5-HYDROXYTRYPTAMINE AND PLASTICITY IN RODENTS

María Guadalupe Flores Cruz*, Alfonso Escobar Izquierdo*

SUMMARY

The attributes that characterize a molecule as neurotransmitter at CNS are: *i.* neuronal synthesis, *ii.* being present at presynapsis, *iii.* Ca^{2+}-dependent release, *iv.* postsynaptic actions mediated by receptors, *v.* an elimination mechanism at synapse. Since 1964, 5-hydroxytryptamine (5-HT) was included as a neurotransmitter and is part of a set of neurotransmitters named *biogenic amines.*

In rodents, the 5-hydroxytryptaminergic system is constituted by nine nuclei at brainstem, and divided in two groups, rostral and caudal by ther localization. The rostral group projects mainly to the telencephalon and diencephalon, while the caudal group does it to the spinal cord. 5-HT innervation to brainstem and cerebellar nuclei have been also described.

The most well-known function of 5-hydroxytryptamine (5-HT) in the CNS is neuromodulation, in processes such as memory, learning, mood, sleep-wake cycle; all of these are regulated by this biogenic amine through a wide family of receptors. All the receptors are metabotropic with the sole exception of $5\text{-}HT_1$, which is an ionotropic receptor.

The 5-HT system differentiates early in ontogenesis; 5-HT immunoreactive neurons are evident in rat fetuses at embryonic day 12 (E12), when almost any other neuronal lineage possesses a cellular commitment. This fact highlights the importance 5-HT has at neurodevelopment.

Scientific works are focused in the 5-HT auto-regulatory signalling for neuropil outgrowth at ontogeny, another remarkable trait of the 5-HT system. In addition, 5-HT releases astrocyte neurotrophic factor S-100 beta, necessary for dendritic maintenance. The 5-HT set point at different stages during ontogeny remains unknown.

Several target structures of the 5-HT system are dependent on the level of 5-HT activity in newborn rodents; e.g. the somatosensory cortex where proper barrel field arrangement requires an active 5-HT innervation.

Moreover, besides the 5-HT level, other factors, such as the level of reelin, are determinant for the proper cytoarchitectonic organization of the neocortex. The use of 5-methoxytryptamine, an unespecific 5-HT agonist, in the prenatal period, which negatively affects the reelin level, leads to cytoarchitectonic derangement, as it has been described to occur in the presubicular cortex.

5-HT and plasticity are also related to neurogenesis in adulthood. Neurogenesis in adulthood is influenced by several factors. Some of them, such as exercise and an enriched environment, increase the rate of newly born neurons in the dentate gyrus and olfactory bulb; while others, such as mood depression (in humans), low 5-HT levels, $5\text{-}HT_{1A}$ receptor blockade by antagonists, or down-regulation, account for a poor neurogenesis rate.

Chronic administration of 5-HT reuptake inhibitors, such as fluoxetine, increases the number of bromodeoxiuridine- labelled (BrdU) granule cells at the dentate gyrus and hilus versus control rats. This means that fluoxetine increases the neurogenesis rate. Newly born granule cells at dentate gyrus are more likely to survive, thus contributing to maintaining the hippocampal volume unchanged.

On the contrary, following chronic 5-HT antagonist administration, specifically $5\text{-}HT_{1A}$ receptor blockade BrdU-labelled granule cells in dentate gyrus are 30% reduced.

Reduced hippocampal volume develops in humans affected by major depression, concomitant in some cases with a decrease in 5-HT neurotransmiter level. Recent studies linking 5-HT neurogenesis stimulation in dentate gyrus explain why plastic phenomena associated to pathology could be reversed by 5-HT reuptake inhibitors like fluoxetine. These works contribute to a better understanding of both depression etiology and clinical approach.

Key words: 5-Hydroxytrypamine, neurodevelopment, neurogenesis, fluoxetine, BrdU.

RESUMEN

Se considera que la 5-hidroxitriptamina (5-HT) como un neurotransmisor en el SNC si cubre los siguientes criterios: *i.* síntesis y vesiculación al interior de la neurona, *ii.* presencia de la molécula en la presinapsis, *iii.* liberación en un mecanismo dependiente de Ca^{+2}, *iv.* acción postsináptica mediada por receptores para la molécula y *v.* presencia de mecanismos de recaptura y degradación.

