, respectively, as previously postulated [306]. Thus, an increased sensitivity of the D_2 receptor-containing striatopallidal GABAergic neurons play an important role in the development of L-DOPA-induced dyskinesia. However, the role of the D_1 receptor-containing striatoentopeduncular GABAergic neurons cannot be understimated. Phenotypical changes of these neurons, including increased expression of prodynorphin mRNA and D_3 receptor upregulation, have also been demonstrated in animal models of Parkinson's disease treated with L-DOPA [305,311,312]. In fact, similar changes have also been suggested to be involved in sensitization to psychostimulants (reviewed in ref. [312]).

Altogheter, the above-mentioned findings allow us to postulate the following working hypothesis: an increased sensitivity of D_{2L} receptors to agonists in the striatopallidal neurons is a common patho-physiological mechanim involved in sensitization to psychostimulants and L-DOPA-induced dyskinesias. As described before, we recently found evidence for co-aggregation, co-internalization and co-desensitization of both A_{2A} and D_{2L} receptors upon agonist exposure [37]. Importantly, under our experimental conditions, D_{2L} receptor stimulation was only associated to significant desensitization upon concomitant stimulation of A_{2A} receptors [37]. Co-stimulation of A_{2A} and D_2 receptors, by inducing desensitization of D_{2L} receptors could be of therapeutic use in sensitization to psychostimulants and L-DOPA-induced dyskinesias. In fact, evidence in favor of this hypothesis has already obtained in animal models. Shimazoe *et al.* found that A_{2A} but not A_1 receptor agonists avoid the development of methamphetamine-induced sensitization [313]. Similarly, we have found that A_{2A} receptor agonist co-treatment avoids the development, but not the expression of L-DOPA-induced behavioral sensitization (turning and AIM) in rats with unilateral striatal dopamine denervation (in preparation). Our results seem to be in contradiction with findings of Freduzzi *et al.* in A_{2A} receptor knockout mice with unilateral striatal dopamine denervation, which do not develop sensitization to L-DOPA-induced turning behavior [314]. The different results could be related to the developmental adaptations of the A_{2A} receptor knockout mouse brain, which includes a functional striatal hypodopaminergic activity (see above and refs. [240,260]. Our preliminary results also indicate that once L-DOPA-induced behavioral sensitization is established, it cannot be reversed by A_{2A} receptor agonists. Thus, although A_{2A} receptor antagonists could be useful in the initial stages of Parkinson's disease (as discussed above), A_{2A} receptor agonists could be useful in late stages, to avoid the development of L-DOPA-induced dyskinesia. Obviously, in this case enough concomitant D_2 receptor agonism should be obtained in order to overcome the A_{2A} receptor agonist-mediated decrease in affinity of D_2 receptors, and, therefore, the decrease in L-DOPA potency (see above). Finally, $mGlu_5$ receptors could also be a therapeutic target in sensitization to psychostimulants and L-DOPA-induced dyskinesias. In fact, there is a marked reduction in the striatal $mGlu_5$ receptor mRNA expression in rats sensitized to the motor activating effects of amphetamine [315].

GENERAL DISCUSSION

The evidence for subtle and complex receptor-receptor interactions suggests a new dimension to neuronal signaling. Two isues will be briefly discussed before introducing new possibilities for drug development.

GPCR Characterization

As mentioned in the Introduction, no clearcut criteria are presently available for an objective classification of GPCRs

into distinct families. The criterion used to date is based on amino acid sequence differences. Obviously this is an important criterion, but from a functional standpoint the important aspect of a protein is its steric structure, which cannot be univocally deduced from its amino acid sequence. Thus, it could be important to propose other criteria based on the tertiary structure of the receptor. We suggest the following two criteria:

The presence of "plastic" domains capable of carrying out a particular function, without considering the amino acid sequence of such domains. To clarify this point, one example is the extracellular domain of Family III GPCR, which forms two distinct lobes facing each other separated by a cavity where the ligand binds. Domains capable of such a function are present in GPCRs that do not have the same amino acid sequence. In fact, it has been found in mGlu, $GABA_B$ and in calcium sensing receptors.

The shared high affinity for some membrane-associated molecules among different GPCRs. Thus, the high affinity interaction of more GPCRs with one and the same membrane-associated molecule could also help in giving a criterion for a "steric" molecular classification. This criterion is similar to the one used for the pharmacological classification of receptors where the high affinity for a ligand, e.g. serotonin, led to the term "serotoninergic receptors", before the knowledge of the molecular structure of the receptors.

These criteria (besides amino acid sequence similarities and pharmacological characterization) could lead to a tentative "taxonomy" of GPCRs that could have great relevance for discovering functionally relevant classes of GPCRs. This taxonomy should distinguish GPCRs by similarities in function and would mainly consider GPCRs to be "analogous" (evolutionary definition of the term: a character shared by a set of species but not present in their common ancestor), instead of "homologous" (evolutionary definition of the term: a character shared by a set of species and present in their common ancestor).

Possible Meaning of Receptor Internalization

The functional importance of the internalization process has not been fully clarified. It may be surmised that cells use different "fingerprints" to allow their intracellular mechanisms to know which receptors or receptor complexes have been activated by the extracellular signals. The first one may be the special "cocktail" of enzymatic cascades activated in a differential way by the ligands acting on the receptors or on the receptor complexes. However, this may lead to incomplete information since similar (but not identical) activations can be triggered by ligands interacting with different receptors or receptor complexes. We put forward the hypothesis that the real "fingerprint" of actively transducing receptors is the internalization process of the receptors and of the receptor complexes bound to the respective ligands. It is suggested that special decoding mechanisms of this intracellular internalized message will be discovered that will be shown to have relevant roles both for the tuning of the neuronal function according to the functional match between the extracellular signals and the receptors on the plasma membrane and, possibly, also for the long-term memory trace formation [316].

Possible New Strategies for Drug Development

This is a vast subject that is still mainly open to speculations and far from a concrete realization [316]. However, some observations can be made on the basis of a few points that have been discussed in the previous parts of this paper. From a general standpoint the target of drug development might not only be any longer the binding pocket for the neurotransmitter. At least five other important strategies can be mentioned:

Pharmacologically-induced alterations of the micro-environments with which the receptors are in contact. These alterations could affect formation or disruption of receptor complexes as well as their interactions with other membrane-associated proteins. Possible therapeutical interventions could take place by acting not directly on the molecular complex, but rather in one or more of the environments in contact with it. Obviously, alterations in the receptor microenvironments could also be studied to discover pathogenetic mechanisms until now ignored.

To prevent the interactions between receptors or receptors and membrane-associated proteins by altering at least one of the two interacting protein interfaces (e.g., by a drug that prevents the match by localizing at the interfaces of the receptors).

To shift the protein-protein interaction towards the formation of a different (inactive) complex (e.g., by favoring the matching between two different interfaces of the two partners).

To favor the interaction of one of the interacting proteins towards the formation of an active complex.

To modulate the process of receptor internalization. If the hypothesis mentioned above has some fine counterpart, this approach may also lead to the development of drugs that modulate neuronal function, and also the formation of long-term memory stores.

Finally, to act in a predictable way on protein-protein interactions is a difficult task. Discontinuous sequences located in distant parts of the proteins seem to be involved in protein-protein interactions. Thus, it is difficult to develop low molecular weight molecules capable of disrupting the protein interfaces that match or capable of favoring a functionally significant matching. The most straightforward strategy to develop a molecule that antagonizes a protein-protein interaction is that of reproducing the essential features of one of the two partner proteins in a smaller protein which consequently interferes with the complex formation. Nevertheless, proteins, even of a reduced size, are still too large to be currently used as drugs. This could be overcome by developing small peptides or peptidomimetic molecules.

ACKNOWLEDGEMENT

The authors want to thank Dr. Roy A. Wise for his critical review of the manuscript.

REFERENCES

[1] Agnati, L.F.; Fuxe, K.; Zini, I.; Lenzi, P.; Hökfelt, T. *Med. Biol.*, **1980,** *58*, 182.

[2] Agnati, L.F.; Ferré, S.; Lluis, C.; Franco, R.; Fuxe, K. *Pharmacol. Rev.*, **2003,** in press.

[3] Fuxe, K.; Agnati, L.F. *Med. Res. Rev.*, **1985,** *5*, 441.

[4] Fuxe, K.; Agnati, L.F. Receptor-receptor interactions. A new intramembrane integrative mechanism, Macmillan Press: London, **1987**.

[5] Ferré, S. *Psychopharmacology*, **1997,** *133*, 107.

[6] Ferré, S.; Fredholm, B.B.; Morelli, M.; Popoli, P.; Fuxe, K. *Trends Neurosci.*, **1997,** *20*, 482.

[7] Ji, T.H; Grossmann, M.; Ji. I. *J. Biol. Chem.*, **1998,** *273*, 17299.

[8] Bockaert, J.; Pin, J.J. *EMBO J.*, **1999,** *18*, 1723.

[9] Kunishima, N.; Shimada, Y.; Tsuji, Y.; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. *Nature*, **2000,** *407*, 971.

[10] Hermans, E.; Challiss, R.A.J. *Biochem. J.*, **2001,** *359*, 465.

[11] Hall, R.A.; Premont, R.T.; Lefkowitz, R.J. *J. Cell Biol.*, **1999,** *145*, 927.

[12] Ferguson, S.S.G. *Pharmacol. Rev.*, **2001,** *53*, 1.

[13] Marinissen, M.J.; Gutkind, J.S. *Trends Pharmacol. Sci.*, **2001,** *22*, 368.

[14] Claing, A.; Laporte, S.A.; Caron, M.G.; Lefkowitz, R.J. *Prog. Neurobiol.*, **2002,** *66*, 61.

[15] Tsao, P.I.; von Zastrow, M. *Pharmacol. Ther.* **2001,** *89*, 139.

[16] Oakley, RH.; Laporte, S.A.; Holt, J.A.; Caron, M.G.; Barak, L.S. *J. Biol. Chem.*, **2000,** *22*, 17201.

[17] Kohout, T.A.; Lin, F.S.; Perry, S.J.; Conner, D.A.; Lefkowitz, R.J. *Proc. Natl. Acad. Sci. USA*, **2001,** *98*, 1601.

[18] Kim, K.M.; Valenzano, K.J.; Robinson, S.R.; Yao, W.D.; Barak, L.S.; Caron, M.G. *J. Biol. Chem.*, **2001,** *40*, 37409.

[19] McDonald, P.H.; Lefkowitz, R.J. *Cell Sign.*, **2001,** *13*, 683.

[20] McPherson, P.S.; Kay, B.K.; Hussain, N.K. *Traffic*, **2001,** *2*, 375.

[21] Ahn, S.; Maudsley, S.; Luttrell, L.M.; Lefkowitz, R.J.; Daaka, Y. *J. Biol. Chem.*, **1999,** *274*, 1185-1188.

[22] Ma, Y.C.; Huang, J.; Ali, S.; Lowry, W.; Huang, X.Y. *Cell*, **2000,** *102*, 635.

[23] Cao, W.; Luttrell, L.M.; Medvedev, A.V.; Pierce, K.L.; Daniel, K.W.; Dixon, T.M.; Lefkowitz, R.J.; Collins, S. *J. Biol. Chem.*, **2000,** *275*, 38131.

[24] Maudsley, S.; Pierce, K.L.; Zamah, A.M.; Miller, W.E.; Ahn, S.; Daaka, Y.; Lefkowitz, R.J.; Luttrell, L.M. *J. Biol. Chem.*, **2000,** *275*, 9572.

[25] Pierce, K.L.; Maudsley, S.; Daaka, Y.; Luttrell, L.M.; Lefkowitz, R.J. *Proc. Natl. Acad. Sci. USA*, **2000,** *97*, 1489.

[26] Kurzchalia, T.V.; Parton, RG. *Curr. Opin. Cell Biol.*, **1999,** *11*, 424.

