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CONCOMITANT DEVELOPMENT OF [^{3}H]-DOPAMINE AND [^{3}H]-5-HYDROXYTRYPTAMINE UPTAKE SYSTEMS IN RAT BRAIN REGIONS

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1 Synaptosomal uptake mechanisms of 5-hydroxytryptamine and dopamine were examined in cerebral cortex, corpus striatum and midbrain plus brainstem of developing rats.

2 In all regions, there was generally a parallel biphasic development of both uptake systems; the most rapid increases occurred in the first two weeks postpartum, followed by a slower rate of increase.

3 Kinetic studies with dopamine indicated that the maturation involved increases in maximal uptake without a change in the substrate K_m, suggesting that there is a change in the number of terminals but not in the uptake system *per se*.

Introduction

Fluorescence histochemistry indicates that central monoamine neurones appear early in gestation in the rat and mouse (Loizou, 1972; Golden, 1972; 1973; Lauder & Bloom, 1975). Biochemical studies also have provided evidence for the presence of monoamine neurones early in development (Baker & Quay, 1969; Coyle & Axelrod, 1972; Coyle & Hendry, 1973). The cell body groups appear to be fully developed at birth, whereas the arborizations of the nerve terminal projections occur primarily postnatally (Loizou, 1972). This increase in monoamine nerve density can be monitored by measuring the *in vitro* uptake or radiolabelled monoamines in brain tissue homogenates (Coyle & Axelrod, 1971; Jonsson, 1976; Nomura, Naitoh & Segawa, 1976; Kirksey, Seidler & Slotkin, 1978), and the developmental pattern for [^{3}H]-noradrenaline uptake in rat brain has been well established (Coyle & Axelrod, 1971). Investigations have also been made of [^{3}H]-dopamine and [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake in neonate and adult brain homogenates or synaptosomes. Although density of both types of neurones has been reported to increase during postnatal development (Nomura *et al.*, 1976), no direct interactions seem to occur between the maturing monoaminergic systems; chemical ablation of catecholaminergic systems does not appear to influence ontogeny of 5-hydroxytryptaminergic neurones, nor does ablation of the latter adversely affect outgrowth of catecholamine systems (Jonsson, Pycock, Fuxe & Sachs, 1972; Jonsson, 1976).

The present study was undertaken to establish the ontogenetic pattern of dopaminergic and 5-hydroxytryptaminergic terminal development in rat brain regions in order to provide a definitive description of the parallelism (or lack thereof) in maturation of the two systems. The uptake of tritiated amine into synaptosomal homogenates was used as an index of innervation. The kinetics of dopamine uptake have also been determined to ascertain whether synaptosomal maturation entails only changes in numbers of terminals or whether alterations also occur in the affinity of the uptake system for substrate.

Methods

Preparation of tissues

Timed pregnant Sprague-Dawley rats and adult rats (175 to 200 g) were obtained from Zivic Miller Laboratories. The pregnant rats were housed in separate breeding cages and allowed food and water *ad libitum*. Following parturition, the neonates were distributed randomly and maintained at 8 to 10 pups per litter. Adult and developing rats were killed by decapitation, and the corpus striatum, midbrain plus brainstem and cerebral cortex dissected according to the method of Glowinski & Iversen (1966). In studies at early points in development, tissues from several animals were pooled to obtain sufficient material for analysis.

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The fresh brain tissues were then weighed and homogenized in 10 volumes (w/v) of 0.3 M sucrose (pH 7.4) in a glass homogenizer fitted with a Teflon pestle (Kontes). Following centrifugation at 1000 *g* for 15 min, the sediment was discarded, the supernatant was diluted four fold with sucrose and the diluted suspension used in subsequent uptake experiments.