Los cuerpos celulares de las neuronas que sintetizan 5-HT se agrupan en nueve núcleos distribuidos en el tallo cerebral. A su vez, estos núcleos se dividen en dos grandes grupos, rostral y caudal, de acuerdo con su localización.

El grupo rostral inerva principalmente el telencéfalo y el diencéfalo, mientras que el grupo caudal hace lo propio con la médula espinal.

La actividad del sistema 5-HT es neuromoduladora, esto es, interviene en la regulación de la actividad neuronal por medio de receptores, todos ellos metabotrópicos, con excepción del recep-

*Depto de Biología Celular y Fisiología. Instituto de Investigaciones Biomédicas. UNAM. Ciudad Universitaria, 04510, México DF.
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tor 5-HT_3, que es ionotrópico. Los procesos relacionados con el sistema 5-HT comprenden la regulación del talante emocional, el aprendizaje, la memoria, la regulación del tono muscular, la ingesta de alimentos, la conducta sexual y la regulación del ciclo sueño-vigilia en humanos.

Durante el desarrollo, las primeras neuronas inmunorreactivas a 5-HT se observan en el día 12 de la gestación de ratas a lo largo del borde entre el metencéfalo y el mielencéfalo rostral, en la región externa de la zona ventricular. El pico de proliferación celular para este fenotipo ocurre en E15 y, pese a que la inervación completa del SNC concluye en la tercera semana de vida post natal, al nacimiento están presentes la totalidad de las neuronas 5-HT, así como las principales proyecciones de este sistema.

La relevancia de la 5-HT es notoria al observarse los procesos con que se relaciona durante el desarrollo. Uno de ellos es la elongación axónica en función de un gradiente de concentración. Al momento en que los neuroblastos intervienen en el nivel celular y se diferencian en neuronas 5-HT, sintetizan y secretan el neurotransmisor. De esta forma, el *milieu* posee un gradiente 5-HT que influye en la elongación axónica por medio de un asa de retroalimentación negativa.

Otra forma en que la 5-HT incide en el neurodesarrollo es al promover la secreción del factor neurotrófico derivado de astrositos: S-100β. Esta proteína estimula el crecimiento neurítico en las neuronas 5-HT y contribuye a mantener la inervación 5-HT a las estructuras blanco.

La administración prenatal de 5-metoxitriptamina, agonista específico 5-HT, provocó alteraciones citoarquitectónicas en la corteza presubicular de las crías evaluadas en P7. Con lo anterior se sugiere, por último, que la 5-HT influye en el desarrollo de sus estructuras blanco.

El vínculo entre 5-HT y plasticidad continúa en la vida adulta, cuando la 5-HT sostiene una estrecha relación con la neurogénesis en el giro dentado.

Trastornos y procesos como el estrés crónico, la depresión (en humanos) y la disminución en el nivel de 5-HT, así como la administración de antagonistas del receptor 5-HT_{1A}, disminuyen la tasa de proliferación neuronal, evaluada mediante el marcaje de células recién generadas con bromodeoxiuridina. Esto lo efectúan en una magnitud similar a la que se observa tras la administración de un inhibidor de la síntesis de 5-HT. Por otra parte, la administración crónica de inhibidores de la recaptura de 5-HT, como la fluoxetina, incrementa la tasa de neurogénesis en ratas adultas.

Estos trabajos resaltan la importancia del sistema 5-HT a lo largo de toda la vida del individuo en los fenómenos de plasticidad.

Palabras clave: 5-Hidroxitriptamina, neurodesarrollo, neurogénesis, fluoxetina, BrdU.

INTRODUCTION

Widespread in the CNS, neurotransmitters modulate the activity of neurons and glia through specialized receptors. Biogenic amines, such as acetylcholine, dopamine, noradrenaline, 5-HT and histamine are examples of neurotransmitters synthesized by a discrete number of neurons but extensively distributed (23).

