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Short Communications

The maturation of cortical serotonin binding sites

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Binding of serotonin to rat brain membranes increased linearly from birth to adulthood, but newborn receptor densities were already 39% of adult levels. These data suggest a postnatal development of serotonin receptors, coincident with synaptic maturation but do not preclude a non-transmitter function for serotonin during early maturation.

For some years, it has been postulated that neurotransmitters existed as chemical messengers long before their neural role evolved[2,8]. A neurohumoral role for serotonin during neural embryogenesis has been especially emphasized[5]. In support of this concept are reports that serotonin levels and the concentration of tryptophan hydroxylase, the rate-limiting enzyme for synthesis of tryptophan, appear relatively early in cerebral ontogenesis[4]. Recently the high affinity binding of serotonin to cortical and subcortical regions was reported to be greater in the 4-day-old rat than in the adult[9]. This observation is the opposite of an earlier report describing a large postnatal increase in serotonin binding[1]. Since the resolution of this discrepancy has implications concerning the non-transmitter functions of serotonin, we have reinvestigated the development of the high affinity binding of serotonin.

Female, Fischer Inbred rats of breeding age, were tested for sexual receptivity with an experienced male. When a female exhibited the lordosis response and other indices of sexual receptivity, the male was left with the female for 24 h. After mating, females remained individually housed until parturition. Twenty-four hours after birth litter size was reduced to 8 pups, but only male offspring were used in the study. At ages 1 day, 10 days, or 21 days, the entire litter was sacrificed. All rats were decapitated and forebrains (minus the striata) were removed and frozen.

Frozen tissue was weighed, homogenized in 19 vols. of 0.32 M sucrose and centrifuged at 20,000 rpm (50,000 $\times$ g_{max}) for 10 min. The pellet was washed in 19 vols. of deionized water and recentrifuged as above. The final pellet was suspended in 19 vols. of 40 mM Tris·HCl, pH 7.4, and stored at —20 °C for determination of binding density. Aliquots of the total homogenate and the soluble supernatants were taken for protein determination[6].

Forebrain membranes (equivalent to 10 mg of original tissue) were incubated in

triplicate for 10 min at 37 °C in 1.01 ml 40 mM Tris·HCl, pH 7.4, containing 3.15 × 10^{-9} M [1,2-^{3}H(N)]serotonin (5-HT) (29.8 Ci/mmol, New England Nuclear, Boston, Mass.), 10 × 10^{-6} M pargyline, 4 × 10^{-3} M $CaCl_2$, and 5.7 × 10^{-3} M ascorbic acid. In order to determine the extent of nonspecific binding, we ran a parallel series of triplicates containing 10^{-6} M serotonin as competitor. Each sample was diluted with 5 ml of Tris buffer, filtered on glass fiber filters (24 mm diameter, 0.3 μm pore size) and washed twice with 5 ml of cold Tris buffer. Filters were dried and counted. Preliminary studies in our laboratory revealed that binding equilibrium was reached with these conditions and that this interaction was both saturable and reversible. The assay was relatively specific since [^{3}H]serotonin binding was depressed only 23 ± 2% in the presence of 10^{-6} M haloperidol.

There was a gradual increase to adult levels of 5-HT binding during postnatal development ($F = 5.67$, df = 3,23, $P < 0.05$) (Fig. 1). Serotonin binding in newborn rats was 39% that of the adult and from birth to adulthood, there was a roughly linear increase in 5-HT binding to forebrain (the correlation between age and pm/g protein was 0.97). Binding in 1-day-old animals was significantly less than 21-day and adult ($P < 0.01$).

The postnatal increase in 5-HT binding is in agreement with Bennett and Snyder's[1] observation of the postnatal development of the 5-HT receptor. However, Uzbekov et al.[9] reported a postnatal decline in the extent of 5-HT receptor binding in various brain regions. The explanation for the discrepancies between the findings of Bennett and Snyder[1] and these of Uzbekov et al.[9] is unclear. Bennett and Snyder[1] expressed their data in terms of brain weight while Uzbekov et al.[9] expressed the 5-HT binding in terms of membrane protein. We therefore considered the possibility that different developmental curves might be the consequence of a differential recovery of brain proteins in the membrane fraction. However, the distribution of total protein between soluble and membrane fractions was consistent across the ages examined. Uzbekov et al.[9] suggested that their declining curve was different from Bennett and

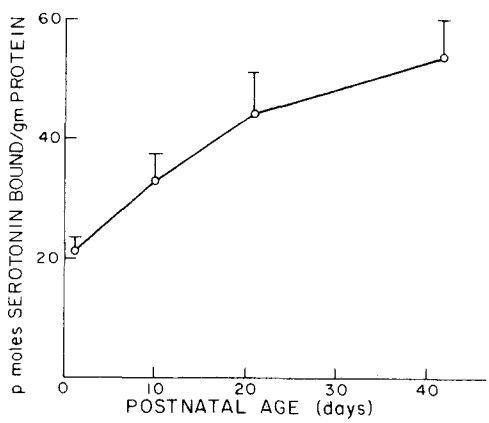

Fig. 1. Development of serotonergic binding capacity in the rat forebrain. Data are means of 6–7 separate analyses. Bars represent standard errors.

Snyder's because finer dissections were examined. While some regional variations undoubtedly exist in receptor maturation, it is not clear how the sum of multiple declining functions can lead to an increasing function.

There were, however, some differences between Uzbekov et al. and our methods. Because Uzbekov et al. used 5-HT of low specific activity (10 Ci/mmol), it was apparently necessary to use as high as 9.3 nM [^{3}H]5-HT. In the present study, we used a higher specific activity serotonin and 3.15 nM for the binding reaction. Our concentration of 3.15 nM is well below the previously reported K_d of binding to the high affinity 5-HT receptor (8 nM) and the value we have found (6.6 nM), but 9.3 nM may be high enough to include a reasonable percentage of binding to the low affinity receptor[7]. Uzbekov et al. used 10^{-5} M 5-HT as cold competitor, as opposed to the 10^{-6} M concentration we used. Thus, they may have used a less rigorous requirement for specific binding than we have used in our measurements. The preliminary low speed spin employed by Uzbekov et al.[9] prior to membrane preparation may have sedimented more synaptic material in the myelinated adult brain than in the neonatal brain. Finally, other variables such as pH, the presence or absence of calcium, and ascorbic acid in the reaction mix also distinguish the two studies.

In summary, 5-HT binding increased during postnatal development. However, newborn receptor densities were already 39% of adult levels. This is particularly high considering that the newborn rat has only around 5% of the presynaptic endings that it will contain as an adult[3]. Whether the early appearance of serotonin binding represents a non-transmitter function for serotonin or early maturation of a population of serotoninergic neurons remains to be determined.

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