[27] Smart, E.J.; Graf, G.A.; McNiven, M.A.; Sessa, W.C.; Engelman, J.A.; Scherer, P.E.; Okamoto, T.; Lisanti, M.P. *Mol. Cell. Biol.*, **1999,** *19*, 7289.

[28] Couet, J.; Belanger, M.M.; Roussel, E.; Drolet, M.C. *Adv. Drug. Deliv. Rev.*, **2001,** *49*, 223-235.

[29] Anderson, R.G.W.; Jacobson, K. *Science*, **2002,** *296*, 1821.

[30] Simons, K.; Ikoken, E. *Nature*, **1997,** *387*, 569.

[31] Harder, T.; Simons, K. *Curr. Opin. Cell Biol.* **1997,** *9*, 534.

[32] Ginés, S.; Ciruela, F.; Burgueno, J.; Casado, V.; Canela, E.I.; Mallol, J.; Lluis, C.; Franco, R. *Mol. Pharmacol.*, **2001,** *59*, 1314.

[33] Burgueno, J.; Enrich, C.; Canela, E.I.; Mallol, J.; Lluis, C.; Franco, R.; Ciruela, F. (submitted).

[34] Kifor, O.; Diaz, R.; Butters, R.; Kifor, I.; Brown, E.M. *J. Biol. Chem.*, **1998,** *273*, 21708.

[35] Murthy, K.S.; Makhlouf, G.H. *J. Biol. Chem.*, **2000,** *275*, 30211.

[36] Matveev, S.V.; Smart, E.J. *Am. J. Cell Physiol.*, **2002,** *282*, C935.

[37] Hillion, J.; Canals, M.; Torvinen, M.; Casado, V.; Scott, R.; Terasmaa, A.; Hansson, A.; Watson, S.; Olah, M.E.; Mallol, J.; Canela, E.I.; Zoli, M.; Agnati, L.F.; Ibanez, C.F.; Lluis, C.; Franco, R.; Ferré, S.; Fuxe, K. *J. Biol. Chem.*, **2002,** *277*, 18091.

[38] Cameron, P.L.; Ruffin, J.W.; Bollag, R.; Rasmussen, H.; Cameron, R.S. *J. Neurosci.* **1997,** *17*, 9520.

[39] Ikezu, T.; Ueda, H.; Trapp, B.D.; Nishiyama, K.; Sha, J.F.; Volonte, D.; Galbiati, F.; Byrd, A.L.; Bassell, G.; Serizawa, H.; Lane, W.S.; Lisanti, M.P.; Okamoto, T. *Brain. Res.*, **1998,** *804*, 177.

[40] Volonte, D.; Galbiati, F.; Li, S.; Nishiyama, K.; Okamoto, T.; Lisanti, M.P. *J. Biol. Chem.*, **1999,** *274*, 12702.

[41] Suzuki, T.; Ito, J.; Takagi, H.; Saitoh, F.; Nawa, H.; Shimizu, H. *Brain Res. Mol. Brain Res.*, **2001,** *89*, 20.

[42] Suzuki, T. *Neurosci. Res.*, **2002,** *44*, 1.

[43] Kennedy, M.B. *Science*, **2000,** *290*, 750.

[44] Scannevin, R.H.; Huganir, R.L. *Nature Rev.*, **2000,** *1*, 133.

[45] Sheng, M.; Kim, E. *J. Cell Sci.*, **2000,** *113*, 1851.

[46] Sheng, M.; Kim, E. *Science*, **2002,** *298*, 776.

[47] Hering, H.; Sheng, M. *Nature Rev. Neurosci.*, **2001,** *2*, 880.

[48] Job, C.; Eberwine, J. *Nature Rev. Neurosci.*, **2001,** *2*, 889.

[49] Rao, A.; Craig, A.M. *Neuron*, **1997,** *19*, 801.

[50] Soderling, T.R.; Derkach, V.A. *Trends Neurosci.,* **2000,** *23,* 75.

[51] Luscher, C.; Xia, H.; Beattie, E.C.; Carroll, R.C.; von Zastrow, M.; Malenka, R.C.; Nicoll, R.A. *Neuron,* **1999,** *24,* 649.

[52] Malinow, R.; Malenka, R.C. *Ann. Rev. Neurosci.,* **2002,** *25,* 103.

[53] Halpain, S. *Trends Neurosci.,* **2000,** *23,* 141.

[54] Matsuzaki, M.; Ellis-Davies, G.C.; Nemoto, T.; Miyashita, Y.; Iino, M.; Kasai, H. *Nature Neurosci.,* **2001,** *4,* 1086.

[55] Star, E.N.; Kwiatkowski, D.J.; Murthy, V.N. *Nat. Neurosci.,* **2002,** *5,* 239.

[56] Conn, P.J.; Pin, J.P. *Ann. Rev. Pharmacol. Toxicol.,* **1997,** *37,* 205.

[57] Lujan, R.; Nusser, Z.; Roberts, J.D.; Shigemoto, R.; Somogyi, P. *Eur. J. Neurosci.,* **1996,** *8,* 1488.

[58] Smith, Y.; Charara, A.; Hanson, J.E.; Paquet, M.; Levey, A.I. *J. Anat.,* **2000,** *196,* 555.

[59] Kornau, H.C.; Schenker, L.T.; Kennedy, M.B.; Seeburg, P.H. *Science,* **1995,** *269,* 1737.

[60] Naisbitt, S.; Kim, E.; Tu, J.C.; Xiao, B.; Sala, C.; Valtschanoff, J.; Weinberg, R.J.; Worley, P.F.; Sheng, M. *Neuron,* **1999,** *23,* 569.

[61] Tu, J.C.; Xiao, B.; Naisbitt, S.; Yuan, J.P.; Petralia, R.S.; Brakeman, P.; Doan, A.; Aakalu, V.K.; Lanahan, A.A.; Sheng, M.; Worley, P.F. *Neuron,* **1999,** *23,* 583.

[62] Doherty, A.J.; Palmer, M.J.; Henley, J.M.; Collingridge, G.L.; Jane, D.E. *Neuropharmacology,* **1997,** *36,* 265.

[63] Awad, H.; Hubert, G.W.; Smith, Y.; Levey, A.I.; Conn, P.J. *J. Neurosci.,* **2000,** *20,* 7871.

[64] Pisani, A.; Gubellini, P.; Bonsi, P.; Conquet, F.; Picconi, B.; Centonze, D.; Bernardi, G.; Calabresi, P. *Neuroscience,* **2001,** *106,* 579.

[65] Alagarsamy, S.; Rouse, S.T.; Gereau, R.W. 4th.; Heinemann, S.F.; Smith, Y.; Conn, P.J. *Ann. N. Y. Acad. Sci.,* **1999,** *868,* 526.

[66] Xiao, B.; Tu, J.C.; Worley, P.F. *Cur. Opin. Neurobiol.,* **2000,** *10,* 370.

[67] Brakeman, P.R.; Lanahan, A.A.; O'Brien, R.; Roche, K.; Barnes, C.A.; Huganir, R.L.; Worley, P.F. *Nature,* **1997,** *386,* 284.

[68] Bottai, D.; Guzowski, J.F.; Schwarz, M.K.; Kang, S.H.; Xiao, B.; Lanahan, A.; Worley, P.F.; Seeburg, P.H. *J. Neurosci.,* **2002,** *22,* 167.

[69] Ango, F.; Prezeau, L.; Muller, T.; Tu, J.C.; Xiao, B.; Worley, P.F.; Pin, J.P.; Bockaert, J.; Fagni, L. *Nature,* **2001,** *411,* 962.

[70] Sato, M.; Suzuki, K.; Nakanishi, S. *J. Neurosci.,* **2001,** *21,* 3797.

[71] Xiao, B.; Tu, J.C.; Petralia, R.S.; Yuan, J.P.; Doan, A.; Breder, C.D.; Ruggiero, A.; Lanahan, A.A.; Wenthold, R.J.; Worley, P.F. *Neuron,* **1998,** *2,* 707.

[72] Ango, F.; Robbe, D.; Tu, J.C.; Xiao, B.; Worley, P.F.; Pin, J.P.; Bockaert, J.; Fagni, L. *Mol. Cell. Neurosci.,* **2002,** *20,* 323.

[73] Roche, K.W.; Tu, J.C.; Petralia, R.S.; Xiao, B.; Wenthold, R.J.; Worley, P.F. *J. Biol. Chem.,* **1999,** *274,* 25953.

[74] Coutinho, V.; Kavanagh, I.; Sugiyama, H.; Tones, M.A.; Henley, J.M. *Mol. Cell. Neurosci.,* **2001,** *18,* 296.

[75] Ciruela, F.; Soloviev, M.M.; McIlhinney, R.A. *Biochem. J.,* **1999,** *341,* 795.

[76] Ciruela, F.; Soloviev, M.M.; Chan, W.Y.; McIlhinney, R.A. *Mol. Cell. Neurosci.,* **2000,** *15,* 36.

[77] Pin, J.P.; Duvoisin, R. *Neuropharmacology,* **1995,** *34,* 1.

[78] Francesconi, A.; Duvoisin, R.M. *J. Biol. Chem.,* **1998,** *273,* 5615.

[79] Francesconi, A.; Duvoisin, R.M. *Proc. Natl. Acad. Sci. USA,* **2000,** *97,* 6185.

[80] Ferraguti, F.; Baldani-Guerra, B.; Corsi, M.; Nakanishi, S.; Corti, C. *Eur. J. Neurosci.,* **1999,** *11,* 2073.

[81] Peavy, R.D.; Chang, M.S.; Sanders-Bush, E.; Conn, P.J. *J. Neurosci.,* **2001,** *21,* 9619.

[82] Ali, D.W.; Salter, M.W. *Curr. Opin. Neurobiol.,* **2001,** *11,* 336.

[83] Boxall, A.R.; Lancaster, B. *Eur. J. Neurosci.,* **1998,** *10,* 2.

[84] Bruno, V.; Battaglia, G.; Copani, A.; Cespedes, V.M.; Galindo, M.F.; Cena, V.; Sanchez-Prieto, J.; Gasparini, F.; Kuhn, R.; Flor, P.J.; Nicoletti, F. *Eur. J. Neurosci.* **2001,** *13,* 1469.

[85] Mannaioni, G.; Marino, M.J.; Valenti, O.; Traynelis, S.F.; Conn, P.J. *J. Neurosci.* **2001,** *21,* 5925.

[86] Heidinger, V.; Manzerra, P.; Wang, X.Q.; Strasser, U.; Yu, S.P.; Choi, D.W.; Behrens, M.M. *J. Neurosci.,* **2002,** *22,* 5452.

[87] Moon, I.S.; Apperson, M.L.; Kennedy, M.B. *Proc. Natl. Acad. Sci. USA,* **1994,** *91,* 3954.

[88] Choe, E.; Wang, J. *Neuroscience,* **2002,** *114,* 557.

[89] Heuss, C.; Scanziani, M.; Gahwiler, B.H.; Gerber, U. *Nat. Neurosci.,* **1999,** *2,* 1070.

[90] Dale, L.B.; Bhattacharya, M.; Seachrist, J.L.; Anborgh, P.H.; Ferguson, S.S. *Mol. Pharmacol.,* **2001a,** *60,* 1243.

[91] Dale, L.B.; Babwash, A.V.; Ferguson, S.S.G. *Neurochem. Int.,* **2002,** *41,* 319.

[92] Mayford, M.; Wang, J.; Kandel, E.R.; O'Dell, T.J. *Cell,* **1995,** *81,* 891.

[93] Wu, G.Y.; Deisseroth, K.; Tsien, R.W. *Proc. Natl. Acad. Sci. USA,* **2001,** *98,* 2808.

[94] Rongo, C. *Bioessays,* **2002,** *24,* 223.

[95] Bayer, K.U.; Schulman, H. *Biochem. Biophys. Res. Commun.,* **2001,** *289,* 917.

