ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 619-636

Evolutionary ancient roles of serotonin: long-lasting regulation of activity and development

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Abstract. Biogenic monoamines (catecholamines, indoleamines and histamine) are evolutionary old and important modulators of long-lasting changes in the functional state of cells. They are found in many protozoans and in almost all metazoans. Monoamines preserve their evolutionary old functions (first of all being intracellular signals and later hormones and growth factors) even in those animals in which they acquired the function of neurotransmitter. The older functions of serotonin, an important member of the family of indoleamines, are reviewed here. Described are: presence of serotonin in organisms at various phylogenetic levels; its role in embryonal, foetal and postnatal development, especially in the development of the central nervous system. It is concluded that in none of these functions serotonin is the only factor, but it is an ubiquitous and important modulator of a vast array of processes and functions taking part in development and plasticity.

Key words: serotonin, monoamines, evolution, development, morphogenesis, plasticity

INTRODUCTION

Development and differentiation of all living organisms is programmed genetically. Therefore, it is obvious that products of timed expressions of certain genes (proteins and peptides) control the pattern and rate of these processes. However, differentiation and, especially, later adaptation of organisms to the existing conditions depend also on compounds that are produced by the expressed proteins. As it was discovered in the last decades, biogenic amines are among the evolutionary oldest and most important of these compounds. In all organisms they are produced from only three amino acids: tryptophan, tyrosine (or, alternatively, phenylalanine) and histidine in one or more enzymatically catalyzed reactions, of which decarboxylation of the amino acid is the obligatory one. Compounds derived from the first two amino acids form the most important families of biogenic amines: indoleamines and catecholamines respectively.

The earliest role of the biogenic amines is that of the intracellular regulator of cell activity. The amines may be produced inside the cell or may enter it from its environment, acting directly on some of the intracellular signalling systems and causing long-lasting changes in the cell functional state, that adapt the organism to its environment. At least in the metazoan this incorporation is active, depending on a specialized membrane protein named SERT (serotonin transport protein).

However, very early in evolution the biogenic amines acquired functions of hormones (or growth-regulating factors), that is molecules of external origin acting on specific receptors localized in the cell membrane. Stimulation of those receptors activates (or inhibits) specific intracellular second messengers that cause long-term changes in the functional state of cells. In this capacity monoamines became important morphogenetic and trophic factors, controlling and coordinating proliferation and differentiation of various cells. The morphogenetic role of amines is still prominent during the embryonal and fetal development of multicellular organisms. The hormonal role of some monoamines is still their main (like adrenaline or melatonin) or one of the most important (like serotonin in blood platelets) functions in vertebrates. Later on in evolution, these amines acquired functions of neuromodulators that are produced and secreted by specific neurones and modulate the general activity of large groups of other neurones or of the whole nervous system - frequently for long periods. This action is exerted through specific receptors that may be placed either within synaptic contacts (both pre- and postsynaptically) or on the unspecialized parts of the cellular membrane. Finally, preserving some of the evolutionary older functions, monoamines took the last and probably newest one, the function of neurotransmitters conveying fast changes. In this capacity they act on receptors activating ion channels that generate fast postsynaptic potentials. Therefore, it seems that the newer functions of monoamines are acquired through externalization and shortening of their original, intracellular and long-lasting modes of action and by limiting their production.

Although the idea that neurotransmitters may act as "morphogens" during development has a long history (for review cf. Lauder 1988), the hypothesis that neurotransmitter monoamines preserved their evolutionary older "prenervous" roles was first formally postulated fifteen years ago by Buznikov and Shmukler (1981), on the basis of their experiments with sea urchin oocytes, that were conducted and published from the late sixties onwards (Buznikov et al. 1968). Serotonin (5-HT) is not unique in this respect, but at present these "prenervous" functions seem to be best documented in its case. Therefore in this review I will try to summarize the evidence for the changing functions of serotonin, other than its function as a classical neurotransmitter.

EVOLUTIONARY HISTORY OF SEROTONIN

Indoleamines, serotonin and the closely related melatonin, appeared very early in evolution. They are present in some protozoans and in almost all metazoans, where they play important roles in de-

velopment and plasticity. From the short list of protozoans investigated so far, serotonin is present in *Tetrahymena* (Csaba 1993) while another indoleamine, melatonin in *Stentor* (Banerjee et al. 1972) and bioluminescent marine Dinoflagellate *Gonyaulax polyedra* (Hardeland 1993). In *Tetrahymena* serotonin acts as an intracellular regulator through the adenylate cyclase and cyclic AMP pathway (Csaba et al. 1978). This protozoan produces both serotonin and monoaminooxydase (MAO), the enzyme decomposing serotonin (Freedman et al. 1977). *Tetrahymena* reacts to the increase of serotonin content in the environment with a sequence of short-term changes in the RNA synthesis (first decrease, then increase) that last up to 30 min; they are followed by the long-lasting increase of RNA synthesis (as measured by tenfold increase of incorporation of ^{3}H-uridine to the RNA), increase of the serotonin content inside the cell and activation of phagocytosis. These changes last for at least 50 consecutive generations of *Tetrahymena* after one short exposure to serotonin (Csaba and Ubornyák 1981). Although all monoamines evoke similar (but not identical) short-lasting changes, the long-lasting effect is unique for serotonin. Application of tryptophan, the precursor of serotonin, decreases serotonin content shortly after its incorporation from the medium (Csaba 1993). Serotonergic receptors were not researched yet in protozoans, but the presence of beta-adrenergic membrane receptors is well documented in these organisms (Quinones-Maldonado 1987, Wyroba 1989).

