Development of the Mesencephalic and Diencephalic Serotoninergic System in Mice and the Role of Serotonin in This Process

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The development of functional activity of the serotoninergic system in the mesencephalon and diencephalon of mice is followed during ontogeny, and it is found that serotoninergic neural elements become capable of specific serotonin uptake and K^+-stimulated serotonin release on day 17 of prenatal life. A single serotonin injection into a female mouse on day 8 of gestation resulted in a drastically reduced specific ^{3}H-serotonin uptake by the brain of its 18-day fetuses.

Key Words: *serotonin; brain; embryo; mouse*

In adult animals, the serotonin (5-HT) elaborated by neurons of the mesencephalic raphe nucleus is transported via their axons to the hypothalamus where it becomes involved in neuroendocrine regulation [3,4]. As shown by histofluorescence [2], immunohistochemical [8,10], and autoradiographic [8] studies, the 5-HT system forms during early ontogeny in rats and mice, with 5-HT neurons being demonstratable in the mesencephalic raphe nucleus on day 13 and 14 of embryonal life, respectively. Morphological studies, however, do not give any concept of how the 5-HT system functions during ontogeny, although 5-HT is regarded as a potential morphogen controlling the differentiation of target cells in the early stages of embryonal development [5].

The present study was undertaken to follow the establishment of the most important functional characteristics of 5-HT neuronal elements, namely, the specific 5-HT uptake and release in health and after a single 5-HT injection into a pregnant female mouse during the critical period of development of the fetal brain.

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MATERIALS AND METHODS

Female CBA and male C57Bl/6 mice were used, including 16-, 17-, and 18-day fetuses, 8- and 9-day-old neonates, and adults. The brain region for study was dissected in the cold and included the mesencephalon and diencephalon for prenatal animals and the hypothalamus for adult ones. Specific uptake and release of ^{3}H-5-HT were determined by the procedure described elsewhere [9]. Brain fragments were placed in flasks containing Krebs—Ringer's bicarbonate buffer (pH 7.4) and preincubated in a carbogen-saturated medium at 37°C with gentle shaking. Thereafter they were incubated with ^{3}H-5-HT (Amersham; specific activity 17-20 Ci/mmol) added to the medium in a concentration of 25×10^{-9} M. Nonspecific binding was determined after adding an inhibitor of neuronal reuptake, cytalopram (Lundbeck) in a concentration of 10^{-5} M. Radioactivity was expressed in cpm/mg tissue. Specific uptake was calculated as the difference between tissue radioactivities after incubation in the presence and absence of the neuronal uptake inhibitor.

For the estimation of 5-HT release, brain fragments incubated with ^{3}H-5-HT were washed and

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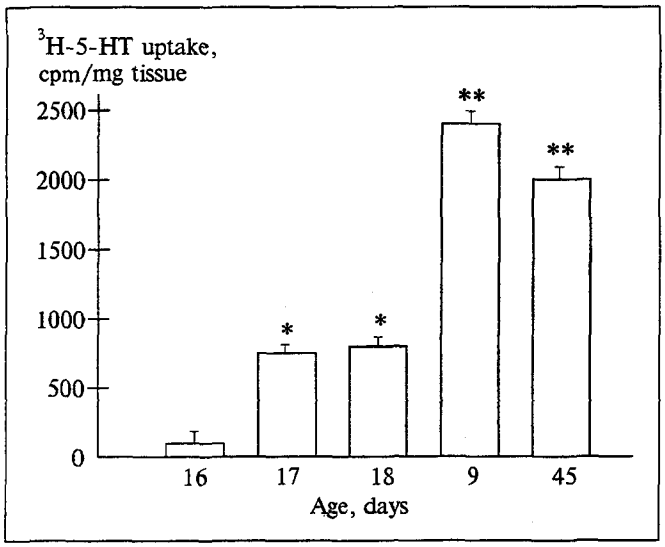

Fig. 1. Specific ^{3}H-5-HT uptake by brain fragments of fetal (days 16, 17, and 18), neonatal (9-day-old), and adult (45-day-old) mice. $p<0.05$: *in comparison with fetal day 16; **in comparison with the preceding day.

transferred to chambers for perfusion, and after 2 minutes four fractions of the perfusate were collected to determine spontaneous 5-HT release. The medium was then changed for a fresh one containing K^+ at elevated concentration (60 mM KCl), and further four fractions were collected — stimulated 5-HT release in response to K^+ depolarization which was carried out for 8 min. Radioactivity was measured by liquid scintillation spectrometry and expressed in cpm/min per 400 μl fraction and per g tissue. Stimulated release was calculated as the difference between radioactivities of the fractions before and after stimulation.

Serotonin (Hungary) was injected intraperitoneally on day 8 of pregnancy in a single dose of 0.9 mg per mouse.

The results were statistically analyzed by Student's *t* test.