In the CNS, nine brainstem nuclei contain the cell bodies of 5-HT neurons. These nuclei were named B1-B9 complex by Dahslström and Fuxe (8), and include raphe nuclei, ventrolateral medulla, paragigantocellular reticular nucleus, caudal linear nucleus, pontis oralis nucleus and supralemniscal region, as shown in Figure 1 (9).

Although raphe nuclei have been classically associated with 5-HT synthesis, 5-HT, substance P and neuropeptide Y are also synthesized in those nuclei (12).

Hydroxylation of the tryptophan amino acid is the rate-limiting step in 5-HT synthesis; the tryptophan hydroxylase (Try-OH) enzyme is responsible for this process and is only contained in 5-HT neurons and in pineal gland, as a step in melatonin synthesis (9).

The higher concentrations of Try-OH are observed in dorsal raphe, with 40% Try-OH immunoreactive neurons from total neuronal population (9, 12).

In adult life, 5-HT exerts modulator functions in several processes such as sleep-wake cycle, learning, memory, mood regulation, food intake, sexual behavior, pain perception and muscular tone (28). Widespread 5-HT distribution in CNS and a high reliability of the neurotransmitter (12) allow this wide and varied set of activities.

5-HIDROXYTRIPTAMINERGIC SYSTEM ONTOGENY

The first 5-HT immunoreactive neurons are detected along metencephalic-myelencephalic flexure at embryonic day 12 (E12) in rats (17, 30); this means that 5-HT neurons are bound to an early functional commitment.

5-HT system development is divided in three developmental stages: *i.* early 5-HT neurogenesis and primitive pathway development (E12-E15), *ii.* late neurogenesis and selective pathway development (E16-E19), *iii.* terminal fields development (E19-P21) (17).

Neurogenesis of 5-HT neurons takes place in the subventricular zone of the basal neural tube, and from this location neurons migrate to their final position at mesencephalic, pontine and medullary tegmentum levels. The primitive pathway development begins at the same time (30).

Morphologically, early axon traits are different in development from those of adults. In rat embryos, axons are thick, unbranched, with smooth curves and low varicose density while in adults, axons are thinner, branched and have a higher varicose density (17).

Compared with other neurotransmitter systems, the characteristic development pattern of the 5-HT system in early ontogeny is shared by all mammals, and is in fact the same in all chordates (2).