It is interesting that the majority of families of vertebrate receptors for serotonin that are present in the cellular membranes (5-HT$_1$, 5-HT$_4$, 5-HT$_6$ and 5-HT$_7$) still modulate adenylate cyclase activity through which they influence the level of cAMP (cf. for review Tecott and Julius 1993, Hoyer and Martin 1996). Therefore, it seems that the main intracellular signalling system of serotonin is very ancient, being preserved throughout the last two billion years of evolution from protozoans to mammals (Nanney 1980). Activation of this system may influence expression of various genes and therefore result in lasting changes of cell activity, allowing serotonin to be an important factor in both development and plastic changes in various organisms. However, other serotonergic receptors, connected to different signalling systems like phospholipase C (5-HT$_2$) or ion channel (5-HT$_3$), are also implicated in development and plasticity.

Serotonin is known to influence regeneration in some of the most primitive metazoans, the *Planaria* (Franquinet 1979). In *Polycelis* it acts through the adenylate cyclase and its absence or application of serotonin antagonists prevents regeneration in the animal (Franquinet et al. 1978, Franquinet 1979). Excess of serotonin produces abnormalities of pigmentation of the body, while another indoleamine, melatonin, has similar effect on the pigmentation of the tail of regenerating *Polycelis*. Distortions of the head pole are caused only by high concentrations of serotonin, while many amines, including serotonin, induce the formation of supernumerary eyes in much lower concentrations (Csaba and Bierbauer 1974).

Serotonin was found in many species of worms. It is present there as both hormone and neurotransmitter in the liver fluke, *Fasciola hepatica* (Mansour et al. 1960), acting through the adenylate cyclase and cAMP-dependent protein kinase (Mansour 1964). Cestodes and round worms synthesize it in both larval and adult stages. In these animals it acts as a hormone influencing development and provoking egg-laying. (Ribeiro and Webb 1983, Martin et al. 1988, Schinkmann and Li 1992, Eriksson et al. 1993).

Serotonergic neurones are present in all investigated arthropods and serotonin is known to act as a neurohormone in the embryonal development of at least some of them. In the lobster *Homarus* and in the crayfish *Cherax* serotonergic neurones appear very early on in the larval stage of *nauplius*, which in these animals develops *in ovo*. They innervate larval olfactory lobes and stimulate their development (Beltz et al. 1992, Helluy et al. 1993). Serotonergic neurones are found in all insects and extensive investigations in *Drosophila* revealed developmental role of serotonin. The enzyme synthesizing serotonin in *Drosophila*, (Drosophila

Tryptophan and Phenylalanine Hydroxylase - DTPH) is similar but functionally distinct from the mammalian tyrosine hydroxylase - TRH (Neckameyer and White 1992). It is ubiquitous in the fruit fly embryo, so the serotonin may influence development of various tissues (Neckameyer and White 1992). *Drosophila* has at least three types of receptors for 5-HT, all of them acting through G proteins and adenylate cyclase (stimulating or inhibiting the enzyme), and one of them activating phospholipase C. Their expression starts in the late embryo and is restricted to neurones (Saudou et al. 1992). In honey bee there is a significant increase of serotonin (and also dopamine) content in the head region during transition from *larva* to *pupa*. Levels of both neurotransmitters continue to increase in the *imago* with age and changing social roles, so they are highest in the oldest bees, that are pollen-collectors (Taylor et al. 1992). Therefore, in insects serotonin may play different roles during different stages of development and life.

In some marine molluscs, like abalone *Haliotis*, the presence of serotonin in the environment is necessary to induce the metamorphosis of pelagic larva into the adult benthic stage. In the environment where serotonin is absent, metamorphosis cannot be induced, even though the larva of abalone has five pairs of serotonergic neurones. One pair of these neurones, innervating velum propelling *larva*, dies during metamorphosis (Barlow and Truman 1992). In the natural environment serotonin is probably secreted by unicellular algae that are being filtered for food by the adult abalone, so serotonin may be an indicator of a place where food resources are adequate to support the sedentary mollusc. Serotonin is also known to trigger meiosis of oocytes in the surf clam *Spisula* (Krantic et al. 1991) through a specific plasma membrane receptor (Krantic et al. 1993). As the function of serotonin in very early embryogenesis of various animals is well documented (Buznikov et al. 1993), it is probable that further investigations will confirm it in many other species.

In most adult snails serotonergic neurones are present in the central ganglia, influencing various functions of the animals, including long-term changes of activity. The best known in this respect is the opistobranch *Aplysia*. In the central nervous system of this mollusc there are five pairs of serotonergic neurones (Nolen and Carew 1994), which play many roles. What is important, serotonin is necessary for consolidation of the long-term synaptic facilitation (Alberini et al. 1994, Walters and Ambron 1995, Wu et al. 1995) and influences the formation of synapses (Zhu et al. 1994).