[96] Chen, H.J.; Rojas-Soto, M.; Oguni, A.; Kennedy, M.B. *Neuron,* **1998,** *20,* 895.

[97] Huber, K.M.; Kayser, M.S.; Bear, M.F. *Science,* **2000,** *288,* 1254.

[98] Huber, K.M.; Roder, J.C.; Bear, M.F. *J. Neurophysiol.,* **2001,** *86,* 321.

[99] Carroll, R.C.; Beattie, E.C.; von Zastrow, M.; Malenka, R.C. *Nat. Rev. Neurosci.,* **2001,** *2,* 315.

[100] Carroll, R.C.; Zukin, R.S. *Trends Neurosci.,* **2002,** *25,* 571.

[101] Snyder, E.M.; Philpot, B.D.; Huber, K.M.; Dong, X.; Fallon, J.R.; Bear, M.F. *Nat. Neurosci.,* **2001,** *4,* 1079.

[102] Xiao, M.Y.; Zhou, Q.; Nicoll, R. *Neuropharmacology,* **2001,** *41,* 664.

[103] Vanderklish, P.W.; Edelman, G.M. *Proc. Natl. Acad. Sci. USA,* **2002,** *99,* 1639.

[104] Gereau, R.W. 4th.; Heinemann, S.F. *Neuron,* **1998,** *20,* 143.

[105] Dale, L.B.; Babwah, A.V.; Bhattacharya, M.; Kelvin, D.J.; Ferguson, S.S. *J. Biol. Chem.,* **2001b,** *276,* 35900.

[106] Cho, K.; Bashir, Z.I. *Trends Neurosci.,* **2002,** *25,* 405.

[107] Latini, S.; Pedata, F. *J. Neurochem.,* **2001,** *79,* 463.

[108] Snyder, S.H. *Ann. Rev. Neurosci.,* **1985,** *8,* 103.

[109] Ferré, S.; Fuxe, K. *Prog. Brain Res.,* **2000,** *125,* 353.

[110] Fredhom, B.B. *Pharmacol. Toxicol.,* **1995**, *76,* 93.

[111] Fredholm, B.B.; Uzerman, A.P.; Jacobson, K.A.; Klotz, K.N.; Linden, J. *Pharmacol. Rev.,* **2001,** *53,* 527.

[112] Schiffmann, S.N.; Jacobs, O.; Vanderhaeghen, J.-J. *J. Neurochem.,* **1991,** *57,* 1062.

[113] Fink, J.S.; Weaver, D.R.; Rivkees, S.A.; Peterfreund, R.A.; Pollack, A.; Adler, E.M.; Reppert, S.M. *Mol. Brain. Res.,* **1992,** *14,* 186.

[114] Hettinger, B.D.; Lee, A.; Linden, J.; Rosin, D.L. *J. Comp. Neurol.,* **2001,** *431,* 331.

[115] Kull. B.; Ferré, S.; Arslan, G.; Svenningsson, P.; Fuxe, K.; Owman, C.; Fredholm, B.B. *Biochem. Pharmacol.,* **1999,** *58,* 1035.

[116] Kull, B.; Svenningsson, P.; Fredholm, B.B. *Mol. Pharmacol.,* **2000,** *58,* 771.

[117] Nishi, A.; Bibb, J.A.; Snyder, G.L.; Higashi, H.; Nairn, A.C.; Greengard, P. *Proc. Natl. Acad. Sci. USA,* **2000,** *97,* 12840.

[118] Svenningsson, P.; Lindskog, M.; Rognoni, F.; Fredholm, B.B.; Greengard, P.; Fisone, G. *Neuroscience,* **1998,** *84,* 223.

[119] Svenningsson, P.; Lindskog, M.; Ledent, C.; Parmentier, M.; Greengard, P.; Fredholm, B.B.; Fisone, G. *Proc. Natl. Acad. Sci. USA,* **2000,** *97,* 1856.

[120] Lindskog, M.; Svenningsson, P.; Fredholm, B.B.; Greengard, P.; Fisone, G. *Neuroscience,* **1999,** *88,* 1005.

[121] Lindskog, M.; Svenningsson, P.; Pozzi, L.; Kim, Y.; Fienberg, A.A.; Bibb, J.A.; Fredholm, B.B.; Nairn, A.C.; Greengard, P.; Fisone, G. *Nature,* **2002,** *418,* 774.

[122] Mayr, B.; Montminy, M. *Nat. Rev.,* **2001,** *2,* 599.

[123] Borsook, D.; Hyman, S.E. *Am. J. Physiol.,* **1995,** *269,* E393.

[124] Evers, B.M.; Wang, X.; Zhou, Z.; Townsend, C.M. Jr.; McNeil, G.P.; Dobner, P.R. *Mol. Cell. Biol.,* **1995,** *15,* 3870.

[125] Hughes, P.; Dragunow, M. *Pharmacol. Rev.,* **1995,** *47,* 133.

[126] Le, F.; Groshan, K.; Zeng, X.P.; Richelson, E. *J. Biol. Chem.,* **1997,** *272,* 1315.

[127] Herdegen, T.; Leah, J.D. *Brain Res. Rev.,* **1998,** *28,* 370.

[128] Wirkner, K.; Assmann, H.; Koles, L.; Gerevich, Z.; Franke, H.; Norenberg, W.; Boehm, R.; Illes, P. *Br. J. Pharmacol.,* **2000,** *130,* 259.

[129] Lee, F.S.; Chao, M.V. *Proc. Natl. Acad. Sci. USA,* **2001,** *98,* 3555.

[130] Sexl, V.; Mancusi, G.; Holler, C.; Gloria-Maercker, E.; Schutz, W, Freissmuth, M. *J. Biol. Chem.,* **1997,** *272,* 5792.

[131] Seidel, M.G.; Klinger, M.; Freissmuth, M.; Holler, C. *J. Biol. Chem.,* **1999,** *274,* 25833.

[132] Schulte, G.; Fredholm, B.B. *Mol. Pharmacol.,* **2002,** *62,* 1137.

[133] Ferré, S.; Karcz-Kubicha, M.; Hope, B.T.; Popoli, P.; Burgueno, J.; Gutierrez, M.A.; Casado, V.; Fuxe, K.; Goldberg, S.R.; Lluis, C.; Franco, R.; Ciruela, F. *Proc. Natl. Acad. Sci. USA,* **2002,** *99,* 11940.

[134] Klinger, M.; Kudlacek, O.; Seidel, M.G.; Freissmuth, M.; Sexl, V. *J. Biol. Chem.,* **2002,** *277,* 32490.

[135] Klinger, M.; Kuhn, M.; Just, H.; Stefan, E.; Palmer, T.; Freissmuth, M.; Nanoff, C. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **2002,** *366,* 287.

[136] Moelling, K.; Schad, K.; Bosse, M.; Zimmermann, S.; Schweneker, M. *J. Biol. Chem.,* **2002,** *277,* 31099.

[137] Stork, P.J.; Schmitt, J.M. *Trends Cell Biol.,* **2002,** *12,* 258.

[138] Vossler, M.R.; Yao, H.; York, R.D.; Pan, M.G.; Rim, C.S.; Stork, P.J. *Cell,* **1997,** *89,* 73.

[139] Schmitt, J.M.; Stork, P.J. *Mol. Cell,* **2002,** *9,* 85.

[140] Kawasaki, H.; Springett, G.M.; Mochizuki, N.; Toki, S.; Nakaya, M.; Matsuda, M.; Housman, D.E.; Graybiel, A.M. *Science,* **1998,** *282,* 2275.

[141] de Rooij, J.; Zwartkruis, F.J.; Verheijen, M.H.; Cool, R.H.; Nijman, S.M.; Wittinghofer, A.; Bos, J.L. *Nature,* **1998,** *396,* 474.

[142] Schmitt, J.M.; Stork, P.J. *J. Biol. Chem.,* **2002,** *277,* 43024.

[143] Morice, C.; Nothias, F.; Konig, S.; Vernier, P.; Baccarini, M.; Vincent, J.D.; Barnier, J.V. *Eur. J. Neurosci.,* **1999,** *11,* 1995.

[144] Zimmermann, S.; Moelling, K. *Science,* **1999,** *286,* 1741.

[145] Rodriguez-Viciana, P.; Warne, P.H.; Dhand, R.; Vanhaesebroeck, B.; Gout, I.; Fry, M.J.; Waterfield, M.D.; Downward, J. *Nature,* **1994,** *370,* 527.

[146] Newman, M.E.; Levitzki, A. *Biochem. Pharmacol.,* **1983,** *32,* 137.

[147] Anand-Srivastava, M.B.; Cantin, M.; Ballak, M.; Picard, S. *Mol. Cell. Endocrinol.,* **1989,** *62,* 273.

[148] Luty, J.; Hunt, J.A.; Nobbs, P.K.; Kelly, E.; Keen, M.; MacDermot, J. *Cardiovasc. Res.,* **1989,** *23,* 303.

[149] Ramkumar, V.; Olah, M.E.; Jacobson, K.A.; Stiles, G.L. *Mol. Pharmacol.,* **1991,** *40,* 639.

[150] Palmer, T.M.; Gettys, T.W.; Jacobson, K.A.; Stiles, G.L. *Mol. Pharmacol.,* **1994,** *45,* 1082.

[151] Chern. Y.; Chiou, J.Y.; Lai, H.L.; Tsai, M.H. *Mol. Pharmacol.,* **1995,** *48,* 1.

[152] Mundell, S.J.; Kelly, E. *Br. J. Pharmacol.,* **1998,** *125,* 1594.

[153] Mundell, S.J.; Luty, J.S.; Willets, J.; Benovic, J.L.; Kelly, E. *Br. J. Pharmacol.,* **1998,** *125,* 347.

[154] Palmer, T.M.; Stiles, G.L. *Biochemistry,* **1997,** *36,* 832.

[155] Olah, M.E. *J. Biol. Chem.,* **1997,** *272,* 3375.

[156] Björklund, A.; Lindvall, O. In *Handbook of Chemical Neuroanatomy*; Björklund, A.; Hökfelt, T. Eds.; Elsevier: Amsterdam, **1984**; Vol. *2*, pp. 55-122.

[157] Missale, C.; Nash, S.R.; Robinson, S.W.; Jaber, M.; Caron, M.G. *Physiol. Rev.,* **1998,** *78,* 189.

[158] Malek, D.; Munch, G.; Palm, D. *FEBS Lett.,* **1993,** *325,* 215.

[159] Lachowicz, J.E.; Sibley, D.R. *Biochem. Biophys. Res. Commun.,* **1997,** *237,* 394.

[160] Filteau, F.; Veilleux, F.; Levesque, D. *FEBS Lett.,* **1999,** *447,* 251.

[161] Ilani, T.; Fishburn, C.S.; Levavi-Sivan, B.; Carmon, S.; Raveh, L.; Fuchs, S. *Cell. Mol. Neurobiol.,* **2002,** *22,* 47.

[162] Liu, Y.F.; Civelli, O.; Grandy, D.K.; Albert, P.R. *J. Neurochem.,* **1992,** *59,* 2311.

[163] Senogles, S.E. *J. Biol. Chem.,* **1994,** *269,* 23120.

[164] Guiramand, J.; Montmayeur, J.P.; Ceraline, J.; Bhatia, M.; Borrelli, E. *J. Biol. Chem.,* **1995,** *270,* 7354.

[165] Fishburn, C.S.; Elazar, Z.; Fuchs, S. *J. Biol. Chem.,* **1995,** *270,* 29819.

[166] Ng, G.Y.; O'Dowd, B.F.; Caron, M.; Dennis, M.; Brann, M.R.; George, S.R. *J. Neurochem.,* **1994**, *63*,1589.

[167] Ito, K.; Haga, T.; Lameh, J.; Sadee, W. *Eur. J. Biochem.,* **1999,** *260,* 112.

