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Regional Distribution of 5-Hydroxytryptamine in Cat Brain *

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The regional distribution of serotonin (5-HT) in the brain of the cat is poorly understood. In this work, serotonin was analyzed fluorometrically along the brain stem and prosencephalon of the cat. The hypothalamus had the highest concentration of serotonin. Serotonin decreased gradually at the mesencephalon, preoptic area, medulla oblongata, hippocampus, pons, visual cortex, spinal cord and frontal cortex. Significant differences were found between the raphe (3 mm thick) and the lateral blocks of the brain stem. The concentration of serotonin is higher in the raphe blocks, though it decreases caudally. There is no significant difference between the raphe (4 mm thick) and the lateral block of the brain stem. The results demonstrate the regional concentration of serotonin in the CNS of a normal cat, the relationships between serotoninergic neuron groups and serotonin concentration, and the probable significance of nerve terminals and varicosities in storing serotonin.

Serotonin (5-HT; 5-hydroxytryptamine) concentration in the central nervous system is known to be related to different phases of sleeping (5, 6) some oligophrenias (11), neuroendocrine mechanisms, and other functions (4). Fluorescence histochemistry has been used sucessfully to detect the main groups of serotoninergic neurons at the brain stem of various species (2). It has been used also for indentification of serotonergic nerve terminals in these regions. Little information is available, however, about regional distribution of 5-HT in the cat brain, particularly, (I) the regional concentration of 5-HT in the CNS of a normal cat; (II) the relationships between regional concentration of 5-HT and distribution of serotoninergic neurons; (III) the main serotoninergic areas at the prosencephalon and (IV) the contribution of serotoninergic terminals to storage of 5-HT.

The purpose of this paper is to obtain some information on the regional distribution of 5-HT in the CNS of the cat, an animal selected for physiological experiments on the sleep-wakefulness cycle.

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5-TH was analyzed by means of quantitative fluorometry. The results have been partially communicated (10).

Materials and Methods

Forty-four adult cats weighing 2-3 kg were killed between 12 AM and 3 PM by intravenous injection of saturated KCl solution. The brain were immediately removed and frozen. In each case the brain stem was dissected from the prosencephalon by making a section from the anterior border of the superior colliculus to the posterior border of the corpus mamillare. The prosencephalon was dissected into the following blocks: hypothalamus, preoptic area, frontal cortex, visual cortex, and hippocampal cortex. To exclude the amygdala, blacks of the hippocampal cortex comprised the posterior half of the temporal lobe and were about 3 mm thick. In all cases, 5-HT assays were performed on pools obtained from equivalent areas at both hemispheres. A second group of animals was used for assays of the brain stem. This region was transversely sectioned into five blocks (fig. 1A); a block enclosing the first segments of the cervical spinal cord was also assayed. The brain stem from a third group of animals was longitudinally sectioned into 3 mm or 4 mm thick medial blocks enclosing the middle line nuclei (fig. 1B). In a fourth group of cats a 3 mm medial block was also obtained from the brain stem but this was transversaly sectioned into mesencephalic, pontine, and medulla oblongata portions. The lateral brain stem portions of these animals were sectioned in the same way (fig. 1C).

Dissection of the prosencephalon was done according to REINOSO-SUÁREZ (14); dissection of the brain stem was performed according to TABER (19). Two cats were used to test the nerve structures enclosed in the blocks submitted to 5-HT assay. Blocks similar to those described

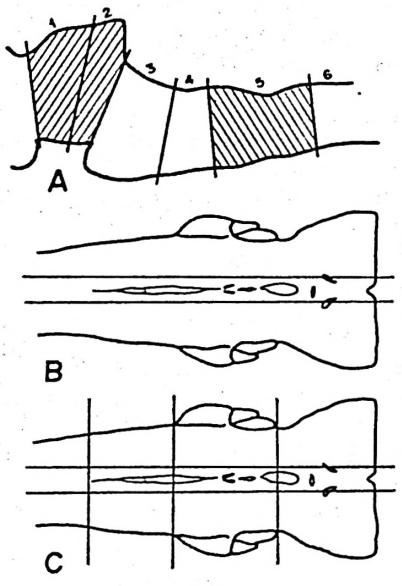

Fig. 1. *Diagrams showing the brain stem (A, B, C) and the sections performed to obtain different tissue blocks for topographical analysis of serotonin.*
Explanation in the text.