The vertebrate pattern of widespread innervation of the whole central nervous system by a small number of serotonergic neurones placed in the nuclei of raphe or close to the wall of the fourth ventricle, if its sides are not sutured, is discernible already in *Cyclostomata* (Pierre et al. 1992) and present in all other orders. In lamprey there are also rostral groups of serotonergic neurones (in the mesencephalon and diencephalon) that are not present in other vertebrate orders. Most of them are placed very close to the midline. In mammals serotonergic neurones are located in the brainstem (most of them in the nuclei of raphe) and divided into two groups. The superior group consists of four main raphe nuclei that innervate most of the cerebrum and cerebellum: caudal linear (CL), median (MR), group B9 and dorsal (DR). The inferior group innervates brainstem and spinal cord. It consists of five nuclei of raphe: obscurus (RO), pallidus (RP), magnus (RM), lateral paragigantocellular (LPG) and intermediate reticular (IR); there are also some serotonergic neurones in the area postrema (for review see Törk 1990, Jacobs and Azmitia 1992). In spite of the common nuclear pattern, there are marked species differences in the topography of serotonergic innervation and receptors (Duncan et al. 1992, for review cf. Jacobs and Azmitia 1992), and also in the function of innervation from different raphe nuclei (Blue et al. 1988). In higher mammals there are signs of further anatomical and functional differentiation of the serotonergic system, with tendency to myelinization of axons and less extensive axonal arbors (Bowker and Abbott 1990).

Although the proportion of serotonergic neurones in mammals is very small (in rat one per million -

Audet et al. 1989) they innervate all parts of the central nervous system, forming the most extensive axonal arborizations of all neuronal systems. Such construction of the serotonergic (and also noradrenergic) systems is suitable for large-scale integration functions, for conveying uniformity of the functional state to the whole nervous system (Brodie and Shore 1957). Yet, differences in the density and positioning of serotonergic terminals, different density and various types of serotonergic receptors may allow for differential regulation of activity and plasticity of various structures during certain functional states and development.

ROLE OF SEROTONIN IN THE DEVELOPMENT OF OOCYTE, EMBRYO AND EARLY STAGES OF THE FOETUS

Indications of the developmental roles of monoamines are frequent transient waves of production of neurotransmitters, their metabolic enzymes and/or receptors in places and times in which there is no neuronal network present. The presence (or absence) of biogenic amines during embryonal development may influence cell proliferation, differentiation and migration.

Serotonin already plays a prominent role in the maturation of oocytes and later on in the initiation of the cleavages of zygote (for review cf. Buznikov 1991). It was found to be one of the important factors at this stage, but it may play different roles in different species. It accelerates maturation of starfish oocytes, but inhibits the maturation of oocytes in amphibians, acting as an antagonist of progesterone (Buznikov et al. 1993). Similar, suppressive functions of serotonin (and also noradrenaline) on the rate of oocyte maturation were found in humans, where monoamines antagonize the action of the luteinizing hormone and prolactin (Bodis et al. 1993b). However, a positive correlation of the content of monoamines in the follicular fluid with the success of *in vitro* insemination shows that at the same time they exert some positive trophic influences on the competence of maturation (Bodis et al. 1993a)

This influence of serotonin ends after a few divisions (Buznikov 1991), to reappear several times at later embryonal stages and in different places. When expressed, serotonin influences the process of differentiation in various tissues, in most cases arresting cell proliferation and inducing differentiation. Earlier data concerning those influences were extensively reviewed by Lauder and Krebs (1978), Lauder (1988, 1990, 1993), and also by Emerit et al. (1992). In short, serotonin appears temporarily in many places that should invaginate during development (like closing neural tube or forming eyecup), being expressed in those parts in which cell proliferation should be arrested (like the fundi of those two structures), and therefore the still proliferating rims of the structures fold up. During formation of the mouth palate serotonin (together with other molecules generally known as neurotransmitters) stimulates cell movements and palate shelf reorientation. Also the heart is shaped by controlled increase of serotonin content (Lauder et al. 1988).

Recently it was shown that serotonergic regulation of the epithelial-mesenchymal interactions plays an important role in the craniofacial morphogenesis (Shuey et al. 1992, Shuey et al. 1993). Critical events of this process in the mouse embryo occur during days 9-12 of gestation. During that period the involved epithelial cells of the facial region transiently express the specific serotonin uptake and binding protein in the areas of low proliferation (Lauder et al. 1988, Shuey et al. 1992, Shuey et al. 1993). If mouse embryos growing in the whole embryo culture are exposed to selective 5-HT uptake inhibitors (sertraline, fluoxetine or amitriptyline) at non-embryotoxic concentrations, they induce craniofacial malformations. The critical period for this effect occurs on days 10-11, that is in the peak of the morphogenetic process of the face formation. Most probably malformations are produced in the effect of a direct action of the inhibitors on the 5-HT uptake sites resulting in serotonin depletion in the developing tissues. This causes decreased proliferation and extensive cell death in the

mesenchyma located 5-6 cell layers deep from the overlying epithelium. As the more peripheral layers of mesenchymal cells showed normal or even elevated levels of proliferation, most probably the serotonin is derived from the fetal environment, so the passive diffusion of serotonin through the epithelium supplied the superficial cells with adequate amounts of serotonin. Ablation of the notochord (which produces the monoamines taken up by the neural tube) leads to a delay in formation of its floor plate (Artinger and Bronner-Fraser 1993). In the above cases serotonin clearly plays its ancient roles, that of the intracellular regulator and hormone or trophic factor.

Intracellular mechanisms of this trophic action of serotonin are only partially understood, but various drugs acting on serotonergic receptors or the 5-HT transporter cause severe deformations in many developing regions during critical periods (Shuey et al. 1993, Moiseivitch and Lauder 1995). Also, stimulatory effect of serotonin on the migration of the neural crest cells to the mesenchyme is mediated by the 5-HT_{1A} receptor temporarily expressed by these cells (Moiseivitch and Lauder 1995). Also the temporarily expressed 5-HT_{2B} receptor is implied in those functions (Choi et al. 1994).