[168] Filtz, T.M.; Artymyshyn, R.P.; Guan, W.; Molinoff, P.B. *Mol. Pharmacol.,* **1993,** *44,* 371.

[169] Zhang, L.J.; Lachowicz, J.E.; Sibley, D.R. *Mol. Pharmacol.,* **1994,** *45,* 878.

[170] Starr, S.; Kozell, L.B.; Neve, K.A. *J. Neurochem.,* **1995,** *65,* 569.

[171] Ng, G.Y.; Varghese, G.; Chung, H.T.; Trogadis, J.; Seeman, P.; O'Dowd, B.F.; George, S.R. *Endocrinology,* **1997,** *138,* 4199.

[172] Khan, Z.U.; Mrzljak, L.; Gutierrez, A.; de la Calle, A.; Goldman-Rakic, P.S. *Proc. Natl. Acad. Sci., USA,* **1998,** *95,* 7731.

[173] Usiello, A.; Baik, J.H.; Rouge-Pont, F.; Picetti, R.; Dierich, A.; LeMeur, M.; Piazza, P.V.; Borrelli, E. *Nature,* **2000,** *408,* 199.

[174] Centonze, D.; Usiello, A.; Gubellini, P.; Pisani, A.; Borrelli, E.; Bernardi, G.; Calabresi, P. *Neuropsychopharmacology,* **2002,** *27,* 723.

[175] Adams, M.R.; Brandon, E.P.; Chartoff, E.H.; Idzerda, R.L., Dorsa, D.M.; McKnight, G.S. *Proc. Natl. Acad. Sci. USA,* **1997,** *94,* 12157.

[176] Lee, K.W.; Hong, J.H.; Choi, I.Y.; Che, Y.; Lee, J.K.; Yang, S.D.; Song, C.W.; Kang, H.S.; Lee, J.H.; Noh, J.S.; Shin, H.S.; Han, P.L. *J. Neurosci.,* **2002,** *22,* 7931.

[177] Mons, N.; Harry, A.; Dubourg, P.; Premont, R.T.; Iyengar, R.; Cooper, D.M. *Proc. Natl. Acad. Sci. USA,* **1995,** *92,* 8473.

[178] Ghahremani, M.H.; Cheng, P.; Lembo, P.M.; Albert, P.R. *J. Biol. Chem.,* **1999,** *274,* 9238.

[179] Banihashemi, B.; Albert, P.R. *Mol. Endocrinol.,* **2002,** *16,* 2393.

[180] Ghahremani, M.H.; Forget, C.; Albert, P.R. *Mol. Cell. Biol.,* **2000,** *20,* 1497.

[181] Hernandez-Lopez, S.; Tkatch, T.; Perez-Garci, E.; Galarraga, E.; Bargas, J.; Hamm, H.; Surmeier, D.J. *J. Neurosci.,* **2000,** *20,* 8987.

[182] Nishi, A.; Snyder, G.L.; Greengard, P. *J. Neurosci.,* **1997,** *17,* 8147-8155.

[183] Faure, M.; Voyno-Yasenetskaya, T.A.; Bourne, H.R. *J. Biol. Chem.,* **1994,** *269,* 7851.

[184] Luo, Y.; Kokkonen, G.C.; Wang, X.; Neve, K.A.; Roth, G.S. *J. Neurochem.,* **1998,** *71,* 980.

[185] Oak, J.N.; Lavine, N.; Van Tol, H.H. *Mol. Pharmacol.,* **2001,** *60,* 92.

[186] Yan, Z.; Feng, J.; Fienberg, A.A.; Greengard, P. *Proc. Natl. Acad. Sci. USA,* **1999,** *96,* 11607.

[187] Brami-Cherrier, K.; Valjent, E.; Garcia, M.; Pages, C.; Hipskind, R.A.; Caboche, J. *J. Neurosci.,* **2002,** *22,* 8911.

[188] Cai, G.; Zhen, X.; Uryu, K.; Friedman E. *J. Neurosci.,* **2000,** *20,* 1849.

[189] Gerfen, C.R.; Miyachi, S.; Paletzki, R.; Brown, P. *J. Neurosci.,* **2002,** *22,* 5042.

[190] Liu, J.C.; Baker, R.E.; Sun, C.; Sundmark, V.C.; Elsholtz, H.P. *J. Biol. Chem.,* **2002,** *277,* 35819.

[191] Jordan, J.D.; Carey, K.D.; Stork, P.J.; Iyengar, R. *J. Biol. Chem.,* **1999,** *274,* 21507.

[192] Dolmetsch, R.E.; Pajvani, U.; Fife, K.; Spotts, J.M.; Greenberg, M.E. *Science,* **2001,** *294,* 333.

[193] Hebert, T.E.; Moffett, S.; Morello, J.P.; Loisel, T.P.; Bichet, D.G.; Barret, C.; Bouvier, M. *J. Biol. Chem.*, **1996**, *271*,16384.

[194] Cvejic, S.; Devi, L.A. *J. Biol. Chem.*, **1997,** *272*,26959.

[195] Ciruela, F.; Mallol, J.; Canela, E .I.; Lluís, C.; Franco, R. *J. Neurosci. Res.*, **1995,** *42*, 818.

[196] Romano, C.; Yang, W.L.; O'Malley, K.L. *J. Biol. Chem.*, **1996,** *271*, 28612.

[197] Romano, C.; Miller, J.K.; Hyrc, K.; Dikranian, S.; Mennerick, S.; Takeuchi, Y.; Goldberg, M.P.; O'Malley, K.L. *Mol. Pharmacol.*, **2001,** *59*, 46.

[198] Zawarynski, P.; Tallerico, T.; Seeman, P.; Lee, S.P.; O'Dowd, B.F.; George, S.R. *FEBS Lett.*, **1998,** *441*, 383.

[199] Robbins, M.J.; Ciruela, F.; Rhodes, A.; McIlhinney, R.A.J. *J. Neurochem.*, **1999,** *72*, 2539.

[200] Ng, G.Y.; O'Dowd, B.F.; Lee, S.P.; Chung, H.T.; Brann, M.R.; Seeman, P.; George, S.R. Biochem. *Biophys. Res. Commun.*, **1996,** *227*, 200.

[201] Bouvier, M. *Nat. Rev. Neurosci.*, **2001**, *2*, 274.

[202] Gouldson, P.R.; Snell, C.R.; Bywater, R.P.; Higg, C.; Reynolds, C.A. *Protein Eng.*, **1998,** *11*, 1181.

[203] Larsen, T.A.; Olson, A.J.; Goodsell, D.S. *Structure*, **1998,** *6*, 421.

[204] Zoli, M.; Agnati, L.F.; Hedlund, P.B.; Li, X.M.; Ferré, S.; Fuxe, K. *Mol. Neurobiol.*, **1993,** *7*, 293.

[205] Enz, R. *FEBS Lett.*, **2002,** *514*,184.

[206] Lin, R.; Karpa, K.; Kabbani, N.; Goldman-Rakic, P.; Levenson, R. *Proc. Natl. Acad. Sci. U.S.A.*, **2001**, *98*, 5258.

[207] Binda, A.V.; Kabbani, N.; Lin, R.; Levenson, R. *Mol. Pharmacol.*, **2002**, *62*, 507.

[208] Shen, L.; Liang, F.; Walensky, L.D.; Huganir, R.L. *J. Neurosci.*, **2000**, *20*, 7932.

[209] Smith, F.D.; Oxford, G.S.; Milgram, S.L. *J. Biol. Chem.*, **1999**, *274*, 19894.

[210] Heimer, L.; Zahm, D.S.; Alheid, G.F. In *The rat nervous system*; Paxinos, G. Ed.; Academic Press: San Diego, **1995**; pp. 579-628.

[211] Gerfen, C.R.; Wilson, C.J. In *Handbook of Chemical Neuroanatomy*; Swanson, L.W.; Björklund, A.; Hökfelt, T. Eds.; Elsevier: Amsterdam, **1996**; Vol. *12*, pp. 371-468.

[212] Shigemoto, R.; Nomura, S.; Ohishi, H.; Sugihara, H.; Nakanishi, S.; Mizuno, N. *Neurosci. Lett.*, **1993,** *163*, 53.

[213] Testa, C.M.; Standaert, D.G.; Landwehrmeyer, G.B.; Penney, J.B.; Young, A.B. *J. Comp. Neurol.*, **1995,** *354*, 241.

[214] Tallaksen-Greene, S.J.; Kaatz, K.W.; Romano, C.; Albin, R.L. *Brain Res.*, **1998,** *780*, 210.

[215] Alexander, G.E.; Crutcher, M.D. *Trends Neurosci.*, **1990,** *13*, 266.

[216] Obeso, J.A.; Rodriguez-Oroz, C.; Rodriguez, M.; Macias, R.; Alvarez, L.; Guridi, J.; Vitek, J.; DeLong, M.R. *Neurology*, **2000,** *55*, S7

[217] Koob, G.F. *Ann. N.Y. Acad. Sci.*, **1999,** *877*, 445.

[218] Agnati, L.F.; Benfenati, F.; Ferri, M.; Morpurgo, A.; Apolloni, B.; Fuxe, K. In *From synapses to rules: Discovering symbolic rules from neural processed data*; Apolloni, B.; Kurfess, F. Eds.; Kluwer Academic/Plenum Press Publisher: New York, **2003**, in press.

[219] Ferré, S.; von Euler, G.; Johansson, B.; Fredholm, B.B.; Fuxe, K. *Proc. Natl. Acad. Sci. USA*, **1991,** *88*, 7238.

[220] Ferré, S.; O'Connor, W.T.; Snaprud, P.; Ungerstedt, U.; Fuxe, K. *Neuroscience*, **1994,** *63*, 765.

[221] Ferré, S.; Popoli, P.; Rimondini, R.; Reggio, R.; Kehr, J.; Fuxe K. *Neuropharmacology*, **1999,** *39*, 129.

[222] Dasgupta, S.; Ferré, S.; Kull, B.; Hedlund, P.B.; Finnman, U.-B.; Ahlberg, S.; Arenas, E.; Fredholm, B.B.; Fuxe, K. *Eur. J. Pharmacol.*, **1996,** *316*, 325.

[223] Dixon, A.K.; Widdowson, L.; Richardson, P.J. *J. Neurochem.*, **1997,** *69*, 315.

[224] Franco, R.; Ferré, S.; Agnati, L.; Torvinen, M.; Gines, S.; Hillion, J.; Casado, V.; Lledo, P.M.; Zoli, M.; Lluis, C.; Fuxe, K. *Neuropsychopharmacology*, **2000,** *23*, S50.

[225] Salim, H.; Ferré, S.; Dalal, A.; Peterfreund, R.A.; Fuxe, K.; Vincent, J.D.; Lledo, P.M. *J. Neurochem.*, **2000,** *74*, 432.

[226] Díaz-Cabiale, Z.; Hurd, Y.; Guidolin, D.; Finnman, U.B.; Zoli, M.; Agnati, L.F.; Vanderhaeghen, J.J.; Fuxe, K.; Ferré, S. *NeuroReport*, **2001,** *12*,1831-1834.

[227] Rimondini, R.; Fuxe, K.; Ferré, S. *NeuroReport*, **1999,** *10*, 2051.

[228] Popoli, P.; Pezzola, A.; Torvinen, M.; Reggio, R.; Pintor, A.; Scarchilli, L.; Fuxe, K.; Ferré, S. *Neuropsychopharmacology*, **2001,** *25*, 505.

[229] Schiffmann, S.N.; Vanderhaeghen, J.J. *J. Neurosci.*, **1993,** *13*, 1080.

[230] Morelli, M.; Pinna A.; Wardas, J.; Di Chiara, G. *Neuroscience*, **1995**, *67*, 49.

[231] Pollack, A.E.; Fink, J.S. *Neuroscience*, **1995,** *68*, 721.

[232] Boegman, R.J.; Vincent, S.R. *Synapse*, **1996,** *22*, 70.