Table I. *Concentration of serotonin in the CNS of the adult cat.*
Concentration differences of 5-HT in the brain stem were significant using the variance test ($p < 0.01$). Differences were significant between prosencephalic areas ($p < 0.005$). 5-HT in the spinal cord correspond to that found at the first cervical segments.

Brain region	Number of animals	Gammas/g (mean±SEM)
Hypothalamus	14	1.32±0.39
Mesencephalon		
Caudal portion	8	1.02±0.08
Rostral portion	8	0.96±0.10
Preoptic area	14	0.93±0.12
Medulla oblongata	8	0.8 ±0.05
Hippocampal cortex	13	0.66±0.04
Pons		
Rostral portion	8	0.66±0.07
Caudal portion	8	0.58±0.05
Visual cortex	8	0.31±0.00
Spinal cortex	8	0.28±0.07
Frontal cortex	12	0.26±0.00

for groups 1 to 4 were fixed in formaldehyde, sectioned in a freezing microtome and the histological preparation were stained with a Nissl method. 5-HT was determined according to UDENFRIEND (20) with about 80 % recovery. Values were calculated for wet tissue and were statistically analyzed by means of the analysis of variance and the Student's t-test.

Results

The highest concentration of 5-HT in the cats' CNS was found in the hypothalamus and the lowest in the frontal cortex (table I). The 5-HT concentration in the brain stem is higher at the 3 mm thick medial block than at lateral zones (table II). Within this medial block, 3 mm thick, 5-HT is concentrated at rostral portion and drops down towards the caudal zone. At lateral zones the pontine portion contains a lower concentration than do the mesencephalic and medullary portions (table III).

Discussion

The present study links biochemistry and morphology; quantitative determination of 5-HT was performed at selected regions of the CNS. Information obtained is basic for further analysis of changes in the regional concentration of 5-HT in relation to different functional studies; for example, the rate of conversion of tryptophan to 5-HT in nerve terminals and neuron perikarya (13) or, more recently, the analysis of tryptophan hydroxylase activity (7, 8). This approach, currently employed, has proved to be very useful.

The above reported data on 5-HT concentration in medulla oblongata and pons are similar to those reported after extraction of the amine in Amberlite columns (17, 18). Differences in results concerning spinal cord may be attributed to differences in dissection techniques. The brain regions analyzed herein are related to the external macroscopic anatomy of the brain and comprise sites frequently selected for physiological and pharmacological studies.

Although 5-HT assays were done only between 12 AM and 3 PM it is assumed that values (table I) at spinal cord, medulla oblongata, pons and hippocampus are constant. Neither circadian rhythms nor ultradian rhythms have been shown to affect 5-HT concentration at these regions (18).

High concentration of 5-HT in synaptic terminals at the caudal portions of the spinal cord have been reported (1). According to present results this would occur also at the brain stem and telencephalon.

Brain stem. We have found that the raphe, which was enclosed in the 3 mm thick medial block, contains higher concentration of 5-HT than lateral regions

Table II. *Serotonin concentration differences between medial and lateral blocks of the brain stem in adult cats (gammas/g).*
NS: not significant. Results show means±S.E.M., with numbers of observations in brackets.

		Raphe block	Lateral block	P
4 mm *				
Pools (2 animals)	(10)	0.80±0.03	0.60±0.04	N.S.
Individuals	(4)	0.71±0.10	0.50±0.00	N.S.
3 mm *				
Individuals	(6)	0.77±0.03	0.59±0.03	< 0.01

* Thickness of the raphe block.