INFLUENCE OF SEROTONIN ON THE FOETAL DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

Serotonergic innervation appears very early, it is probably the first long-distance projection in the brain. In the rat the first neurones producing serotonin appear between days 12 and 14 of gestation (Lauder and Bloom 1971). Neurones of the superior group contain serotonin already by the 12-th day of gestation (E12) whereas in the inferior group it is found 2 days later (Wallace and Lauder 1983). Serotonergic axons of the superior group appear in the medial forebrain bundle at E14 and innervate thalamus, hippocampus and neocortex by E18. By E19 projections of all raphe nuclei reach their targets (Lidov and Molliver 1982, Aitken and Törk 1988), but in many structures it evolves for a long time (Lidov and Molliver 1982, Azmitia et al. 1983).

Development of the serotonergic properties of raphe neurones depends on positive feedback influences, that is, small amounts of serotonin present in the tissue initiate its own synthesis and stimulate development of other serotonergic properties, sometimes even in neurones that would not normally produce serotonin (De Vitry et al. 1986). This mechanism may be responsible for both rapid development of serotonergic neurones (Lauder and Krebs 1978, Azmitia and Whitaker-Azmitia 1987) and transient presence of serotonin in some neurones during development (Shuey et al. 1993).

When cultures of embryonic rat serotonergic neurones were continuously treated with cAMP analogues (which mimicked the increased cAMP levels), the neuronal morphology was changed towards that typical for mature serotonergic neurones (Foguet et al. 1993). This change was accompanied by the induction of expression of tryptophan hydroxylase, the rate-limiting enzyme in serotonin production, and the down-regulation of expression of the inhibitory autoreceptor, 5-HT_{1A}. Therefore, increased cAMP concentrations affect the development and cause a prolonged activation of serotonergic transmission. However these facts should be interpreted cautiously; since 5-HT_{1A} receptors inhibit cAMP formation, their down-regulation in this paradigm differed from observations *in vivo* where they act in a negative feedback control of the serotonergic system (Jacobs and Azmitia 1992).

Both 5-HT_{1A} and 5-HT_2 receptors are expressed prenatally (Hellendall et al. 1993) in the rat brain and the 5-HT_{1A} agonists can stimulate the cholinergic parameters of primary cultures of rat fetal septal neurones (Emerit et al. 1992). These interactions exist also in adult animals, as altanserin (a serotonergic antagonist) produces profound changes in acetylcholine release in the brain of baboon (Dewey et al. 1993). The accelerating influence of stress to the mother on the development of serotonergic neurones in rat foetuses shows that glucocorticoids

are also involved in the modulation of their development (Lauder and Krebs 1978).

Maturation of the serotonergic system is also dependent on some local growth factors and the neuronal activity (Azmitia and Zhou 1986, Azmitia et al. 1988, Ching et al. 1994, Eaton et al. 1995). One of the important mechanisms of the trophic influence of serotonin is stimulation of glia to production of trophic factors. This effect was first described by Whitaker-Azmitia and Azmitia (1989) who showed that serotonin added to the astroglia cultured *in vitro* stimulates glial production of trophic factors that are secreted into the culture medium and may regulate the growth of serotonergic axons. Liu and Lauder (1992) presented similar results. In their experiments serotonin was added to the co-culture of the E14 raphe or substantia nigra neurones with radial glia/astrocytes derived from the same or opposite nucleus. Serotonin stimulated both types of monoaminergic neurones more significantly when they were co-cultured with glia derived from mesencephalon (where the substantia nigra is placed). The increased survival of the serotonergic neurones in co-culture with both types of glia suggests that factors secreted by both raphe and mesencephalic glia contribute to the support of the raphe neurones. Further studies showed that serotonin acts on the astroglial 5-HT_{1A} receptor that stimulates release of the growth factor S100B from astroglia (Bell et al. 1992, Akbari et al. 1994). As there are local differences in the trophic activity of glia, it is probable that serotonin may also stimulate glial production of some other factors influencing neuronal development.

The level of serotonin in the developing brain is precisely regulated. Therefore, the increased availability of tryptophan, the substrate from which tryptophan hydroxylase produces serotonin, delays outgrowth of the 5-HT axons and reduces serotonin synthesis. If pregnant rats are fed on a tryptophan-enriched diet, neurochemical markers of 5-HT innervation in 5 day old pups (content of 5-HT, activity of TRH and high-affinity 5-HT uptake) are reduced. The postnatal increase of these markers in the pups is retarded. The precise mechanism of this negative feedback is not yet known. All these changes were more pronounced in the cerebral cortex than in the brainstem (Huether et al. 1992). It is interesting that mesencephalic glial factors, in conjunction with their trophic influence, increase the inhibitory effects of serotonin on the outgrowth of raphe neurones (Liu and Lauder 1992). In the intact animal these mechanisms may prevent hyperinnervation of a brain structure by the serotonergic axons.