Tritiated amine uptake

Aliquots of the brain homogenates (100 µl) containing 2.5 mg of tissue were added to 0.9 ml of modified Krebs-Henseleit medium containing final concentrations of 1.25 µM iproniazid, 2 µM ascorbic acid and 50 nM [^{3}H]-dopamine (New England Nuclear, 19.6 Ci/mmol) or 50 nM [^{3}H]-5-hydroxytryptamine (New England Nuclear, 25.8 Ci/mmol). The low substrate concentrations were chosen because earlier studies concentrations were chosen because earlier studies have shown that dopamine can enter 5-hydroxytryptaminergic terminals and 5-HT can enter catecholaminergic terminals if micromolar concentrations are used (Snyder, Kuhar, Green, Coyle & Shaskan, 1970). Samples were incubated for 5 min at 37°C, while duplicate samples were maintained on ice to serve as blanks. Uptake was stopped effectively by placing the tubes on ice and adding 3 ml of ice-cold Krebs-Henseleit medium. This was followed by rapid vacuum filtration on cellulose acetate filter paper, pore size 0.2 µm (Gelman); the papers containing the labelled particulate fraction were then washed twice with 3 ml of Krebs-Henseleit medium, placed in a scintillation vial containing 10 ml of fluor and counted. Samples incubated at 0°C and carried through the procedure served as blanks. Active uptake was defined as the difference between uptake at 0°C and uptake at 37°C. For kinetic determinations, the tritiated amines were diluted isotopically with either unlabelled dopamine (Calbiochem) or 5-HT (Sigma) so as to achieve substrate concentrations in the range of 50 to 400 nM.

All data are given as mean values and standard errors. In double reciprocal plots, straight lines are fitted by least-squares analysis and intercepts compared by the two-tailed Student's *t* test.

Results

Development of brain region weight, [^{3}H]-dopamine and [^{3}H]-5-hydroxytryptamine uptake

Over the course of development, cerebral cortex wet weight increased six fold, from about 100 mg at one day postpartum to 700 mg at approximately 6 weeks of age, at which point weights were indistinguishable from those of adult (50 to 60 day old) rats (Figure 1a). The greatest rate of increase in weight was seen in the first 2 weeks of development. The developmental