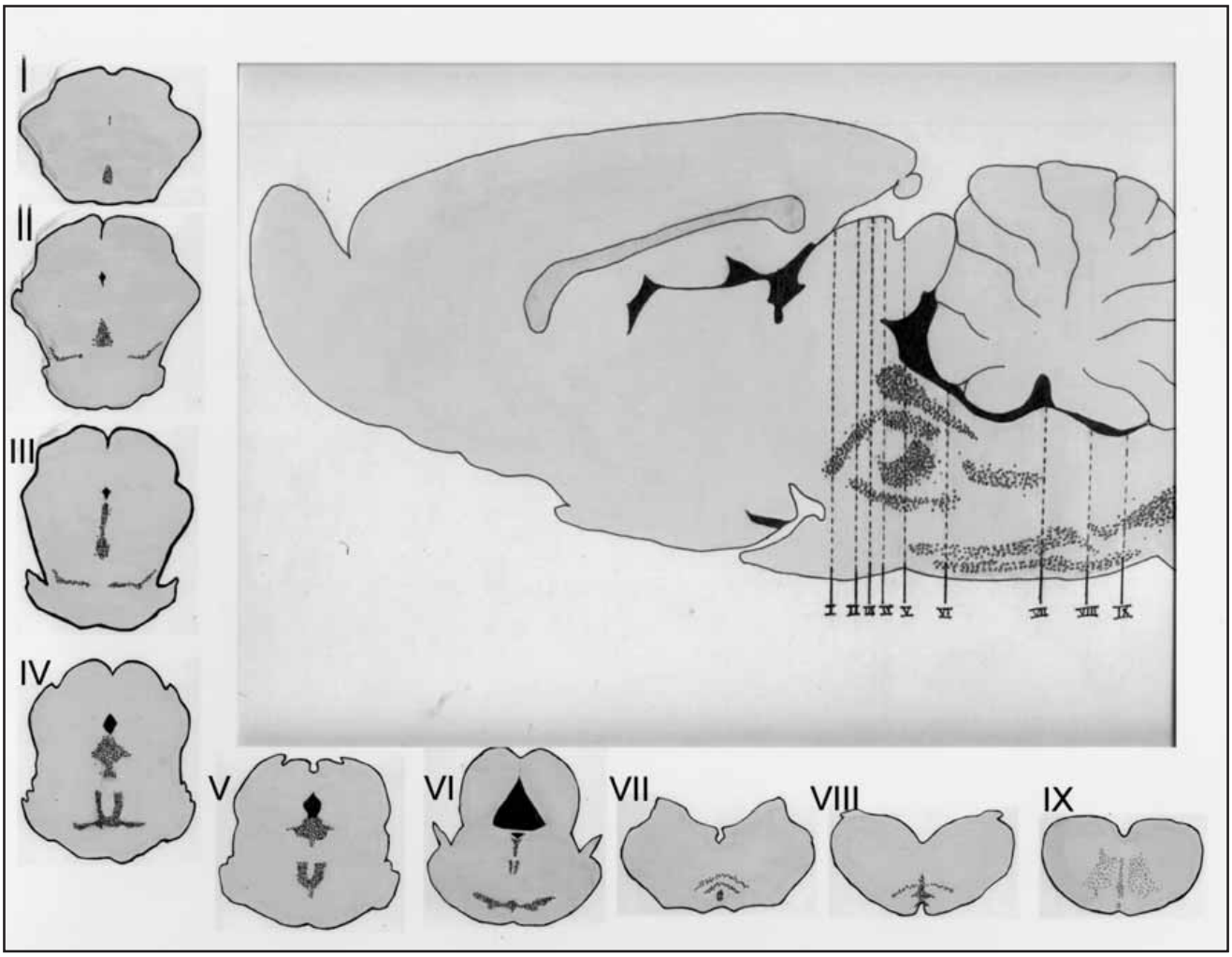

Fig. 1. Distribución de los núcleos serotonérgicos en el tallo cerebral. Vistas lateral y coronales.

5-HYDROXYTRYPTAMINE AND NEUROGENESIS

In addition to events associated to 5-HT, as previously mentioned in the text, 5-HT is also related to adult neurogenesis (3-5, 19, 26) and neurotrophic activity during neurodevelopment (35, 36).

It is known that in the brain of mammals two regions exhibit cellular proliferation in adulthood. The first one is the subventricular zone (SVZ), located in the walls of the lateral ventricle; from the SVZ, new interneurons migrate to the olfactory bulb along the rostral migratory stream. The second CNS region with neuronal proliferation during adulthood is the subgranular layer in the dentate gyrus, where granular neurons proliferate (3).

At these places neurogenesis is regulated by physiological stimuli and phatophysiological conditions. Exercise, enriched environments, trophic factors –such as epidermic growth factor and neurotransmitter signalling, e.g., 5-hydroxytryptamine- are some of the physiological stimuli for adult neurogenesis (3, 16). Pathophysiological conditions that negatively affect neurogenesis include stress or stress hormones exposure, malnutrition, and NMDA receptor up-regulation. These conditions decrease cellular proliferation (11, 19).

Some pathophysiological conditions, such as depression, decrease 5-hydroxytripatminergic neurotransmission and down-regulate 5-HT_{1A} receptors (11). This argument leads to bearing in mind the possibility that 5-HT might be related to adult neurogenesis.

Evidence supporting this hypothesis was offered by the significant increase of bromodeoxiuridine-immunolabelled (BrdU) granular neurons at dentate fascia and hilus from chronic administration of antidepressant fluoxetine (a 5-HT selective reuptake inhibitor) to treated rats versus controls (19).