[233] Pinna, A.; Wardas, J.; Cristalli, G.; Morelli, M. *Brain Res.*, **1997,** *759*, 41.

[234] Pinna, A.; Wardas, J.; Cozzolino, A.; Morelli, M. *Neuropsychopharmacology*, **1999,** *20*, 44.

[235] Richardson, P.J.; Kase, H.; Jenner, P. *Trends Pharmacol. Sci.*, **1997,** *18*, 338.

[236] Svenningsson, P.; Fourreau, L.; Bloch, B.; Fredholm, B.B.; Gonon, F.; Le Moine, C. *Neuroscience*, **1999,** *89*, 827.

[237] Ward, R.P.; Dorsa, D.M. *Neuroscience*, **1999,** *89*, 927.

[238] Chen, J.F.; Moratalla, R.; Impagnatiello, F.; Grandy , D.K.; Cuellar, B.; Rubinstein, M.; Beilstein, M.A.; Hackett, E.;

Fink, J.S.; Low, M.J.; Ongini, E.; Schwarzschild, M.A. *Proc. Natl. Acad. Sci. USA*, **2001,** *98*, 1970.

[239] Zahniser, N.R.; Simosky, J.K.; Mayfield, R.D.; Negri, C.A.; Hanania, T.; Larson, G.A.; Kelly, M.A.; Grandy, D.K.; Rubinstein, M.; Low, M.J.; Fredholm, B.B. *J. Neurosci.*, **2000,** *20*, 5949.

[240] Dassesse, D.; Massie, A.; Ferrari, R.; Ledent, C.; Parmentier, M.; Arckens, L.; Zoli, M.; Schiffmann, S.N. *J. Neurochem.*, **2001,** *78*, 183.

[241] Barraco, R.A.; Martensm K.A.; Parizon, M.; Normile, H.J. *Brain Res. Bull.*, **1993,** *31*, 397.

[242] Rimondini, R.; Ferré, S.; Ogren, S.O.; Fuxe, K. *Neuropsychopharmacology*, **1997,** *17*, 82.

[243] Rimondini, R.; Ferré, S.; Gimenez-Llort, L.; Ogren, S.O.; Fuxe, K. *Eur. J. Pharmacol.*, **1998,** *347*, 153.

[244] Kanda, T.; Shiozaki, S.; Shimada, J.; Suzuki, F.; Nakamura, J. *Eur. J. Pharmacol.*, **1994,** *256*, 263.

[245] Malec, D. *Pol. J. Pharmacol.*, **1997,** *49*, 323.

[246] Shiozaki, S.; Ichikawa, S.; Nakamura, J.; Kitamura, S.; Yamada, K.; Kuwana, Y. *Psychopharmacology*, **1999,** *147*, 90.

[247] Aoyama, S.; Kase, H.; Borrelli, E. *J. Neurosci.*, **2000,** *20*,5848.

[248] Ferré, S.; Popoli, P.; Giménez-Llort, L.; Rimondini, R.; Müller, C.E.; Strömberg, I.; Ögren, S.O.; Fuxe, K. *Parkinsonism Relat. Disord.*, **2001,** *7*, 235.

[249] Hauber, W.; Neuscheler, P.; Nagel, J.; Muller, C.E. *Eur. J. Neurosci.*, **2001,** *14*, 1287.

[250] Wardas, J.; Konieczny, J.; Lorenc-Koci, E. *Synapse*, **2001,** *41*, 160

[251] Kanda, T.; Jackson, M.J.; Smith, L.A.; Pearce, R.K.; Nakamura, J.; Kase, H.; Kuwana, Y.; Jenner, P. *Ann. Neurol.*, **1998,** *43*, 507.

[252] Grondin, R.; Bedard, P.J.; Hadj Tahar, A.; Gregoire, L.; Mori, A.; Kase, H. *Neurology*, **1999,** *52*, 1673.

[253] Ungerstedt, U. *Acta Physiol. Scand. Suppl.*, **1971,** *367*, 69.

[254] Pinna, A.; di Chiara, G.; Wardas, J.; Morelli, M. *Eur. J. Neurosci.*, **1996,** *8*, 1176.

[255] Strömberg, I.; Popoli, P.; Müller, C.E.; Ferré, S.; Fuxe, K. *Eur. J. Neurosci.*, **2000,** *12*, 4033.

[256] Fenu, S.; Pinna, A.; Ongini, E.; Morelli, M. *Eur. J. Pharmacol.*, **1997,** *321*, 143.

[257] Kanda, T.; Jackson, M.J.; Smith, L.A.; Pearce, R.K.; Nakamura, J.; Kase, H.; Kuwana, Y.; Jenner, P. *Exp. Neurol.*, **2000,** *162*, 321.

[258] Koga, K.; Kurokawa, M.; Ochi, M.; Nakamura, J.; Kuwana, Y. *Eur. J. Pharmacol.*, **2000,** *408*, 249.

[259] Popoli, P.; Reggio, R.; Pezzola, A. *Neuropsychopharmacology*, **2000,** *22*, 522.

[260] Chen, J.F.; Beilstein, M.; Xu, Y.H.; Turner, T.J.; Moratalla, R.; Standaert, D.G.; Aloyo, V.J.; Fink, J.S.; Schwarzschild, M.A. *Neuroscience*, **2000,** *97*, 195.

[261] Berretta, S.; Parthasarathy, H.B.; Graybiel, A.M. *J. Neurosci.*, **1997,** *17*, 4752.

[262] Parthasarathy, H.B.; Graybiel, A.M. *J. Neurosci.*, **1997,** *17*, 2477.

[263] Sgambato, V.; Pages, C.; Rogard, M.; Besson, M.J.; Caboche, J. *J. Neurosci.*, **1998,** *18*, 8814-8825.

[264] Ossowska, K.; Konieczny, J.; Wolfarth, S.; Wieronska, J.; Pilc, A. *Neuropharmacology*, **2001,** *41*, 413.

[265] Deutch, A.Y.; Lee, M.C.; Iadarola, M.J. *Mol. Cell. Neurosci.*, **1992,** *3*, 322.

[266] Robertson, G.S.; Fibiger, H.C. *Neuroscience*, **1992,** *46*, 315.

[267] Merchant, K.M.; Dorsa, D.M. *Proc. Natl. Acad. Sci. USA*, **1993**, *90***,** 3447.

[268] Robertson, G.S.; Jian, M. *Neuroscience*, **1995,** *64*, 1019.

[269] Díaz-Cabiale, Z.; Vivo, M.; Del Arco, A.; O'Connor, W.T.; Harte, M.K.; Müller, C.E.; Martínez, E.; Fuxe, K.; Popoli, P.; Ferré, S. *Neurosci Lett.*, **2002,** *324*, 154.

[270] Sills, T.L.; Azampanah, A.; Fletcher, P.J. *Psychopharmacology.*, **2001,** *156*, 187.

[271] Hutchison, A.J.; Webb, R.L.; Oei, H.H.; Ghai, G.R.; Zimmerman, M.B.; Williams, M. *J. Pharmacol. Exp. Ther.*, **1989,** *251*, 47.

[272] Mathôt, R.A.A.; Cleton, A.; Soudijn, W.; Ijzerman, A.P.; Danhof, M. *Brit. J. Pharmacol.*, **1995,** *114*, 761.

[273] Webb, R.L.; Sills, M.A.; Chovan, J.P.; Peppard, J.V.; Francis, J.E. *J. Pharmacol. Exp. Ther.*, **1993,** *267*, 287.

[274] Rowlett, J.K.; Mattingly, B.A.; Bardo, M.T. *Synapse*, **1995,** *20*, 209.

[275] Muscat, R.; Iravani, M.M.; Kruk, Z.L. *Neuroscience*, **1996,** *75*, 1175.

[276] Henry, D.J.; Hu, X.T.; White, F.J. *Psychopharmacology*, **1998a,** *140*, 233.

[277] Vanderschuren, L.J.; Kalivas, P.W. *Psychopharmacology*, **2000,** *151*, 99.

[278] Rouge-Pont, F.; Usiello, A.; Benoit-Marand, M.; Gonon, F.; Piazza, P.V.; Borrelli, E. *J. Neurosci.*, **2002,** *22*, 3293.

[279] Nestler, E.J.; Terwilliger, R.Z.; Walker, J.R.; Sevarino, K.A.; Duman, R.S. *J. Neurochem.*, **1990,** *55*, 1079.

[280] Robinson, T.E.; Becker, J.B. *Brain Res.*, **1986,** *396*, 157.

[281] Antelman, S.M.; Eichler, A.J.; Black, C.A.; Kocan, D. *Science*, **1980,** *207*, 329.

[282] Akimoto. K.; Hamamura, T.; Kazahaya, Y.; Akiyama, K.; Otsuki, S. *Brain Res.*, **1990,** *507*, 344.

[283] Badiani, A.; Anagnostaras, S.G.; Robinson, T.E. *Psychopharmacology*, **1995,** *117*, 443.

[284] De Vries, T.J.; Schoffelmeer, A.N.; Binnekade, R.; Raaso, H.; Vanderschuren, L.J. *Neuropsychopharmacology*, **2002,** *26*, 18.

[285] Khroyan, T.V.; Barrett-Larimore, R.L.; Rowlett, J.K.; Spealman, R.D. *J. Pharmacol. Exp. Ther.*, **2000,** *294*, 680.

[286] Laruelle, M. *Brain Res. Brain Res. Rev.*, **2000,** *31*, 371.

[287] Sato, M.; Chen, C.C.; Akiyama, K.; Otsuki S. *Biol. Psychiatry*, **1983,** *18*, 429.

[288] Sato, M.; Numachi, Y.; Hamamura, T. *Schizophr. Bull.*, **1992,** *18*, 115.

[289] Laruelle, M.; Abi-Dargham, A.; van Dyck, C.H.; Gil, R.; D'Souza, C.D.; Erdos, J.; McCance, E.; Rosenblatt, W.; Fingado, C.; Zoghbi, S.S.; Baldwin, R.M.; Seibyl, J.P.; Krystal, J.H.; Charney, D.S.; Innis, R.B. *Proc. Natl. Acad. Sci. USA*, **1996,** *93*, 9235.

[290] Breier, A.; Su, T.P.; Saunders, R.; Carson, R.E.; Kolachana, B.S.; de Bartolomeis, A.; Weinberger, D.R.; Weisenfeld, N.; Malhotra, A.K.; Eckelman, W.C.; Pickar, D. *Proc. Natl. Acad. Sci. USA*, **1997,** *94*, 2569.

[291] Tong, Z.Y.; Overton, P.G.; Clark, D. *Brain Res.*, **1995,** *674*, 63.

[292] Wolf, M.E.; Dahlin, S.L.; Hu, X.T.; Xue, C.J.; White, K. *Neuroscience*, **1995,** *69*, 417.

[293] Weinberger, D.R. *Neuropsychopharmacology*, **1996,** *14*, 1S.

[294] Saunders, R.C.; Kolachana, B.S.; Bachevalier, J.; Weinberger, D.R. *Nature*, **1998,** *393*, 169.

[295] Metman, L.V.; Konitsiotis, S.; Chase, T.N. *Mov. Disord.*, **2000,** *15*, 3.

[296] Nutt, J.G.; Obeso, J.A.; Stocchi, F. *Trends Neurosci.*, **2000,** *23*, S109.

[297] Creese, I.; Burt, D.R.; Snyder, S.H. *Science*, **1977,** *197*, 596.

[298] Geurts, M.; Hermans, E.; Cumps, J.; Maloteaux, J.M. *Brain Res.*, **1999,** *841*,135.

[299] Terasmaa, A.; Andbjer, B; Fuxe, K.; Rinken, A. *Neuroreport*, **2000,** *11*, 2691.

[300] Cai, G.; Wang, H.Y.; Friedman, E. *J. Pharmacol. Exp. Ther.*, **2002,** *302*, 1105.

