CHAPTER 1.2:

“Studies on Histidine Decarboxylase Activity”

1.2. Studies on Histidine Decarboxylase Activity

In the previous paper entitled "Quantitative Radioisotopic Determination of Histidine Decarboxylase Using High-Perfomance Liquid Chromatography" (Analytical Biochemistry, **280**: 111-117, 2000) has been described a new technique which significantly improve histidine decarboxylase activity determination using a radioisotopic methodology. To asses the optimal function of this method, we have shown different experiments which demonstrate its high selectivity, sensibility and accuracy.

To further confirm the validity of this technique we have reproduced several experiments studying HDC activity which have been previously studied with other methods.

1.2.1. Determination of HDC activity in different rat brain regions and rat stomach: (Table 1)

Male sprague-dawley rats of 250-300g were decapitated and brains were chilled on ice cold buffer (10mM potassium phosphate buffer, pH=7.4). Working on a cold environment coronal sections of 1mm thick were obtained using a McIlwain chopper. Regions were dissected according to the atlas of Paxinos and Watson. Each region was homogenized in 15% (w/v) in the phosphate buffer at pH=7.4. For the tuberomammillary dissections, a chunk of posterior hypothalamus was obtained based on the shape of the adjacent third ventricle.

Frontal hypothalamus presented the highest HDC activity in rat brain because it is the most histaminergic nerve-endings innervated region. Tuberomammillary nucleus also presented high HDC activity because in this region the histaminergic neuron cell bodies are located. In contrast, cerebellum showed the lowest activity of the histamine synthesizing enzyme because of its insignificant histaminergic nerve-endings innervation. Finally, stomach presented very high HDC activity. It is known that the enterochromaffin-like cells of the gastric wall have the highest histamine levels and HDC activity of the whole organism. The relative activities compared among different regions are in agreement with those previously obtained using other methodologies. Given the different substrate concentration used by different procedures (Taylor and Snyder, 1972; Baudry et al., 1973), a comparison of absolute activity values can not be made. As a comparison with our results, in table 1 we show results obtained by Baudry et al (1973).

TABLE 1.
DISTRIBUTION OF HDC ACTIVITY IN DIFFERENT RAT BRAIN REGIONS AND STOMACH

Regions	Total activity (fmolHA /mg prot x h)	Total activity * (nmolHA/g tissue x h)
Cortex	45.0 ± 5.6 (19)	0.66 ± 0.1 (3)
Striatum	46.7 ± 5.8 (6)	0.65 ± 0.06 (3)
frontal Hypothalamus	285.5 ± 20.7 (19)	2.88 ± 0.4 (2)
Tuberomammillary nuclei	164.0 ± 31.0 (6)	--- (--)
Cerebellum	5.8 ± 0.3 (3)	0.33 ± 0.003 (5)
Stomach	680.3 ± 2.0 (2)	--- (--)

Results are means ± SD. Number of experiments is indicated in brackets. * Comparative results obtained from Baudry et al., 1973.

1.2.2. Regional distribution of soluble and particulate HDC activity: (Table 2)

In all regions studied, we have found that the main percentage of HDC activity is located in supernatant fraction (approximately 80% of the total activity). This suggests that the histamine synthesizing enzyme is predominantly located in the cytosol as previously reported (Toledo A., 1988). Otherwise, our grup had previously demonstrated that part of HDC is located in brain membranes (Toledo et al., 1988). In rat stomach, we have obtained HDC activity predominantly in cytosolic fraction.

TABLE 2. – REGIONAL DISTRIBUTION OF SOLUBLE AND PARTICULATE HDC ACTIVITY IN RAT BRAIN AND STOMACH

Regions	Supernatant (fmolHA/mg prot x h)	(% of total)	Particulate (fmolHA/mg prot x h)	(% of total)
Cortex	32.35	86.7	4.95	13.3
Striatum	62.28	84.2	11.71	15.8
Hypothalamus	298.42	78.6	81.29	21.4
Cerebellum	2.77	92.5	0.23	7.5
Stomach	526.66	97.4	11.22	2.1

Each region was homogenized in 15% (w/v) 10mM potassium phosphate buffer at pH=7.4. After centrifugation at 100.000 x g for 1h, the enzyme activities were determined in supernatant and pellet, respectively. Results are Means of duplicate samples.

1.2.3. HDC activity in subcellular fractions of whole brain homogenates: (Table 3)

The rat brain was homogenized in 10% (w/v) 0.32M sacarose in 5mM tris buffer at pH=7.4 . Subcellular fractions (P1, P2 and S2) were obtained as described by Whittaker (1968). Results of HDC activity in each fraction were in agreement with previous published data (Picatoste F., 1978; Palacios J.M., 1975; Baudry et al., 1973).

The distribution of the HDC activity was similar to that of other enzymes involved in neurotransmitter synthesis like DOPA decarboxylase or acetylcholine-transpherase (Whittaker et al., 1969; Baudry et al., 1973). The proportions of HDC activities obtained in the crude nuclear (P1), crude mitochondrial (P2) and microsomal plus soluble (S2) fractions were 8.1%, 44.5% and 47.5%, respectively. These results are in complete agreement with previously reported results obtained in our laboratory using a fluorimetric method (Palacios J.M., 1975; Picatoste F., 1978; Toledo A., 1988). Simultaneously to the HDC activity determination, we studied the protein percentage in P1, P2 and S2 to control the quality of subcellular fractionation. The data are in good agreement with previously published observations suggesting that the subcellular fraction had been correctly obtained.

In conclusion, these results indicate that a major part of histidine decarboxylase in rat brain is held in the cytoplasm of nerve terminals (synaptosomes) whereas another important fraction is located in cytoplasm of neurones or glial cells.