Table III. *Serotonin distribution along the brain stem.*
The medial bolck (raphe) was 3 mm thick. Homologous blocks of two animals were pooled for 5-HT assay. 5-HT concentration differences between mesencephalic raphe and caudal portions of the brain stem raphe were significant ($p < 0.005$). Differences were not significant (NS) between pons raphe and medulla oblongata raphe. Number of experiments, 14 for each group. Results show means ± S.E.M.

Levels	Raphe block	Lateral block	P
Mesencephalon	0.897 ± 0.07	0.739 ± 0.006	N.S.
Pons	0.605 ± 0.03	0.337 ± 0.004	< 0.02
Medulla oblongata	0.524 ± 0.01	0.637 ± 0.020	N.S.

(table I). This suggests that the raphe nuclei, where most serotoninergic neurons are located (2), are the main storage sites of 5-HT in the brain stem. It is interesting, however, that higher concentrations of 5-HT at the level of the raphe nuclei occur at the rostral part of our medial block (table III). It is likely, therefore, that 5-HT concentration is not necessarily related to distribution of tryptaminergic neuronal groups. In this regard it is of interest that large tryptaminergic nuclei occur at the pons, i.e., centralis superior, raphe pontis and magnus. This is the case also for the medulla oblongata (raphe pallidus and obscurus). In such regions, however, concentrations of 5-HT are lower. The block of mesencephalic raphe encloses only the so called nucleus dorsalis raphe (B 7) by DAHLSTROM and FUXE (2) and the group described by PIN *et al.* (13) between the nuclei of the third cranial nerve. It is therefore suggested that either neuronal perikarya at these nuclei contain the higher concentration of 5-HT at the brain stem or that numerous serotoninergic afferents occur at the mesencephalic raphe. Up to now, the latter have not been described, however.

It is interesting to note that lateral region of the mesencephalon and medulla oblongata (tabla III) contain large amounts of 5-HT. The existence of scarce neurons of «B 9» and «B 3» in such lateral sites does not explain their high 5-HT content. It is likely that «B 9» of the rat (2) corresponds to a group described caudally to the ruber nucleus in the cat (12). Present results suggest that storage of 5-HT at lateral zones of the brain stem occur mainly within nerve terminals or varicosities of bypassing axons in addition to serotoninergic nerve cell bodies. Storage capacity of varicosities and terminals might be as important as that of neuron perikaria.

Terminals at lateral zones of medulla oblongata and pons might correspond to raphe neurons of short axons projecting horizontally to neighboring regions (21).

Prosencephalon. Results of determination of 5-HT at the prosencephalon are in agreement with those reported already. Ascending projections from medianus and dorsalis raphe nuclei have been detected with fluorescence histochemistry (3) as well as with degeneration techniques (9, 15, 16). Present results suggest that many of these projections would leave both collaterals and terminals at the hypothalamus and preoptic areas in their course within the medial forebrain bundle (3). Moreover, «B 7» and «B 8» groups would project also to the hippocampus in the rat (15). It is of interest that visual cortex contains higher 5-HT concentration than frontal cortex (sensory-motor cortex). However, analysis of silver stained material has shown ascending projections from dorsalis and medianus raphe to frontal cortex (16) but not to visual cortex. In present observation care was taken to dissect out the visual cortex from tissue of

neighboring circumvolutions which might contain serotoninergic terminals (gyrus cinguli).