Inhibition of the growth of developing serotonergic neurones seems to be one of the important factors in neurobehavioural abnormalities resulting from prenatal exposure to cocaine (Fantel and Macphail 1982, Azmitia 1992). This inhibition was shown both *in vitro* (Azmitia and Whitaker-Azmitia 1987) and *in vivo* (Akbari et al. 1992, Akbari et al. 1994). Prenatal exposure to cocaine (days E13 till parturition) decreased the amount of the trophic factor S-100B and resulted in lower 5-HT uptake and lower serotonergic fibres density in the cortex and hippocampus during the first postnatal week of life of the exposed pups. It may also induce microcephaly. One month old pups showed substantial recovery, with the exception of hippocampal fields CA1 and CA2 where the density of serotonin-containing fibres was still low. Interestingly, pups that were further injected with cocaine on postnatal days 1-5 recovered faster, so at one week 5-HT uptake in their brains was comparable to that in normal pups and in the cortex it was even higher. The same results were obtained with postnatal administration of ipsapirone, the 5-HT_{1A} receptor agonist (Akbari et al. 1992, 1994).

These facts are probably explained by the finding that regulation of development of the ascending serotonergic neurones is achieved through the balance of receptors exerting reciprocal effects (Bell et al. 1992). Cocaine, acting on SERT could have increased the serotonin level, which activated the negative feedback through the release-regulating 5-HT_{1A} autoreceptor (Sikich et al. 1990, Huether et al. 1992, for review cf. Chojnacka-Wojcik 1995) leading to underdevelopment of the serotonergic system. Withdrawal of cocaine in such system may

cause delayed postnatal maturation because development of the serotonergic fibres requires activation of the positive feedback loop by serotonin acting on the astroglial 5-HT_{1A} receptor that stimulates release of the growth factor S100B from astroglia (Bell et al. 1992, Akbari et al. 1994). Also, the level of Ca^{2+} ions in the serotonergic axons could have been far from optimal, which could inhibit the neurite expansion (Cohan et al. 1987). As the level of serotonin in the brain after cocaine withdrawal is lower than the physiological norm, so the positive loop is not activated adequately and the growth of the fibres is retarded. Therefore, small postnatal doses of cocaine, releasing more serotonin, or injections of ipsapirone that directly stimulated the glial 5-HT_{1A} receptor allowed for faster maturation of the serotonergic system. If this hypothesis is true, opposite effects of postnatal injections of much higher doses of cocaine on the growth of serotonergic axons might be predicted. Also, in normal rat pups postnatal cocaine should retard growth, even in the lower doses. Foetal exposure to ethanol causes developmental abnormalities of the cortical projections of serotonergic neurones in rats. These abnormalities seem also to be dependent in part on the low foetal 5-HT content and may be overcome by *in utero* treatment with a 5-HT_{1A} agonist (Tajuddin and Druse 1993).

Development of the descending serotonergic projection seems to depend on somewhat different factors. In the experiments of Bell et al. (1992), pregnant rat dams were treated with drugs selectively activating receptors 5-HT_{1A} (8-OH-DPAT) or 5-HT_{1B} (TFMPP) or releasing serotonin (fenfluramine) from gestation day 12 until birth. Pups born from these pregnancies were tested on postnatal days 10 and 30 for alterations in development of nociceptive pathways (tail-flick latency) and serotonergic axons density in the spinal cord (^{3}H-paroxetine binding). The applied drugs had no effect on the development of spinal serotonergic pathways, whereas such influences were exerted by way of the 5-HT_3 receptors, as the prenatal treatment with the 5-HT_3 agonist (phenylbiguanide) increased latency of the tail-flick reflex, while treatment with the 5-HT_3 antagonist, MDL 72222, decreased the latency. Surprisingly, both substances significantly increased the density of serotonergic innervation of the spinal cord when tested on postnatal day 18. Receptors 5-HT_3 are connected to potassium channels and have relatively high permeability to calcium ions. Through this channel significant amounts of calcium may enter the cytoplasm (Tecott and Julius 1993), and this may be the mechanism by which serotonin influences development of the nociceptive pathway.

Liu and Lauder (1991) showed that serotonin significantly improves the survival, cell body size and neurite outgrowth of embryonic (E14) dopaminergic neurones of substantia nigra by co-culturing them with the raphe neurones of the same age.

TROPHIC FUNCTIONS OF SEROTONIN IN THE POSTNATAL DEVELOPMENT AND PLASTICITY OF THE CENTRAL NERVOUS SYSTEM

Evidences of serotonin participation in development and plasticity are known from teleosts. In the coho salmon (*Oncorhynchus kisutch*) the smolt transformation (change from the juvenile fresh-water form to the form living in salt water, that occurs between the 17-th and 18-th month of life) is associated with sequential surges of several neurotransmitters in the brain, including serotonin. Also, in the 17 month old fish (and only at that stage) there emerges a transient population of serotonin-immunoreactive neurones in the lateral preoptic area and in the lateral part of the dorsal right habenular nucleus (Ebbeson et al. 1992). At this stage the density of serotonergic fibres in the telencephalon is higher than in the preceding or following stages, but the transient serotonergic structures do not appear simultaneously with the surge of 5-HT concentration in the brain, so they do not seem to be the source of 5- HT during the surge. They may influence some other developmental changes in the brain of salmon associated with the smolt transformation. Serotonin may also stimu-

late gonadotropin release from the pituitary of mature Atlantic croaker, so it participates in reproduction (Khan and Thomas 1992). Social interactions (establishment of domination) may also lead to permanent changes in serotonergic activity (Winberg et al. 1992).

In mammals the final density and placement of serotonergic terminals is shaped during postnatal maturation of the central nervous system, which in rat may take weeks or even months (Lidov and Molliver 1982, Azmitia et al. 1983, Wallace and Lauder 1983). In some structures serotonergic innervation passes through stages of temporal developmental hyperinnervation or temporal expression of some 5-HT receptors, which may be the substrate of serotonin participation in both development and plasticity of the central nervous system.