In a study, the 5-HT neurotoxin 5,7-dyhydroxitryptamine, was administered to young rats,

which were sacrificed eight days after a single BrdU injection. BrdU-immunolabelled cells were counted and 35% less neurons were observed in the neurotoxin administration group compared with controls. The same results –a 40% reduction of BrdU-immunolabelled granule cells– were observed after 5-HT synthesis inhibitor *para*-clorophenylalanine administration. These results lead to the conclusion that 5-HT neurotransmission is necessary to ensure adult cellular proliferation phenomena (4).

5-HT receptors are essentially G-protein-coupled (6 types) and just one cationic channel-coupled receptor (9). 5-HT_{1A} receptor antagonists, 4-iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-benzamide (p-MPPI), 1-(2-methoxyphenyl)-4(4-(2-phtalimido)butyl)piperazine (NAN-190) and N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635), were administered to adult rats. Thirty minutes later, a single BrdU injection was provided, and rats were sacrificed two hours later. Antagonist treatment lead to a 30% reduction in the cellular proliferation in dentate gyrus versus control, assessed by BrdU-immunolabelled granule cell count. p-MPPI, NAN-190 and WAY-100635 effects over the reduction were the same, concluding that 5-HT_{1A} receptor activity is related with cellular proliferation (26).

5-HT_{1A} receptor inhibits adenylate cyclase activity, decreasing cyclic adenosin monophosphate. Kinase protein C is also reduced, which decreases cAMP response element-binding transcription (CREB). 5-HT_{1A} receptor activates a K^+ inward current that hyperpolarizes the neuron (9). 5-HT_{1A} receptor agonist promotes microtubular tubulin assemble during cellular maturation as mitotic spindle assemble during cellular division (2). However, the cellular proliferation 5-HT-mediated mechanism remains to be clarified.

5-HT FUNCTIONAL RELEVANCE DURING CNS ONTOGENY

At ontogeny, 5-HT exerts influence over two plastic phenomena: 5-HT effects over 5-hidroxytryptaminergic neurones ontogeny and maturation and 5-HT target structure development (36).

Prenatal thalidomide or valproic acid injections at E9 were given to ascertain the effect of increased 5-HT level at development. Systemic hyperserotonemia, higher 5-HT concentrations at hippocampus, a caudal shift in the dorsal raphe position and sparse 5-HT process were observed in the whole brain at E15 & E18 in thalidomide/valproic acid-exposed rats (14, 22).

5-Hydroxytryptaminergic innervation is controlled by negative auto-regulation, 5-HT_{1B} receptors in the tip of 5-HT growth cones; higher 5-HT concentrations inhibits elongation toward that zone, while low concentrations promote elongation (35). Thalidomide/valproic acid induced hyperserotonemia acts as negative signal for 5-HT growth cone elongation, leading to a poorly innervated brain.

Monoamineoxidase inhibitors(MAOi), deprenyl (MAOi-A) and chlorgyline (MAOi-B) increased 150% 5-HT process density in hippocampus, 200% at caudate nucleus and showed a 50% decrease in neocortex of prenatal exposed rats, observed at P5. At P30, hippocampus and caudate nucleus resemble the controls but sparse 5-HT processes at neocortex of MAOi rats remains (32).

Another auto-regulatory pathway for 5-HT development mediated by astrocyte-released growth factor is S-100b (35, 36). This protein is a member of a wide family of 100% ammonia sulphate soluble proteins (S-100). From this set, only the beta variety affects both development and adult plasticity in CNS; S-100β is released by astrocytes after 5-hydroxytryptaminergic and glutamatergic stimulation (36).

S-100β acts as a growth factor for 5-HT processes, as seen in a mesencephalic cell culture from E14 rats. In that study, 5-HT mesencephalic neurons were cultured with one protein from the set: S-100β, calmodulin, insulin, nerve growth factor and epithelial growth factor. After three days, [H^3]5-HT was added and scintillation counted. 3.2 ng/mL S-100β increased 171% [H^3]5-HT reuptake, while 1 pg/mL S-100β increased the reuptake 150% as compared with the control (1).

5-HT agonist ipsaperone was administered to an astrocyte culture; the cultured media were then added to a 5-HT neurons cell culture with or without a S-100 antibody. After three days, [H^3]5-HT was placed in the cell culture and scintillation measured. 5-HT neurons cultured with astrocyte-ipsaperone presented a scintillation slightly higher than the S-100 antibody added to the culture; no other cell culture was different from the control (33).