TABLE 3.
HDC ACTIVITY IN SUBCELLULAR FRACTIONS OF WHOLE BRAIN HOMOGENATES

	Protein		HDC activity			
Regions	(mg prot/g tissue)	(%)	(fmolHA/g tissue x h)	(%)	(nmolHA/g tissue x h) [a]	(%)*
HG	118.9 ± 0.32 (3)	---	3384.4 ± 119.6 (3)	---	1.83 ± 0.05 (5)	---
P1	27.32 ± 0.26 (3)	27.6	206.02 ± 9.2 (3)	8.05	0.17 ± 0.01 (5)	8.81
P2	45.12 ± 0.4 (3)	45.6	1137.6 ± 90.9 (3)	44.48	0.72 ± 0.03 (5)	37.3
S2	26.4 ± 0.12 (3)	26.7	1213.7 ± 62.8 (3)	47.51	1.04 ± 0.07 (5)	53.87

Results are means ± SD. Number of determinations is indicated in brackets. % correspond to the percentage of total recovered material. Abbreviations: Homogenate (HG), crude nuclear (P1), crude mithocondrial (P2) and microsomal plus soluble (S2) fractions. [a] Results obtained from Toledo A. (1988)

1.2.4. Effect of the presence of Protease Inhibitors on HDC activity: (Table 4)

As it has been previously reported that histidine decarboxylase from several tissues could be proteolysed by several proteases (Yamada et al., 1980; Tanaka et al., 1995; Ichikawa et al., 1998), we studied whether the presence of proteases inhibitors in the incubation medium could modify the enzyme activity. The additon of a mixture of protease inhibitors (including PMSF, aprotinin and leupeptin) to the incubation medium produced an increase on HDC activity of +53.7% versus controls.

In conclusion, these results suggest that the presence of protease inhibitors in the incubation medium prevents the proteolysis/inactivation of HDC.

TABLE 4.
EFFECT OF PROTEASE INHIBITORS ON RAT BRAIN HDC ACTIVITY

	HDC activity (fmolHA/mg prot x h)	
	Control	+ Protease Inhibitors
Rat brain homogenate	17.55 ± 1.15 (16)	**28.1 ± 1.7 (7)

Rat brain was homogenized in 15% (w/v) 10mM potassium phosphate buffer at pH=7.4. Protease inhibitors were added to the medium at the begining of incubation period. Protease inhibitors included: PMSF (1mM), Aprotinin (10µg/ml) and Leupeptin (10µg/ml). Results represent means ± SD. Number of experiments is indicated in brackets. ** P<0.01 compared with control (t-Student test).

1.2.5. Effect of Pyridoxal 5'-phosphate on HDC activity in rat brain homogenates: (Figure 1)

As pyridoxal 5'-phosphate (P-5P) is the cofactor for HDC, we studied the effect of exogenous P-5P on histidine decarboxylase activity on whole rat brain homogenates using our radioisotopic assay. The results indicate that histamine synthesis increases by the presence of the cofactor in a concentration-dependent manner. The maximal stimulatory effect was obtained in the presence of 1 x 10^{-3} M of the cofactor. In these conditions, it was obtained an increase on HDC activity of +84.4% versus homogenates without P-5P. At high concentrations (2 x 10^{-3} M), we observed a saturation of the effect, probably due to the saturation of the enzyme by its cofactor.

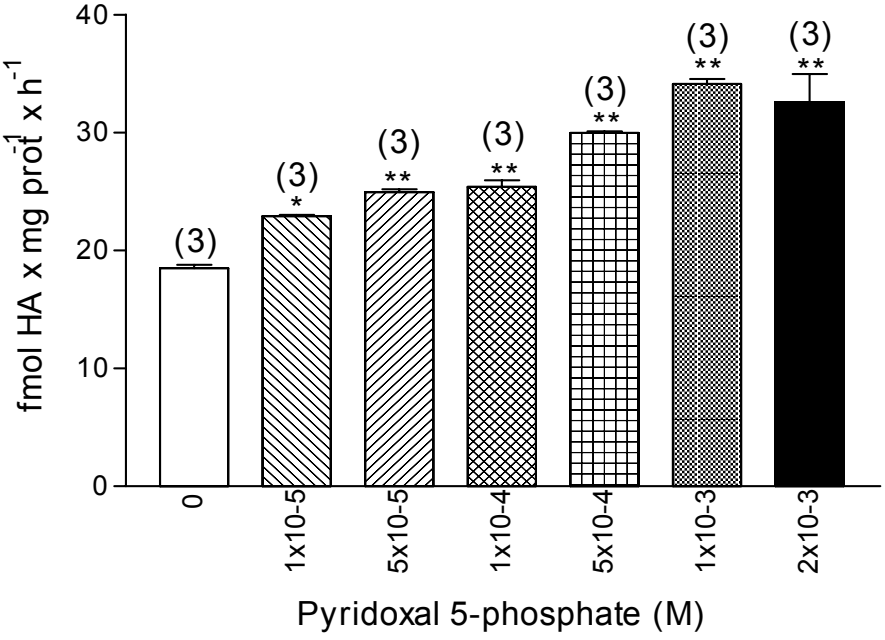

Figure 1. Pyridoxal 5'-phosphate stimulates HDC activity on whole rat brain homogenates. Rat brain was homogenized in 15% (w/v) 10mM potassium phosphate buffer at pH=7.4. Results represents means ± SD. Number of experiments is indicated in brackets above the columns. ** P<0.01 and * P<0.05 compared with samples incubated without P-5P.