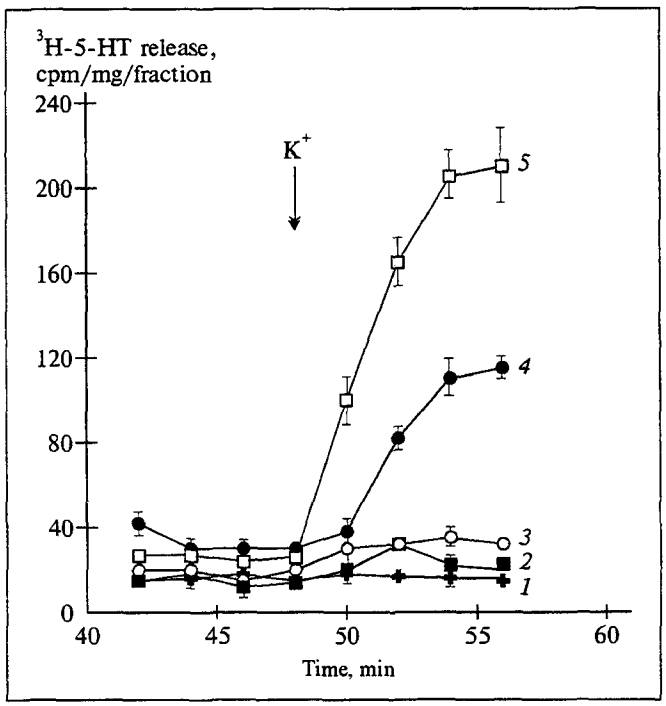

Fig. 2. Spontaneous and K^+-stimulated release of ^{3}H-5-HT from brain fragments of 16-, 17-, and 18-day fetal (*1*, *2*, and *3*), neonatal (day 9) (*4*), and adult (*5*) mice.

RESULTS

On day 16 of prenatal life, the mesencephalic and diencephalic nerve tissues were not yet capable of specific ^{3}H-5-HT uptake. Neuronal reuptake was first detectable in 17-day fetuses and persisted at this level for 24 h. On postnatal day 9, a sharp rise in ^{3}H-5-HT uptake, exceeding even that in adult animals, was recorded (Fig. 1).

Tests for the ability of neural elements of murine brain to release the taken up ^{3}H-5-HT showed that a response to K^+ depolarization (increase in the rate with which the labeled amine was released) first appeared in 17-day fetuses and remained at the same level in 18-day fetuses. A sharp rise in the K^+-stimulated release occurred on day 9 of postnatal life and amounted to about 50% of its level in adult mice (Fig. 2).

Our results are consistent with the morphological evidence that fluorescent neurons are detectable in small numbers in the mesencephalic region of 16-day mouse fetuses, that they increase in number by day 17, and that no further increase occurs during the following 24 h [2]. It should be noted that a very marked rise in specific ^{3}H-5-HT uptake was observed to occur between days 16 and 18 of development in our previous study designed to find out how the 5-HT system forms in rat fetuses [9].

A response to depolarization on day 17 first arose coincidentally with the occurrence of specific ^{3}H-5-HT uptake. The most likely explanation for the observed sharp increase in ^{3}H-5-HT uptake in 9-day-old and adult mice is that 5-HT innervation of the diencephalon (hypothalamus) develops at the end of the prenatal period [2].

The role of 5-HT in the development of the 5-HT system was demonstrated in our experiment where 5-HT was injected on day 8 of pregnancy. This resulted in a drastic fall of specific ^{3}H-5-HT uptake by neuronal elements of the brain in 18-19-day fetuses as compared to its level in the fetuses of intact females (Fig. 3). Since specific uptake is regarded as an index of innervation [1], such a fall may be interpreted as reflecting autoregulation of the development of the system under consideration.

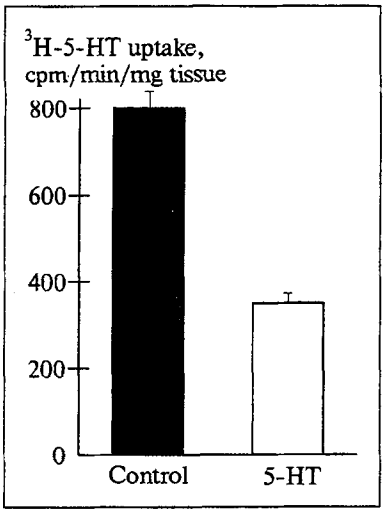

Fig. 3. Effect of single 5-HT injection into pregnant mice (day 8 of pregnancy) on the specific ^{3}H-5-HT uptake by brain tissue of their 18-day fetuses.

Indeed, it was shown earlier that chronic dosing of female mice with a 5-HT agonist from day 8 of gestation onward resulted in reduced numbers of 5-HT receptors in the brainstem and forebrain of their offspring on the first postnatal day [12], and that the agonist added to a culture of rat embryonal neurons inhibited their growth [11].

To summarize, starting with day 17 of development, the mesencephalon and diencephalon of murine fetuses contain 5-HT neural elements characterized by specific 5-HT uptake and depolarization-stimulated 5-HT release. The sharp increases in uptake and release between prenatal day 18 and postnatal day 9 appears to be associated with intensive development of an axonal network in the hypothalamus. 5-HT injection into a pregnant female brings about a drastically reduced specific uptake of labeled amine by the brain of its 18- and 19-day fetuses as compared with the fetuses of an intact female, which is most likely to be a manifestation of autoregulation of the 5-HT system development.

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