In vitro experiments with the neonatal rat hippocampal and cortical explants showed that the presence of serotonin improves the survival of neurones. Serotonin also accelerates the neuronal differentiation, myelinization of axons, differentiation of synaptic contacts and development of neuropile. However, serotonin could also inhibit development of the growth cones and limit branching of axons (Chumasov et al. 1980, Gromova et al. 1983, Chubakov et al. 1986). The same authors found that the spontaneous electrical activity of the majority of neurones in the explants was much higher if serotonin was present in the culture medium. This effect contrasted with the predominant net inhibitory action of serotonin in most structures of the central nervous system of the adult animal (cf. for review Jacobs and Azmitia 1992). However, serotonin may also inhibit information transfer during development of the cortex (Dyck and Cynader 1993, see below for details).

Transient developmental serotonergic hyperinnervation or innervation different from the adult pattern was described in many brain structures. It was found in some subcortical structures, like in the comissure of the superior colliculi of the neonatal hamster, through which many serotonergic fibres cross from one side to another. Almost all of them are eliminated in the first two postnatal weeks (Rhoades et al. 1990b, Bennet-Clarke et al. 1991b). But the clearest examples of transient serotonergic hyperinnervation were found in the cerebral cortex.

The developmental serotonergic hyperinnervation in the neocortex was first found in the somatosensory area of the neonatal mouse (Fujimiya et al. 1986), and later in all primary sensory areas of neocortex in the neonatal rat. Other areas of the neocortex were not hyperinnervated (D'Amato et al. 1987). In the somatosensory area serotonergic axons formed patches that were coextensive with the vibrissal barrels. The densest innervation was found in layers IV and VI (D'Amato et al. 1987). Further investigations (Rhoades et al. 1990a, Blue et al. 1991) showed that the hyperinnervation, consisting of fine caliber fibres developed between birth and the fourth or fifth postnatal day. In the somatosensory area it was visible from the first postnatal day, in the tangential cortical sections being shaped in the form of rattunculus. Until the end of the second postnatal day (or for the first 60 hours after birth – Bennet-Clarke et al. 1994b) it was uniform, only later forming patches that were visible for the next two weeks. After that, there formed the adult pattern of much scarcer serotonergic innervation, where the highest density of the serotonergic axons was found in layers I and II. The serotonergic patches in layer IV of the barrel field formed 0.5- 1 day later than the layer was innervated by the thalamic axons, therefore the patches may not be the developmental signal for the formation of barrels, but rather the serotonergic axons were induced by the thalamic axons (Rhoades et al. 1990a).

Although the transient serotonergic innervation forms a very well defined topographic pattern, its source (the set of serotonergic neurones in the raphe nuclei) is not any more topographically organized than in the adult animal (Bennet-Clarke et al. 1991a, 1994a), therefore the whole patterning of this hyperinnervation is probably shaped within the cortex, in the interactions of the growing serotonergic axons with some factors acting in the developing cortex. During the transient 5-HT hyperinnervation receptors of the 5-HT_{1B} type are present on the growing thalamic axons, which may be the mech-

anism through which serotonin influences formation of the axon terminals (Bennet-Clarke et al. 1993).

Nakazawa et al. (1992) investigated the transient serotonergic hyperinnervation in the rat visual cortex and showed that it evolves in three stages. The primary aggregation first appeared in the subplate and moved upward, keeping up with the progress of laminar differentiation until it reached the presumptive layer IV, where it formed the secondary aggregation shaped in an evenly spread layer. From there the serotonergic axons extended to the upper layers in column-like structures (the tertiary aggregation), which in the layer I formed a lattice-like pattern. Development of this transient 5-HT innervation began in the anterior part of the visual cortex on postnatal day 2 (PND 2) and progressed posteriorly (in correspondence with the direction of differentiation of the visual cortex) until it was completed on PND 11. The hyperinnervation disappeared on PND 15.

In the lateral septum, development of the serotonergic innervation parallels neuronal differentiation in this area. Varicosities of the serotonergic axons in newborn animals are in close association with neuronal elements, without any intervening neuroglial processes; towards the end of the first postnatal week almost all of them (95%) exhibit well-defined synaptic specializations (Dinopoulos et al. 1993).

Information about developmental changes in the serotonergic receptors is very far from satisfactory, but even such incomplete patchy data provide interesting information (Whitaker-Azmitia 1991). In rat it was found that the density of the 5-HT_{1A} receptors (as measured by the level of binding of its selective ligand, ^{3}H-8-OH-DPAT) in the brain markedly changes during development (Daval et al. 1987). In the cerebral cortex and hippocampus the density of these receptors increased postnatally until it reached the adult level around the third postnatal week, without showing any transient overproduction. In contrast, in the cerebellar cortex that develops exclusively postnatally, the density of the 5-HT_{1A} receptors was very high at birth. It was gradually reduced by almost 90%, falling down to the adult levels at PND 14. In fact, the peak of density of the 5-HT_{1A} receptors is reached in the late fetal period in both rat and human (Whitaker-Azmitia et al. 1990, Bar-Peled et al. 1991).