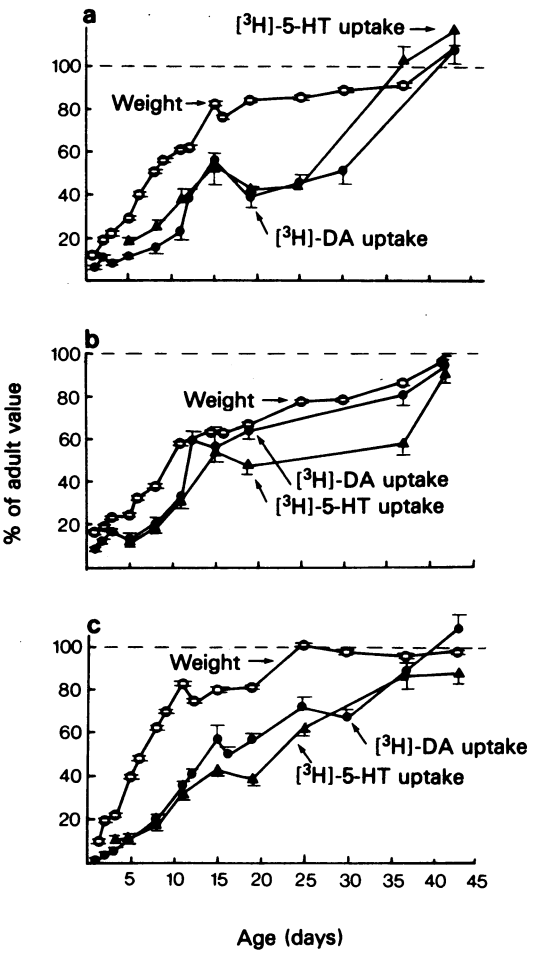

Figure 1 Development of synaptosomal uptake of dopamine and 5-hydroxytryptamine (5-HT) in brain regions. Data are plotted per brain region. In cerebral cortex (a) adult [^{3}H]-dopamine ([^{3}H]-DA, ●) uptake was 0.34 ± 0.02 nmol/g wet wt. or 0.25 ± 0.01 nmol/region, adult [^{3}H]-5-HT (▲) uptake was 0.47 ± 0.04 nmol/g wet wt. or 0.30 ± 0.03 nmol/region and adult tissue wet wt. (○) was 0.69 ± 0.02 g. In midbrain plus brainstem (b), adult [^{3}H]-dopamine (●) uptake was 0.36 ± 0.02 nmol/g wet wt. or 0.18 ± 0.01 nmol/region, adult [^{3}H]-5-HT (▲) uptake was 0.61 ± 0.02 nmol/g wet wt. or 0.45 ± 0.02 nmol/region, and adult tissue wet wt. (○) was 0.76 ± 0.02 g. In corpus striatum (c) adult [^{3}H]-dopamine (●) uptake was 2.5 ± 0.1 nmol/g wet wt. or 0.28 ± 0.01 nmol/region, adult [^{3}H]-5-HT (▲) uptake was 0.90 ± 0.05 nmol/g wet wt. or 0.102 ± 0.006 nmol/region and adult tissue wet wt. (○) was 0.108 ± 0.002 g. [^{3}H]-dopamine and [^{3}H]-5-HT concentration in the medium was 0.05 µM. Points and bars represent means and standard errors of 5 to 6 determinations. Adult values are from 30 animals.

curves for [^{3}H]-dopamine and [3H]-5-HT uptake were similar, with both systems demonstrating a biphasic characteristic with the most rapid increases occurring in the first 2 weeks; with both, maturity was reached by approximately 6 weeks of age.

In the midbrain plus brainstem (whole brain minus cerebellum, corpus striatum and cerebral cortex), tissue wet weight increased from 120 mg at one day postpartum to 750 mg at 6 weeks of age, the latter weight not being statistically different from adult values (Figure 1b). [^{3}H]-dopamine uptake in the midbrain plus brainstem increased from 0.015 nmol/region on day 1 to 0.17 nmol/region on day 43. Uptake of [^{3}H]-5-HT was 0.12 nmol/region on day 3 and also reached adult levels (0.43 nmol/region) by day 43. Again, development of both [^{3}H]-dopamine and [^{3}H]-5-HT uptakes were biphasic with the most rapid increase occurring in the first 14 days postpartum and reached levels of uptake indistinguishable from adult animals by 6 weeks of age. However, there were noticeable differences in the two uptakes during the late phase of development, with dopamine uptake generally higher than 5-HT uptake.

Tissue weights of corpus striata from developing animals reached adult levels at approximately 4 weeks of age, increasing from 12 mg on day 1 postpartum to 107 mg on day 43 (Figure 1c). [^{3}H]-dopamine uptake in striatal homogenates increased 15 fold over the course of development from 1 to 43 days of age and [^{3}H]-5-HT uptake increased eight fold from 5 days of age to adulthood. The same characteristics were found as with cerebral cortex: parallel biphasic development of the two transmitter systems and achievement of adult levels by 6 weeks of age.

Kinetics of [^{3}H]-dopamine uptake

Uptake in the cortex was saturable at all ages tested (Figure 2). The K_m values in the neonatal animals did not differ significantly from the K_m (200 nM) observed in adult cerebral cortex. In contrast, the maximum uptake increased significantly with maturation from 0.67 ± 0.02 nmol/g wet wt. at day 6 to 0.90 ± 0.10 nmol/g at day 23, and to 1.6 ± 0.2 nmol/g in adults.

Uptake of [^{3}H]-dopamine in striatal homogenates of developing animals was also saturable at all ages examined (Figure 3). The maximal uptake increased from 1.6 ± 0.1 nmol/g at day 2 to 11.0 ± 1.0 nmol/g in adult animals, while the K_m values in developing animals were not significantly different from the adult value (200 nM).

The maximum uptake value of [^{3}H]-dopamine into homogenates of the striatum was seven fold greater than that observed in the cerebral cortex homogenates; the two regions displayed no significant differences in K_m.