[301] Lee, T.; Seeman, P.; Rajput, A.; Farley, I.J.; Hornykiewicz, O. *Nature*, **1978,** *273*, 59.

[302] Ichise, M.; Kim, Y.J.; Ballinger, J.R.; Vines, D.; Erami, S.S.; Tanaka, F.; Lang, A.E. *Neurology*, **1999,** *52*, 1206.

[303] Ferré, S.; Casas, M.; Cobos, A.; Garcia, C.; Jane, F.; Grau, J.M. *Psychopharmacology*, **1987,** *1*, 254.

[304] Engber, T.M.; Susel, Z.; Kuo, S.; Gerfen, C.R.; Chase, T.N. *Brain Res.*, **1991,** *552*, 113.

[305] Cenci, M.A.; Lee, C.S.; Bjorklund, A. *Eur. J. Neurosci.*, **1998,** *10*, 2694.

[306] Engber, T.M.; Susel, Z.; Juncos, J.L.; Chase, T.N. *Eur. J. Pharmacol.*, **1989,** *168*, 291.

[307] Carey, R.J. *Brain Res.*, **1991,** *568*, 205.

[308] Henry, B.; Crossman, A.R.; Brotchie, J.M. *Exp. Neurol.*, **1998,** *151*, 334.

[309] Kashihara, K.; Manabe, Y.; Shiro, Y.; Warita, H.; Abe, K. *Neurosci. Res.*, **2000,** *38*, 273.

[310] Lundblad, M.; Andersson, M.; Winkler, C.; Kirik, D.; Wierup, N.; Cenci, M.A. *Eur. J. Neurosci.*, **2002,** *15*, 120.

[311] Bordet, R.; Ridray, S.; Carboni, S.; Diaz, J.; Sokoloff, P.; Schwartz, J.C. *Proc. Natl. Acad. Sci., USA*, **1997,** *94*, 3363.

[312] Joyce, J.N. *Pharmacol. Ther.*, **2001,** *90*, 231.

[313] Shimazoe, T.; Yoshimatsu, A.; Kawashimo, A.; Watanabe, S. *Eur. J. Pharmacol.*, **2000,** *388*, 249.

[314] Fredduzzi, S.; Moratalla, R.; Monopoli, A.; Cuellar, B.; Xu, K.; Ongini, E.; Impagnatiello, F.; Schwarzschild, M.A.; Chen, J.F. *J. Neurosci.*, **2002,** *22*, 1054.

[315] Mao, L.; Wang, J.Q. *Synapse*, **2001,** *41*, 230.

[316] Agnati. L.F.; Franzén, O.; Ferré, S.; Franco, R.; Fuxe, K. *J Neural Transm.*, **2003**, in press.

Regulation of heptaspanning-membrane-receptor function by dimerization and clustering

Rafael Franco[1], Meritxell Canals[1], Daniel Marcellino[1], Sergi Ferré[2], Luigi Agnati[3], Josefa Mallol[1], Vicent Casadó[1], Francisco Ciruela[1], Kjell Fuxe[4], Carmen Lluis[1] and Enric I. Canela[1]

[1]Department of Biochemistry and Molecular Biology of the University of Barcelona, Martí Franquès 1 Barcelona E-08028, Spain
[2]Preclinical Pharmacology Section, Department of Health and Human Services, NIH, NIDA, IRP, Baltimore, MD 21224, USA
[3]Section of Physiology, Department of Biomedical Sciences, University of Modena, 41100 Modena, Italy and Department of Rehabilitation, Ludes, Paradiso, Switzerland
[4]Department of Neuroscience, Division of Cellular and Molecular Neurochemistry, Karolinska Institutet, S-171 77 Stockholm, Sweden

G-protein-coupled receptors form homomers and heteromers; agonist-induced conformational changes within interacting receptors of the oligomer modify their pharmacology, signalling and/or trafficking. When these receptors are activated, the oligomers rearrange and cluster and a novel mechanism of receptor-operation regulation by oligomer intercommunication is possible. This intercommunication would be assisted by components of the plasma membrane and by scaffolding proteins. Receptor cross-sensitization, cross-desensitization and novel, integrated receptor responses can then develop between oligomeric receptor complexes of the cluster without direct contact between them. This concept gives a new perspective to the understanding of neurotransmission and neuronal plasticity.

Previously, heptaspanning membrane, or G-protein-coupled, receptors were considered monomeric proteins that interact only with G proteins. However, it has become clear over the past four years that heptaspanning-membrane receptors are oligomeric structures formed not only by receptor homo- and hetero-dimers, but also a variety of proteins that interact both along the plane of the membrane (horizontal interactions) and across the plane of the membrane (from the extracellular to the intracellular side; vertical interactions). Furthermore, there is clear evidence that agonists induce a marked rearrangement of heptaspanning-membrane receptors in the plasma membrane.

Consideration of oligomerization and ligand-induced rearrangement (aggregation or clustering) of receptors provides new insight into our understanding of the mechanisms underlying the regulation of receptor function. Oligomeric complexes that come together in protein clusters form supramolecular structures in the plasma membrane that, together with scaffolding proteins, condition the behaviour (e.g. pharmacology, signalling and trafficking) of individual oligomeric receptor complexes.

In this process it should be considered that the plasma membrane is not an isomorphic structure, but a structure made by membrane domains with defined composition and chemical–physical characteristics (e.g. lipid rafts). In this article we comment on the existence of receptor regulation through direct protein–protein interactions within the oligomers, and propose intercommunication between proteins in oligomers that are not interacting directly but are present in the same cluster.

The conformational changes that are transferred through direct receptor–receptor and receptor–protein interactions constitute the first level of regulation of receptor function (Fig. 1). The second level of regulation is proposed to occur via indirect interactions between different oligomeric receptor complexes, and are modulated by agonist-induced aggregation of such complexes (Fig. 2). This regulation between non-interacting receptors in clusters is proposed to take place through intramembrane lipids and scaffolding proteins, involving conformational changes in a set of molecules in the membrane

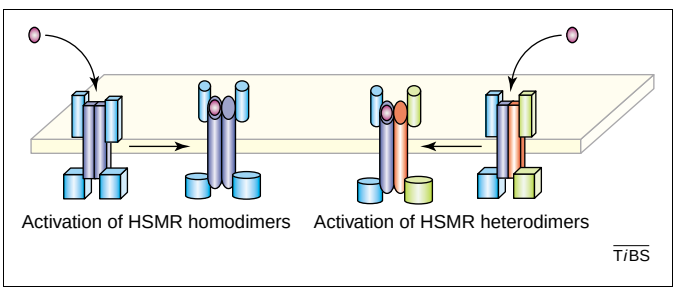

Fig. 1. First level of regulation. The ligand (red) for one type of receptor (dark blue) can change the conformation of heptaspanning-membrane receptor proteins by interacting directly with it as constitutive homodimers or heterodimers, as well as changing the conformation of associated proteins on the extracellular or intracellular side of the membrane. Different conformations of receptors are shown in dark blue and orange; different conformations of interacting proteins are shown in light blue and light green.

Corresponding author: Rafael Franco (r.franco@bq.ub.es).

http://tibs.trends.com 0968-0004/03/$ - see front matter doi:10.1016/S0968-0004(03)00065-3

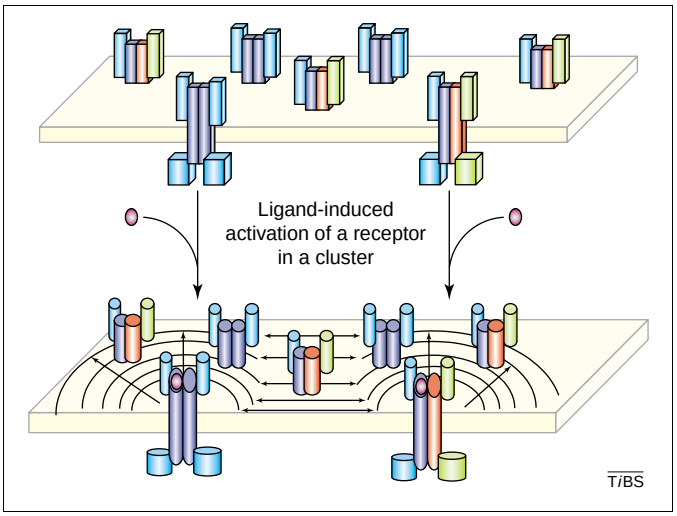

Fig. 2. Second level of regulation. The ligand (red) for one type of receptor (dark blue) of a homo- or hetero-dimer can change the conformation of proteins that are not necessarily interacting with them. This is possible by crosstalk established within proteins that are clustered together in an area of membrane, as illustrated here for homomeric and heteromeric receptor complexes.

domain (or patch) with the formation of a molecular circuit.

Oligomerization: the first level of regulation

Heteromeric complexes

The dimerization of membrane receptors (which was first demonstrated for the tyrosine kinase receptor superfamily) can be essential for signal transduction, and involves autophosphorylation and enhancement of agonist affinity [1,2]. It was long assumed that heptaspanning-membrane receptors were isolated monomers despite the hypothesis that they interacted [3–6].

Interactions between heptaspanning-membrane receptors – which can be homo- or hetero-meric – are crucial to understand the varied functional cross-talk that has been observed, especially for neurotransmitter receptors. These functional interactions can be either antagonistic or agonistic, that is, signalling through one receptor can be enhanced or depressed while, simultaneously, the other receptor is being activated [5,7–9]. This has key physiological consequences, especially in neurological diseases (see [5]).

Heptaspanning-membrane receptors also interact with intracellular components; the best-characterized heterotypic interaction is with β-arrestins. In most instances, β-arrestins are required for receptor desensitization and internalization. The interaction between β-arrestins and these receptors also triggers the extracellular signal-regulated kinases (ERK) cascade in which β-arrestins can behave both as agonist-regulated molecular adapters and as scaffolds [10], thus allowing activated ERKs to target specific subcellular domains [11]. An increasing number of proteins also behave as modulating (receptor-activity modifying proteins; RAMPs) or scaffolding proteins for heptaspanning-membrane receptors [5,7–9], but their description is beyond the scope of this article.

The interaction between a receptor and the enzyme that degrades its physiological ligand is paradigmatic; two examples of receptor–enzyme interactions have been revealed. The chemokine receptor CXCR4 interacts with a type II membrane protein with dipeptidyl peptidase IV activity. This protein – also known as activation antigen CD26 – metabolizes the chemokine recognized by CXCR4, thus modulating the signal [12,13]. CD26 cleaves the two N-terminal amino acids of the ligand, SDF1α, to render another ligand with different chemo-attractant and anti-HIV activity. This suggests that among the variables influencing the final activity of SDF1α, the amount of enzyme present, the K_M (for peptidase activity), the K_D (for receptor binding) and the initial concentration of the ligand are the most relevant. It would also be useful to find out whether the CD26–CXCR4 interaction modifies the dipeptidyl peptidase IV activity of CD26. Such regulation would lead to an altered availability of the ligand at the level of the receptor micro-environment, with reduced presence upon increases of peptidase activity. The second example is the interaction of adenosine deaminase (ADA) with two subtypes of adenosine receptors (A_1 and A_{2B}) [14,15]. Two aspects have to be considered in this interaction: one dependent and the other independent of the enzymatic activity (see below).

Adenosine A_1 receptors as a paradigm

Here, we mainly discuss receptor homomers and heteromers that, through conformational changes, modulate the binding properties, G-protein coupling and receptor trafficking of one another [5,7–9].