In the same study, 5-HT_{1A} astrocyte receptors were assessed by double immunostaining the 5-HT_{1A} and glial fibrillar acidic protein. Double marked cells were found in higher concentrations in the dentate gyrus polymorphic layer and lateral septal nucleus and in lower concentrations in the cingulate gyrus, amygdala and intermediate layers of temporal neocortex of adult rats (31).

Cell cultures of raphe nuclei, obtained from Nagoya polydactyl mutant rats end co-cultured with the hippocampus and neocortex, exhibited sparse

scintillation following [H^3]5-HT addition versus control. The neocortex and hippocampus of Nagoya polydactyl mutant rats co-cultivated with wild type raphe nuclei increased 250% scintillation, compared with raphe nuclei neurons cultured alone (31). The Nagoya polydactyl mutant rat is characterized by S-100β absence. These findings support the hypothesis that S-100β is necessary to ensure proper 5-HT system development, independent of the target tissue (29).

5-HT TARGETING STRUCTURES

5-HT system target structure development studies are mainly focused in cortical structures (10, 13, 18). Cortical marginal zone receives 5-hydroxytryptaminergic afferents, making synaptic contact with Cajal-Retzius cells (CRc)(13). CRc are cortical pioneer neurons, which synthesize and release *reelin* protein; the number of CRc decreases dramatically after birth, being practically absent in adult cortex (20). *Reelin* is a stop signal protein for neurons migrating to the cortex, its absence causes an «inverted» cortex, as seen in *reeler* mutant mice (27).

Increased 5-HT activity through prenatal 5-metoxytryptamine administration, a 5-HT non-selective agonist, leads to a disrupted laminar and columnar pattern in presubicular cortex at hippocampal formation, evaluated at P7 and decreased *reelin* levels at P0. These results suggest that 5-HT signalling is necessary to ensure proper laminar and columnar development (13). In rats, the somatosensory cortex 5-HT level must also be constant to prevent barrel field disruption; if 5-HT level increases, barrels are absent (10, 18).

5-HT SYSTEM DISRUPTIVE EVENTS

The 5-hydroxytryptaminergic system is highly vulnerable to a vast set of events, including ethanol exposure (34), cocaine, opiates, hypoxia, tryptophan disposability (5-HT precursor) (32), stress (24, 25, 32) and stress hormones administration (21, 25).

The hypothalamus-pituitary-adrenal axis (HPA) presents a 5-HT regulation; a central function of this system is the regulatory signal provided by corticotrophin releasing factor (CRF) neurons in the hypothalamic paraventricular nucleus. This nucleus receives inputs from magnus raphe nucleus (15) and dorsomedial hypothalamic nucleus *pars compacta* (12).

Bilateral adrenalectomy (ADX) in adult rats increases 5-HT_{1A} mRNA expression at hippocampus, while corticosterone administration after ADX returns 5-HT_{1A} mRNA expression to pre-ADX levels, just like controls (6).

5-HT synthesis is regulated by free tryptophan serum levels. At basal conditions free fatty acids bind tryptophan. In these conditions, the tryptophan transporter is incapable of taking it into the CNS. However, after corticosteroids are released, lipolysis increases free tryptophan serum concentrations and a higher tryptophan concentration crosses blood brain barrier, and 5-HT synthesis increases (7).

CONCLUSION

5-Hydroxytryptaminergic pathways are distributed in the whole CNS and modulate chemical neurotransmission in a wide set of processes during adult life. Recent investigations revealed a 5-HT regulation in plasticity phenomena through the whole life cycle: from ontogeny to adulthood. During ontogeny, 5-HT promotes both the auto-regulation of its own system development by negative feedback and the development of the target structure; in adulthood, it promotes cellular proliferation in dentate gyrus.

5-HT-mediated plasticity highlights the necessity to consider 5-HT as an active neurotransmitter influence at developmental stages to promote adequate wiring and targets structure assemblage.

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