In contrast, recent investigations of the developmental pattern of the 5-HT_{1B} receptors in rat showed that they form a transient, barrel-like pattern in the somatosensory cortex of the developing rat (Leslie et al. 1992, Bennet-Clarke et al. 1993). They are expressed for the first two postnatal weeks by the developing terminals of the thalamocortical axons from the ventral posteromedial nucleus (VPM). Different densities of several serotonergic receptors result in different action of serotonin on cortical neurones (Rhoades et al. 1994). During the first two weeks of life serotonin strongly inhibits EPSP's evoked in the cortical neurones by the discharges of thalamic axons. Yet, serotonin had no effect on the membrane potential or depolarization induced by direct application of the glutamic acid to the neurones. Agonists of the 5-HT_{1B} receptor mimicked this influence of serotonin, while antagonists blocked it. This effect was significantly reduced in animals older than 2 weeks, which was in good correlation with the timing of developmental reduction of the 5-HT_{1B} receptors. Therefore, in rat there seems to exist a special mechanism allowing serotonin to exert its developmental function, different from its function in adult animal.

Somewhat different results were found in the visual areas of cat's cerebral cortex, where the postnatal changes of density of the type 1A, 1C, 2 and 3 serotonergic receptors were assessed with the *in vitro* binding of specific radioactive ligands (Dyck and Cynader 1993). Specific labelling of the 5-HT_3 receptors was not found at any age. Each of the remaining receptors exhibited unique temporal, regional and laminar patterns of expression in the cortical areas 17, 18, 19 and the lateral suprasylvian (LS) area. Receptors 5-HT_{1A} showed transient overproduction between the PND 10 and 30, while the 5-HT_{1C} receptors were most abundant between the PND 40 and 75 and the receptors 5-HT_2 between the PND 40 and 120. Receptors 5-HT_{1A} were expressed in all visual areas, predominantly in their

supra- and infragranular layers. The highest concentrations of receptors 5-HT_{1C} were found in the area 17, where they formed a columnar pattern in the layer IV and the lower part of layer III. As the size of the columns was 400 μm and their centre-to centre spacing was 900 μm, it is probable that they have been coextensive with the ocular dominance columns. Receptors 5-HT_2 were found in all visual areas, showing highest density in the layer IV. In the area 17 they formed patches that were found in the same vertical columns as the receptors 5-HT_{1C}.

Still different developmental changes of serotonergic (and other monoaminergic) receptors were found in primates, where they proceed for very long time. Lidow and Rakic (1992) using quantitative *in vitro* autoradiography studied the density of the serotonergic (5-HT_1 and 5-HT_2), dopaminergic (D1 and D2) and noradrenergic (alpha 1, alpha 2, and beta) receptors in the cortex of rhesus monkeys from birth till 60 months of age. The density of all the investigated receptors increased during first two postnatal months in a highly coordinated manner, reaching level twice higher than that in the adults. After the fourth month, receptor density began to fall, to stabilize at the adult levels around the time of puberty. The course of developmental changes was similar in all cortical layers and in all investigated areas. However, the magnitude of overproduction of each type of receptor varied depending on the cortical layer and area. In the adult animal all monoaminergic receptors were concentrated in the superficial cortical layers. There is still great need of precise data on the developmental changes of expression of various serotonergic receptors in different species. Taking into account fast changes in the classification of serotonergic receptors, recent discovery of their new types and development of new, more selective ligands (for recent review cf. Hoyer and Martin 1996) it seems that certain data will have to be confirmed with the new pharmacological tools.

The developmental pattern of serotonergic hyperinnervation of the sensory cortical areas is so striking, that many experiments were conducted to elucidate its role. Neonatal serotonin depletion in rat with injections of serotonergic neurotoxins PCA or 5,7-DHT delayed development of the barrel field pattern in the somatosensory cortex for 1-2 days (Blue et al. 1991). This delay allowed for longer preservation of the capacity for plastic changes after lesions of vibrissae in mice Osterheld-Haas et al. 1994). The permanent effect of the neonatal serotonergic lesion was the atrophy of the thalamic axons, that was showed by the 30% reduction of area covered by the axon bundles stained with carbocyanine dye DiI and similar reduction of cytochrome oxidase patches in the barrel field (Bennet-Clarke et al. 1994b). The size of the zone activated by a vibrissa stimulation (as measured by ^{3}H-deoxyglucose incorporation) was reduced similarly (Turlejski et al. 1995). Interestingly, density of cortical receptors 5-HT_{1A} and 5-HT_{1B} did not seem to be changed after lesion of the serotonergic axons with 5,7-DHT (Verge et al. 1986). Another line of evidence, derived from the neonatal lesions of vibrissal afferents, suggests that the serotonergic axons may actually require the presence of normally functioning thalamic axons to be able to form barrel-like hyperinnervation pattern (Rhoades et al. 1990a, Bennet--Clarke et al. 1994a). However, inactivation of the somatosensory cortex spike activity with tetrodotoxin for the first 7 or 11 postnatal days in rat did not disrupt the transient barrel-like pattern of serotonergic innervation, and neither formation of the normal morphology of the barrel field (Chiaia et al. 1992). Interdependence of those two axonal systems needs to be further investigated.

Different results were obtained in the visual cortex. Transient developmental aggregation of the serotonergic axons in the cortex of rat may be regulated primarily by the intrinsic factors, while extrinsic factors only modulate this process (Nakazawa et al. 1992). Neither monocular nor binocular neonatal enucleation significantly affected the structure of the serotonergic innervation of the visual cortex in rat, with the exception of irregularities of secondary aggregations in both contralateral and ipsilateral cortices. After monocular deprivation the density of 5-HT fibres in the contralateral binocular area (Oc1B) increased. Similar results were obtained in

primates (Rakic and Lidow 1995). Binocular foetal enucleation (days E60 - E81) in the rhesus monkey did not affect distribution of the serotonergic (5-HT_1, 5-HT_2), noradrenergic or dopaminergic receptors in the animals surviving either 2 or 12 months after birth. However, blocking of the 5-HT_1 and 5-HT_2 serotonergic receptors during the period of developmental plasticity in cat showed that both types of the receptors are needed for the plastic change to occur (Gu and Singer 1995). Therefore, intracortical infusion of the 5,7-DHT or a mixture of ketanserine and methysergide (but not of each of the antagonists alone) reduced plastic changes evoked in the visual cortex by the monocular deprivation during the critical period.