Along the plasma membrane (horizontal plane), the most recently described protein–protein interactions involve homo- or hetero-dimers. Non-linear Scatchard plots, which are used to analyze ligand binding to heptaspanning-membrane receptors, have been explained by assuming various degrees of coupling between monomeric receptors and G proteins. Because receptors are now known to form dimers by directly interacting with each other, it is no longer necessary to include the interacting G proteins in the equation. The binding behaviour of heptaspanning-membrane receptors can be easily interpreted assuming that there is negative (or positive) cooperativity in binding, that is, after the binding of one ligand molecule to a receptor, the next molecule binds to the second receptor of the dimer with less (or higher) affinity (Fig. 1). In fact, the presence of dimers justifies why agonists apparently modify the percentage of receptors with high affinity [16]. This is the case in the cooperativity displayed by oligomeric enzymes, which results from the conformational changes that occur when the ligand is bound to one of its active sites. A similar hypothesis can be suggested for heterodimers formed by two heptaspanning-membrane receptors (Fig. 1). In fact, we have shown in cells co-expressing adenosine A_1 receptors (A_1R) and dopamine D_1 receptors (D_1R) that agonists for A_1R inhibit the binding of dopamine analogues to D_1R [17]. In analogy, the adenosine A_{2A} receptor ($A_{2A}R$) agonist decreases agonist binding to D_2 dopamine receptors (D_2R) in the $A_{2A}R$–D_2R heteromers [18]. Antagonism or synergism (e.g. in A_1R–metabotropic glutamate receptor 1α heteromers [19]) can, in part, be caused by cross-talk at the level of second messengers. In fact, the physiological adenosine–dopamine antagonism existing in striatum

(e.g. demonstrated in enhancement of dopamine-receptor-mediated locomotion by treatment with adenosine-receptor antagonists [17]) can be partly because activation of D_1R leads to increases in cAMP, whereas activation of A_1R leads to decreases in cAMP. However, the effect on the kinetics of ligand binding, which is also relevant to understand the physiological antagonisms detected, can be attributed almost exclusively to conformational changes transmitted within the intercommunicating receptor molecules. Heteromerization can also modify the pharmacology of interacting receptors [5,20], which offers great potential for designing novel drugs.

Across the membrane (vertical to the plane of the membrane), A_1Rs interact with both intracellular proteins that are not directly involved in signalling cascades and also with proteins that have an extracellular topology. Are these direct interactions relevant for receptor function? The heat-shock cognate protein hsc73 was identified as a cytosolic component that interacts with the third intracellular loop of A_1R. As demonstrated by affinity chromatography and a biosensor-based technique (surface plasmon resonance), purified A_1Rs interact specifically with hsc73 with a nanomolar dissociation constant (0.5 ± 0.1 nM) [21]. The hsc73–A_1R interaction markedly reduces the affinity of A_1R agonist and antagonist ligands, and prevents the activation of G proteins, as deduced from [^{35}S]GTPγS binding assays. The effect on A_1R-agonist binding was stronger than that exerted by GTP analogues (which uncouple receptors from G proteins), and was completely prevented by ADA (a protein that interacts with the extracellular domains of A_1R). Therefore, the binding of ADA to the extracellular side of A_1R leads to conformational changes that either prevent the binding of the hsc73 to the intracellular side of A_1R, or block the effects of the hsc73–A_1R binding.

A_1R and caveolin also interact. In several cell lines, A_1R are homogeneously distributed in the absence of ligand, but in the presence of agonist they concentrate in rafts that are cholesterol-rich specialized regions of the membrane [22,23]. The C-terminal region of A_1R has a consensus motif for interaction with caveolin (a protein enriched in caveolae), which are flask-shaped rafts. It is not known whether this interaction affects ligand binding, but it is crucial for receptor internalization – at least, it is in cells in which A_1R internalization does not occur via clathrin-coated vesicles [22,23]. Although it has long been believed that heptaspanning-membrane receptors internalize via clathrin-enriched vesicles, recent evidence indicates that these receptors can also follow a clathrin-independent, caveolin-dependent internalization pathway [22–24]. It is of interest to note that A_1R and ADA can co-internalize via a caveolin-dependent mechanism that ends up in the endosomal compartment, where differential sorting takes place followed by independent recycling towards the cell surface [23,25]. Details of this trafficking mechanism are being examined to fully understand the physiological role of the interaction between A_1R and caveolin.

In addition, A_1R interact with ADA across the plane of the membrane. The binding of [^{3}H]-2-chloroadenosine to A_1R in rat brain membranes was first studied in two laboratories; binding in the absence or presence of ADA – single (low-affinity) or two binding (low- and high-affinity) sites, respectively [26–29]. The appearance of a high-affinity-binding site in the presence of ADA was explained either by the disappearance of endogenous adenosine – which behaves as a competitor of A_1R agonists – or by the assumption that ADA has an extracatalytic high-affinity-binding site for 2-chloro-adenosine – which is not consistent with the X-ray structure of the enzyme [30]. Now, sufficient evidence supports a functional coupling between A_1R and ADA. In fact, without this direct interaction A_1R display a low affinity for agonists and can not be activated. The enzymatic activity of ADA is not necessary for this co-stimulatory role because the effect is still detected when adenosine deaminase activity is completely abolished. It should be noted that the A_1R–D_1R-mediated dopamine–adenosine antagonism requires interaction between A_1R and ADA. Therefore, ADA regulates the binding of dopamine to D_1R through A_1R without directly contacting D_1R [31].

It remains to be investigated whether ADA enzymatic activity is modified by this ADA–A_1R interaction. A recent report points to strong and variable changes in ADA-specific activity upon binding to dipeptidylpeptidase IV, which can also anchor ADA in membranes [32]. A similar mechanism of enzyme regulation in ADA–A_1R complexes might be particularly interesting because ADA and A_1R are internalized together by a caveolae-dependent mechanism. As reported by Lefkowitz and colleagues, signalling is often maintained in internalized vesicles that contain heptaspanning-membrane receptors [11,24]. Thus, a reduction in ADA activity might lead to the maintenance of the signal produced by adenosine until both A_1R and ADA reach the endosomal compartment, where they are differentially sorted and separately recycled to the cell surface.

Clustering: the second level of regulation

Interactions among multiple heteromeric complexes

Oligomers containing heptaspanning-membrane receptors, enzymes and other RAMPS, redistribute in the membrane and give rise to clusters when activated by hormones or neurotransmitters [17,33–35]. Clusters arise from a marked redistribution of proteins to an area of the plasma membrane. There is a controversy as to whether the state of aggregation of receptors, for example, formation of heterotrimers or heterotetramers, increases with clustering. It seems that in cells expressing recombinant receptors, the degree of heteromerization does not vary with clustering, although this remains to be confirmed in unmodified cells. Irrespective of this, our hypothesis is that individual heptaspanning-membrane receptors present in the heteromers intercommunicate with each other through membrane lipids and intracellular scaffolds. Although the event of clustering has not received sufficient attention to date, we postulate that it constitutes a higher level of heptaspanning-membrane receptor and membrane enzyme regulation. Receptors (or enzymes interacting with them) might be regulated by other receptor molecules in the interacting complexes and also by other molecules that do not interact physically, but communicate with them in the cluster.

The occurrence of clustering clearly suggests that heptaspanning-membrane receptors generate high-order molecular structures, in which multimers of the receptors form functional complexes. Clusters define a nested hierarchical level of organization in which a more refined behaviour can be envisioned. Thus, the regulation produced by these superstructures is at a higher level than that associated with an 'isolated' heteromeric-receptor complex system [36].

I. Ligand binding

We believe that the ligand-induced conformational changes of a receptor in a cluster transmit information within the cluster that slightly alters the thermodynamic properties (and so the K_D) of all proteins in that cluster. These conformational changes reflect the energy landscape of the clustered proteins (Fig. 2).

In 1996, we proposed a model that takes into account that various heptaspanning-membrane receptor molecules undergo, as a result of ligand binding, a cascade of discrete conformational transitions that affect their affinity. The cluster-arranged cooperative model (CACM) [33] is based on the hypothesis that heptaspanning-membrane receptors are not isolated proteins, but that they communicate with each other by components of the membrane and scaffolds. This intercommunication between molecules was regarded, at that time, as the basis of the observed cooperativity, and its participation in the multiple processes involved in ligand-induced desensitization was suggested. The validity of the model was shown with A_1Rs [33], for which clustering probably alters the conformation of the receptor in such a way that it decreases the affinity for the agonist. This is also the reason why the model was named the cluster-arranged cooperative model, and why all the molecules were assumed to intercommunicate within a patch in the membrane. Therefore, the CACM might be a suitable model for how higher-order structures affect receptor and enzyme functions.

Let us consider the heteromeric complexes A, B and C in a given cluster. When the first ligand binds to a receptor in A, not only are other enzyme or receptor molecules in A affected (by negative or positive cooperativity), but the kinetics for ligand binding to, for instance, receptors present in B and C, also change, as do their K_D values. These slight modifications in K_D for receptors in B and C are a function of the concentration of the first agonist interacting with the cluster. When a receptor in A is activated within the cluster, it can be assumed that this first ligand interaction decreases the K_D for a receptor in B (and/or C). A lower concentration of specific ligands for that receptor in B (and/or C) would be then sufficient to trigger its signalling cascade. In this way, sensitization could occur. Obviously, the opposite could also happen: if activation of a receptor in A increases the K_D for a receptor in B (and/or C), a higher concentration of agonists for that receptor in B (and/or C) is needed to trigger signalling. In these circumstances, desensitization or even anergy (no signalling) could take place. Furthermore, it is possible that the situation could be even more complex. Consider, for instance, that the first agonist decreases the K_D for a receptor in B, and increases the K_D for a receptor in C. This would be a mechanism for an integrative response (specialization) because a hormone or neurotransmitter would simultaneously sensitize a receptor in B and desensitize a receptor in C, which might represent a process of learning (Fig. 3). According to this model, the first signal arriving at the membrane cluster would condition the future behaviour of all receptors in the cluster. This could help explain the molecular basis of complex phenomena such as neuronal plasticity at the membrane level (i.e. information handling among heteromeric receptor complexes in the agonist-induced receptor cluster).

II. Molecular rearrangements

Apart from changing affinity, signalling could also be conditioned by the constraints of clustering. In a given area of the cell surface, the two-dimensional 'concentration' of a receptor in the absence of the ligand could be low because the receptors are homogeneously distributed. In response to the agonist, the two-dimensional 'concentration' and distribution of receptors in the area of membrane change as the cluster forms. This was shown for A_1R [33,34], A_1R–D_1R heterodimers [17] and A_{2A}R–D_2R heterodimers [35] in cell lines and neurones. This molecular rearrangement might be produced by two events: conformational changes in the proteins at the horizontal level and the action of scaffolding proteins such as β-arrestin, caveolin, and others (e.g. those linking receptors to the cytoskeleton), which guide receptors in this clustering journey. Depending upon the cell (each cell has it own set of receptors expressed on the membrane) and the type of activated receptors, cluster composition and molecular rearrangement (geometry and distances) can be postulated to change. This leads to differences in

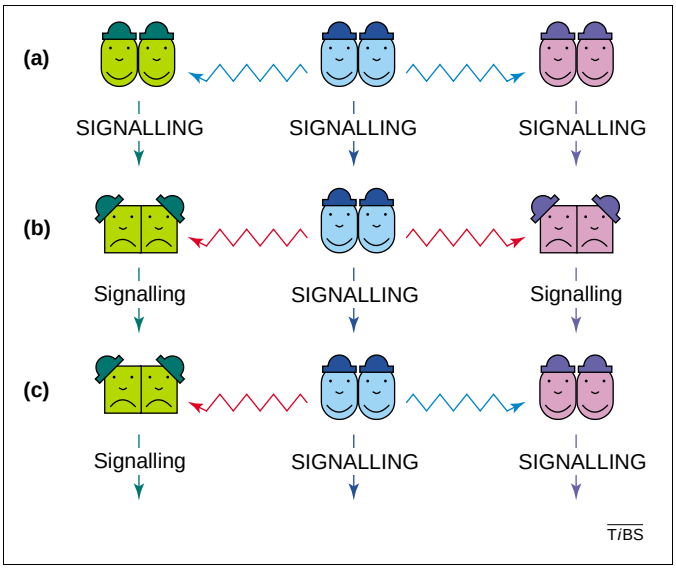

Fig. 3. Scheme of how an agonist ligand (symbolized by a hat) for one receptor (symbolized by a face) might sensitize or desensitize other receptors for their transmitters in a cluster. According to the text, different types of receptors (light green, light blue and pink) exist in distinct homo-oligomeric complexes clustered in the same membrane area. 'Happy', round receptors with hats on would be those having a high affinity for the agonist and, thus, high signalling. 'Sad', square receptors with tilted hats would be those having a low affinity for the agonist and low signalling.

