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Homovanillic Acid Levels in Corpus Striatum, Limbic System and Diencephalon of Male and Female Rats

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The HVA levels in corpus striatum, limbic system and diencephalon in male and female rats during the postnatal period have been measured.

The HVA levels in corpus striatum and diencephalon differed significantly when both sexes were compared, whereas in limbic system significant differences were not found. A decrease in the levels of HVA in all areas studied was observed.

Key words: Homovanillic acid, Corpus striatum, Limbic system, Diencephalon, Rat.

Dopamine (DA) is one of the most important neurotransmitters in mammals, and its action is mainly located in the brain (1). Homovanilic acid (HVA) is one of its principal metabolites, and it appears in the brain with the same distribution as the amine does. This is the reason why its concentration is usually studied on basal ganglia (2, 5, 13, 20).

When dopamine acts at synoptic level, it binds to a specific receptor transmitting a chemical message. It is catabolized and therefore inactivated by the corresponding enzymes which are found in great quantities in basal ganglia (5). As a consequence of its degradation, DA changes mainly into HVA which is poured into the cerebrospinal fluid and afterwards into the urine, these two latter fluids being most significant for analysis (1, 10, 18).

In this work, the HVA levels have been measured as an index on the dopaminergic activity in the brain areas (corpus striatum, limbic system and diencephalon) on male and female rats along with the postnatal development.

Materials and Methods

The present work was performed on Sprague-Dawley rats. All the animals belonging to the same litter were picked

for each experiment, weaning after 23 days. When the animals were 10, 15, 30 and 60 days old they were beheaded by means of a guillotine and the encephalon was extracted and dissected using the CARLSSON and LINDQVIST method (4), placing the brain on a cold surface. The time employed in both cases never exceeded three minutes. In order to avoid possible circadian variations, the animals were always beheaded between 9 a.m. 11 a.m.

The pieces of tissue obtained were immediately weighed and frozen a temperature of —20° C. The samples did not stay frozen for more than three weeks because after this time, it was observed that important catecholamines losses occur.

In the HVA extracted out of the brain tissue the MURPHY *et al.* technique (15) was employed, using n-butil acetate (Merck) as an organic phase, and trisphosphate buffer (pH 7.5) as aqueous phase. The HVA was determined using the ANDÉN *et al.* technique (1, 6) in a Perkin-Elmer spectrofluoremeter mod. 2000.

For the comparison of the results obtained in the present work, two types of tests have been used: Intrapopulation: a) Arithmetic mean as a centralized measure for all data of each group; b) Standard deviation as a dispersion measured for values in respect to the mean. Interpopulation: Student-t test, in order to compare male and female groups.

Results and Discussion

Table I represent the HVA levels in corpus striatum limbic system diencephalon, along with the posnatal development in both sexes. A decrease of the compound level to be studied within an age range is observed in the three representations. This decrease takes place both in males and females and is stronger in diencephalon than in the other regions studied. BJÖRKLUND and NOBIN (3) have demonstrate that the tyrosine hydroxylase activity is maximal, with clear zonal differences, at the neonatal period. Other authors (12, 14, 16, 19) postulate an increase in DA and NA levels, to reach adult values, as the development progress. They also show zonal increasse rate variation among the different parts of the brain. The maduration of DA presinaptic receptors occurs several weeks after in mesolimbic region than in striatum (17). Noradrenergic transmision seems to be faster than dopaminergic one (12). The decrease of the HVA levels may be due do the activation of the norepinephrine pathway. This was brougth forth by COYLE and HENRY (7) who observed an increase in the NE synthesis since birth until adult age. Therefore, a decrease in the use of the DA to HVA catabolic pathway may exist.

On the other hand, referring to the level of this metabolite, a great difference is found when males and females are compared. This makes one think about a functional brain difference in relation to sex, in the same way a GORSKI (9) mentione a great morphological difference in the brain of rats in relation to sex. These differences are significant until the age of 30 days, while they disappear at the age of 60 days. We ignore the reason why, being a motor centre, this difference exist in the first three periods studied (table I).

Meanwhile, the differences found in diencephalon are more outstanding and, perhaps, easier to explain. They take place from the very first periods studied up to that of 60 days. Being this latter period ulterior to the sexual development of the rat, it can almost be assured that the DA catabolism difference in diencephalon will continue during the rest of the life.

Table I. *HVA levels (ng/g fresh tissue) in corpus striatum, limpic system and diencephalon in rats relation with age (in days).*

	10 day	15 day	30 day	60 day
Corpus striatum:				
males (n = 11)	0.624 ± 0.12 (n = 3)**	1.085 ± 0.17 (n = 2)*	0.942 ± 0.03 (n = 3)**	0.726 ± 0.23 (n = 3) N.S.
females (n = 15)	0.907 ± 0.17 (n = 3)	0.905 ± 0.19 (n = 3)	1.085 ± 0.15 (n = 4)	0.677 ± 0.15 (n = 5)
Limbic system:				
males (n = 12)	1.300 ± 0.12 (n = 3) N.S.	1.262 ± 0.11 (n = 3) N.S.	1.243 ± 0.37 (n = 3) N.S.	0.857 ± 0.16 (n = 3) N.S.
females (n = 13)	1.339 ± 0.05 (n = 3)	1.356 ± 0.14 (n = 3)	1.184 ± 0.11 (n = 3)	0.979 ± 0.23 (n = 4)
Diencephalon:				
males (n = 11)	0.525 ± 0.08 (n = 2)****	1.011 ± 0.21 (n = 3)*	0.872 ± 0.16 (n = 3)**	0.163 ± 0.06 (n = 3)****
females (n = 15)	0.986 ± 0.15 (n = 4)	0.726 ± 8.10^{-4} (n = 2)	0.658 ± 0.15 (n = 4)	0.393 ± 0.04 (n = 5)

Student-t: *, $p < 0.050$; **, $p < 0.025$; ***, $p < 0.010$; ****, $p < 0.005$. N.S., not significative.

In this way we could explain this phenomenon following Gorski's theory. He refers to a difference, at the morphological level, of certain hypothalamic nucleus related to sexual behaviour.

Resumen

Se valoran las concentraciones de ácido homovanílico en cuerpo estriado, sistema límbico y diencéfalo, en ratas macho y hembra durante el período postnatal.

Los niveles encontrados en cuerpo estriado y diencéfalo difieren significativamente entre machos y hembras, no apareciendo diferencias significativas en el sistema límbico. Las concentraciones de HVA van disminuyendo a lo largo del período postnatal en las tres regiones del cerebro estudiadas.

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