Figure 2 shows that the highest concentrations of 5-HT in the CNS correspond to basal area of the brain. This region reaches rostrally the preoptic area and comprises the whole hypothalamus and the mesencephalon. Another CNS region richly supplied with 5-HT is the medulla oblongata. Though 5-HT concentration is higher at hypothalamic levels, no significant differences between the above mentioned zones were observed. Inasmuch as serotoninergic perikarya would occur only at the brain stem (2) the topographical distribution of 5-HT in the prosencephalon does correspond to distribution of serotoninergic terminals and varicosities. It might be the case, however, that serotoninergic nerve cell bodies would occur at basal brain zones but these might not have been detected with fluorescent histochemistry. Pitfalls in such techniques are known to occur frequetly, moreover good microscopic preparations of basal zones are difficult to obtain.

The higher concentration of 5-HT at lateral zones of the brain stem, described herein, correspond to regions containing both serotoninergic terminals and perikarya. In this regard, topographical determination of soluble tryptophan hydroxylase activity might be of some interest.

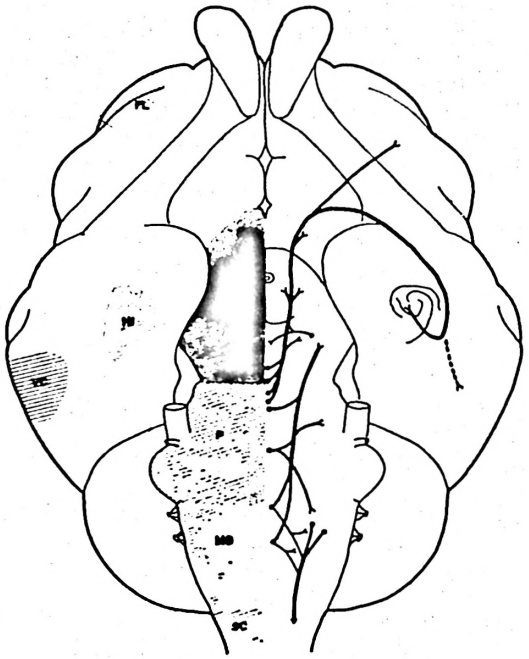

Fig. 2. *Diagram of serotonergic projection in cat brain.*
PA: preoptic area; H: hypothalamus; M: mesencephalon; P: pons; MO: medulla oblongata; SC: spinal cord; FL: sensory-motor cortex; VC: visual cortex; HI: hippocampus. Density of lines indicates 5-HT concentration. Neuronal path at the right half of the diagram illustrates probable distribution of perikarya, axons and terminals responsables for differences in 5-HT concentration between the above mentioned areas.

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Resumen

Se determina por fluorometría la 5-hidroxitriptamina (serotonina) en áreas del encéfalo de gato normal utilizadas con frecuencia en estudios fisiofarmacológicos. La mayor concentración de 5-HT se encuentra en el hipotálamo y disminuye progresivamente en el mesencéfalo, área preóptica, medulla oblongata, hipocampo, puente, corteza visual, medulla spinalis y corteza frontal. Se observa diferencia significativa entre el bloque medial del tronco del encéfalo, que incluye a los núcleos del rafe (3 mm de grosor) y los bloques laterales. La concentración es mayor en el bloque del rafe, pero disminuye en sentido rostro-caudal. No hay diferencia significativa entre la concentración de 5-HT en un bloque medial de 4 mm de grosor y los laterales del tronco del encéfalo.

Los resultados demuestran la concentración de serotonina en algunas áreas de interés del SNC en el gato normal para estudios funcionales; se discute la relación entre estas concentraciones y la presencia de grupos neuronales serotonérgicos; y el posible papel en el almacenamiento de 5-HT de varicosidades y terminales.

Addendum

When this study was accepted for publication two works, using the retrograde axonal transport of the horseradish peroxidase, have confirmed in cat direct connections from the raphe brain stem (serotonergic groups) and locus coeruleus (catecholaminergic group) to sensory-motor cortex (LLAMAS *et al.*, Brain Research, 89, 331-336, 1975) and hippocampus (PASQUIER, D. A., Proceedings 10th. Int. Cong. Anat., p. 139, Tokyo, 1975). These data are in accord with our results.

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