Cerebellum is innervated by two systems of serotonergic axons: mossy fibres and fine, beaded axons (the diffuse system). Neonatal X-ray irradiation caused cerebellar cortical agranularity and elimination of the mossy fibres, while the diffuse system was hypertrophied (Beaudet and Sotelo 1981). Serotonergic hyperinnervation was also found in other denervated structures of central nervous system (Jackson and Abercrombie 1992).

Another interesting results were obtained in the experiments in which development proceeded in the presence of the high level of serotonin in the brain. Neonatal administration of 5,7-DHT in hamsters brings paradoxical results: although it depletes serotonin in the cerebral cortex, it causes in the same time hypertrophy of serotonergic innervation in the superior colliculus, with markedly increased percentage of serotonergic terminals forming synapses. This condition was found to cause permanent abnormalities of the visual afferentation of superior colliculus and change transmission of the visual information (Rhoades et al. 1993, Arce et al. 1995, Crnko et al. 1996). Very high serotonin levels accumulate also in the brain of transgenic mice with a deficient gene encoding monoamine oxidase A (MAOA). In such mice barrel structure of the somatosensory cortex does not form at all. The effect was reproduced by transient pharmacological inactivation of the MAOA in normal newborn mice (Cases et al. 1996). However, no changes in the brain structure were observed in another mouse mutant that also accumulates high levels of serotonin (Son et al. 1994).

Serotonin participates in the development of sexual differentiation (Frankfurt et al. 1985). In rat during the second week of life concentrations of 5-HT in the hypothalamus are higher in females than in males and this difference is testosterone-dependent. In this period injections of pCPA depleting 5-HT enhanced, and injections of 5-HT reduced the changes evoked by testosterone injections. Thus, 5-HT may normally exert an inhibitory control on the action of neonatal testosterone (Wilson et al. 1992). Serotonin is also one of the mediators of the adaptation to stress, as it was shown to regulate the glucocorticoid receptor (GluR) density in the *in vitro* cultures of hippocampal neurones. This action of serotonin requires induction of changes in the cAMP level (Mitchel et al. 1992, Flügge 1995, cf. also Lauder and Krebs 1978). This may be one of the neurochemical mechanisms by which environmental stimuli acting during development, such as handling, shape adult responses to stress.

While most of the developmental signals and mechanisms cease to be present and active in the adult animal, serotonin makes a notable exemption in this rule. Serotonergic axons of the mature animal are unique in preserving extensive regenerative capacity. Therefore, they reinnervate their targets, innervate neighboring regions where serotonergic fibres were destroyed and form excessive innervation in structures that were deprived of their specific input in adult animal (Azmitia et al. 1978, Frankfurt and Azmitia 1983, Frankfurt et al. 1985, Frankfurt and Beaudet 1987, 1988, for review cf. Jacobs and Azmitia 1992, Marlier et al. 1992). Selective serotonergic agents capable of activating or blocking specific spinal cord serotonergic receptor subtypes were also foud to be useful in the treatment of certain specific pain diseases, such a phantom limb pain that is caused by limb amputations in adult animals or humans (Feria et al. 1992).

An extensive literature has accumulated over the past three decades implicating alterations in serotonergic neurotransmission in the pathophysiology

of depression (for review cf. Risch and Nemeroff 1992). Investigations showed that in the brains of many depressed patients concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) is decreased. Frequent alterations in both presynaptic and postsynaptic CNS serotonergic receptors were found in these patients. Similar alterations in the peripheral serotonergic system such as platelet serotonin uptake or serotonergic receptor densities suggest that these changes may have genetic background.

CONCLUDING REMARKS

The ancient modulatory roles of serotonin, mechanisms of which were already developed in protozoans, are preserverd throughout evolution, coordinating both development and plasticity in various species. In mammals this ability is present throughout life. Serotonin is not unique in this respect. Although investigations of other monoamines were not so extensive, similar points could have been made for noradrenaline, dopamine or melatonin. From other neurotransmitter-like substances acetylcholine and GABA seem to be equaly old modulators. Different actions of these compounds and also multiple receptors conveying different influences allow for great flexibility of regulation of various functions. Future research will probably concentrate on: functions of various receptors, with the use of modern methods of molecular biology (Lucas and Hen 1995), interdependence of actions of different amines, developmental functions of neuropeptides cocolocalized in the aminergic neurones and on various trophic factors that may be transiently expressed in these neurones.

ACKNOWLEDGEMENTS

I am greatly indebted to Professor J. Vetulani, Professor Oderfeld-Nowak, Dr. M. Skup, Dr. E. Wyroba and M. Gierdalski MSc., for useful remarks and suggestions, and to A. Rydz for improvement of English. Supported by KBN grant 0392/P2/94/06.

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Received 15 March 1996, accepted 3 April 1996

This paper is dedicated to Professor Stella Niemierko on the occasion of her 90th birthday, with esteem and admiration