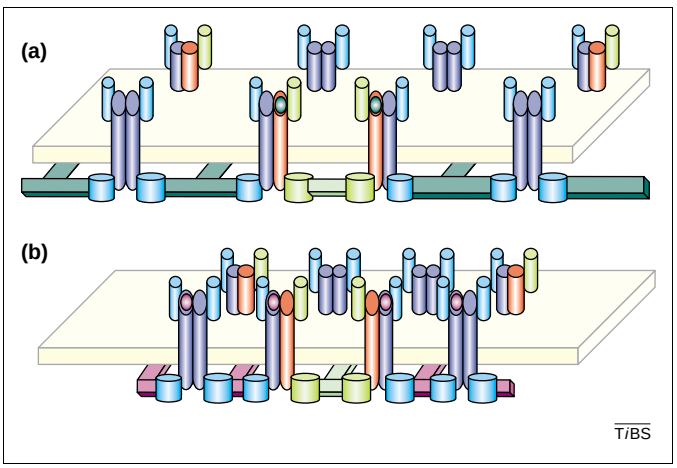

Fig. 4. Scheme showing how molecular rearrangements in a cluster can condition receptor signalling. Two arrangements for receptor homo- and hetero-dimers in a cluster triggered by different agonists are shown, (a) one activating one type of receptors (dark green) and (b) another activating another type of receptor (red). Differentially activated adaptor/scaffolding proteins guide the rearrangement of the interacting proteins and the underlying signalling machinery into a variety of shapes, (different 'crystallization' patterns of molecular networks in the patch). This might lead to qualitatively different signalling pathways indicated as overall signalling (a) or (b). It should be noted that only two examples of simple arrangements with only eight receptor dimers are provided, but more complex distributions of receptors can be expected.

signalling from cell to cell and even within the same cell when activated by distinct sets of hormones or neurotransmitters. This redistribution should be relevant for integration of signalling, which would vary according to the location of the activated receptors in the membrane (Fig. 4).

It should be noted that at this second level of regulation, the plasma membrane itself transduces conformational changes in neighbouring elements. This can be achieved, at least in part, by modifications, for example, in membrane lipids, which condition receptor pharmacology; this is important to understand changes in signalling in cells whose membrane fluidity is altered when they become tumorigenic. Moreover, there is evidence that agonists acting on adenosine receptors alter membrane microviscosity in myelin [37] and neutrophils [38]. Because each cell has its own set of receptors and interacting molecules, clustering and ligand-induced changes in membrane structure give rise to specific molecular rearrangements of the receptors, interacting proteins and underlying signalling machinery. The variety of molecular networks in the clusters might be described as several systems of 'crystallization' in the patch. Different processes of freezing the receptors within those molecular networks might lead to the formation of memory traces in the membrane [39].

In a system of interconnected binary elements, some of the elements might freeze in fixed states of activity (either active or inactive) [40]. According to our hypothesis, a cluster of frozen elements in a receptor mosaic might represent a mechanism for the maintenance of a constant input of a neurone and, hence, might play a role in the learning process (where the input of a neurone is likely to remain constant for a period of time) [39], which sometimes is simply represented by a phenomenon of sensitization and desensitization. Even for a single cell, the arrangement of the receptors (or enzymes) in the cluster might depend on the type of receptor (or enzyme) that 'guides' the clustering, that is, arrangement is dependent on the type of receptor activated first, which receptor is activated second and so on. It seems evident that the location of the receptors in the membrane after activation and the clustering influences the physiological effect. Thus, the concentration, the degree of activation and the arrangement of receptors in the clusters could condition signalling in such a way that signalling might differ from cell to cell and even within the same cell (among distinct locations in a given cell) depending on the spatial–temporal course of activation. This novel concept provides a new perspective in understanding neurotransmission and neuronal plasticity.

Acknowledgements

This work was supported by grant QLG3-CT-2001–01056 from the European Community and grants BIO1000-0601-C02-02, SAF2002-03293 and SAF2001-3474 from Ministerio de Ciencia y Tecnología, 02/056-00 from Fundació la Caixa and 01/012710 from Fundació Marató of Catalonian Telethon.

References

1 Schlessinger, J. and Ullrich, A. (1992) Growth factor signalling by receptor tyrosine kinases. *Neuron* 9, 383–391
2 Ullrich, A. and Schlessinger, J. (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell* 20, 203–212
3 Agnati, L.F. *et al.* (1980) Aspects on receptor regulation and isoreceptor identification. *Med. Biol.* 58, 182–187
4 Fuxe, K. *et al.* (1983) Evidence for the existence of receptor-receptor interactions in the central nervous system. Studies on the regulation of monoamine receptors by neuropeptides. *J. Neural Transm. Suppl.* 18, 165–179
5 Agnati, L.F. *et al.* Molecular mechanisms and therapeutical implications of intramembrane receptor–receptor interactions among heptahelical receptors with examples from the striato-pallidal GABA neurons. *Pharmacol. Rev.* (in press)
6 Lefkowitz, R.J. (2000) The superfamily of heptahelical receptors. *Nat. Cell Biol.* 2, E133–E136
7 Milligan, G. and White, J.H. (2001) Protein–protein interactions at G-protein-coupled receptors. *Trends Pharmacol. Sci.* 22, 513–518
8 Marshall, F.H. (2001) Heterodimerization of G protein coupled receptors in the CNS. *Curr. Opin. Pharmacol.* 1, 40–44
9 Bouvier, M. (2001) Oligomerization of G-protein-coupled transmitter receptors. *Nat. Rev. Neurosci.* 2, 274–286
10 Miller, W.E. and Lefkowitz, R.J. (2001) Expanding roles for β-arrestins as scaffolds and adapters in GPCR signalling and trafficking. *Curr. Opin. Cell Biol.* 13, 139–145
11 Luttrell, L.M. and Lefkowitz, R.J. (2002) The role of β-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J. Cell Sci.* 115, 445–465
12 Herrera, C. *et al.* (2001) Comodulation of CXCR4 and CD26 in human lymphocytes. *J. Biol. Chem.* 276, 19532–19539
13 Lambeir, A.M. *et al.* (2001) Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. *J. Biol. Chem.* 276, 29839–29845
14 Saura, C.A. *et al.* (1996) Adenosine deaminase interacts with A1 adenosine receptors in pig brain cortical membranes. *J. Neurochem.* 66, 1675–1682
15 Herrera, C. *et al.* (2001) Adenosine A2B receptors behave as an alternative anchoring protein for cell surface adenosine deaminase in lymphocytes and cultured cells. *Mol. Pharmacol.* 59, 127–134
16 Casadó, V. *et al.* (1991) The binding of [3H]R-PIA to A1 adenosine receptors produces a conversion of the high- to the low-affinity states. *FEBS Lett.* 286, 221–224
17 Ginés, S. *et al.* (2000) Dopamine D1 and adenosine A1 receptors assemble into functionally interacting heteromic complexes. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8606–8611
18 Salim, H. *et al.* (2000) Activation of adenosine A1 and A2A receptors

modulates dopamine D2 receptor-induced responses in stably transfected human neuroblastoma cells. *J. Neurochem.* 74, 432–439
19 Ciruela, F. *et al.* (2001) Metabotropic glutamate 1α and adenosine receptors assemble into functionally interacting complexes. *J. Biol. Chem.* 276, 18345–18351
20 Rios, C.D. *et al.* (2001) G-protein-coupled receptor dimerization: modulation of receptor function. *Pharmacol. Ther.* 92, 71–87
21 Sarrio, A. *et al.* (2000) The heat shock cognate protein hsc73 assembles with A1 adenosine receptors to form functional modules in the cell membrane. *Mol. Cell. Biol.* 20, 5164–5174
22 Ginés, S. *et al.* (2001) Involvement of caveolin in ligand-induced recruitment and internalization of A(1) adenosine receptor and adenosine deaminase in an epithelial cell line. *Mol. Pharmacol.* 59, 1314–1323
23 Escriche, M. *et al.* Ligand-induced caveolae-mediated internalisation of A1 adenosine receptors. Morphological evidence of endosomal sorting and receptor recycling. *Exp. Cell Res.* (in press)
24 Pierce, K.C. *et al.* (2002) Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* 3, 639–650
25 Saura, C.A. *et al.* (1998) Adenosine deaminase and A1 adenosine receptors internalize together following agonist-induced receptor desensitization. *J. Biol. Chem.* 273, 17610–17617
26 Wu, P.H. *et al.* (1980) Specific binding of 2-[3H]chloroadenosine to rat brain cortical membranes. *Can. J. Physiol. Pharmacol.* 58, 576–579
27 Wu, P.H. and Phillis, J.W. (1982) Adenosine receptors in rat brain membranes: characterization of high affinity binding of [3H]-2-chloroadenosine. *Int. J. Biochem.* 14, 399–404
28 Williams, M. and Risley, E.A. (1980) Biochemical characterization of putative central purinergic receptors by using 2-chloro[3H]adenosine, a stable analog of adenosine. *Proc. Natl. Acad. Sci. U. S. A.* 77, 6892–6896
29 Williams, M. and Risley, E.A. (1980) High affinity binding of 2-chloroadenosine to rat brain synaptic membranes. *Eur. J. Pharmacol.* 64, 369–370
30 Wilson, D.K. *et al.* (1991) Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. *Science* 252, 1278–1284
31 Torvinen, M. *et al.* (2002) Interactions among adenosine deaminase, adenosine A1 receptors and dopamine D(1) receptors in stably cotransfected fibroblast cells and neurons. *Neuroscience* 113, 709–719
32 Ben-Shooshan, A. *et al.* (2002) On the regulatory role of dipeptidyl peptidase IV (MCD26Madenosine deaminase complexing protein) on adenosine deaminase activity. *Biochim. Biophys. Acta* 1587, 21–30
33 Franco, R. *et al.* (1996) The cluster-arranged cooperative model: a model that accounts for the kinetics of binding to A1 adenosine receptors. *Biochemistry* 35, 3007–3015
34 Ciruela, F. *et al.* (1997) Ligand-induced phosphorylation, clustering and desensitization of A1 adenosine receptors. *Mol. Pharmacol.* 52, 788–797
35 Hillion, J. *et al.* (2002) Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. *J. Biol. Chem.* 277, 18091–18097
36 Changeaux, J-P. and Dehaene, S. (2000) Hierarchical neuronal modeling of cognitive functions: from synaptic transmission to the Tower of London. *Int. J. Psychophysiol.* 35, 179–187
37 Casadó, V. *et al.* (1991) Adenosine receptors in myelin fractions and subfractions: the effect of the agonist (R)-phenylisopropyladenosine on lyelin membrane microvscosity. *J. Neurochem.* 57, 1623–1629
38 Cronstein, B.N. *et al.* (1989) Adenosine, a cytoprotective autacoid: effects of adenosine on neutrophil plasma membrane microviscosity and chemoattractant receptor display. *Biochim. Biophys. Acta* 987, 176–180
39 Agnati, L.F. *et al.* (2002) Molecular basis of learning and memory: modelling based on receptor mosaics. In *From Synapses to Rules: Discovering Symbolic Rules from Neural Processed Data* (Apolloni, B. and Kurfess, F., eds) Kluwer Academic Publisher
40 Kauffman, S.A. (1993) *The Origin of Order*, Oxford University Press.