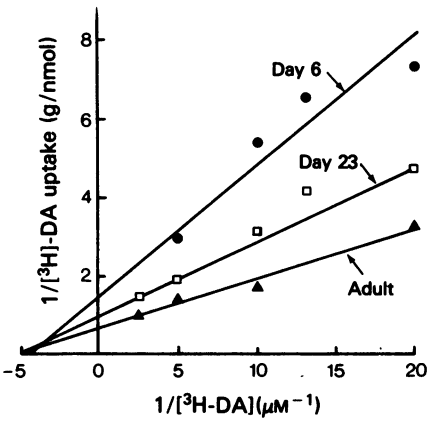

Figure 2 Kinetics of uptake/g of [^{3}H]-dopamine in cerebral cortex of developing rats. Points represent means of 5 to 6 determinations.

Age	K_m (*nM*)	*Maximum uptake (nmol/g wet wt.)*
Day 6	220 ± 50	0.67 ± 0.02*
Day 23	180 ± 20	0.92 ± 0.12*
Adult	200 ± 20	1.60 ± 0.20

* Uptake significantly different from the adult value, $P < 0.05$ or better.

Discussion

Measurement of tritiated amine accumulation in tissue homogenates was used in this study to quantitate ontogenetic changes in the relative number of monoamine nerve terminals. In the first few days postpartum, the ability of the cerebral cortex, midbrain plus brainstem, and corpus striatum homogenates to accumulate actively [^{3}H]-dopamine and [^{3}H]-5-HT per brain region was approximately 10% of that of the adult regions. During the subsequent postnatal maturation, the two uptake processes increased in similar fashions, achieving values indistinguishable from adults by 5 to 6 weeks of age. At all ages examined, the uptake of [^{3}H]-dopamine in the homogenates of cerebral cortex and corpus striatum displayed saturation kinetics with no significant changes in K_m. In contrast, the maximum uptake increased significantly with maturation. Similar observations have been made in kinetic studies of [^{3}H]-noradrenaline (Coyle & Axelrod, 1971) and [^{3}H]-5-HT (Nomura *et al.*, 1976) uptake development; neonatal K_m values were indistinguishable from adult values, while the maximal uptakes in adults were consistently greater than the neonatal values. These data suggest that the observed developmental changes in mono-

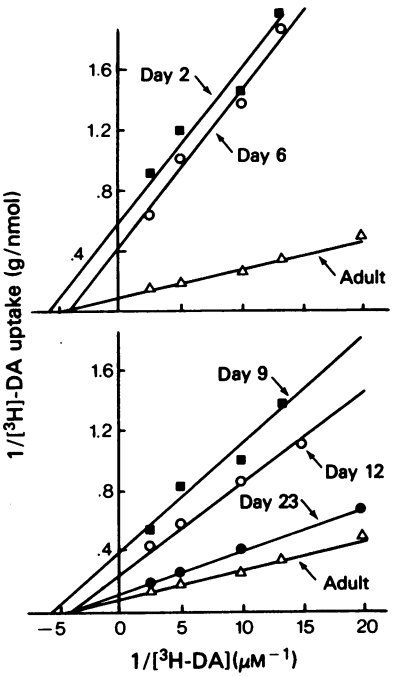

Figure 3 Kinetics of uptake per g of [^{3}H]-dopamine in corpus striatum of developing rats. Points represent means of 4 to 6 determinations.

Age	K_m (*nM*)	*Maximal uptake* (*nmol/g wet wt.*)
Day 2	160 ± 10	1.6 ± 0.1*
Day 6	280 ± 50	2.4 ± 0.4*
Day 9	150 ± 10	2.3 ± 0.2*
Day 12	232 ± 5	3.8 ± 0.1*
Day 23	240 ± 10	8.4 ± 0.4
Adult	200 ± 20	11.0 ± 1.0

* Uptake significantly different from adult, $P < 0.05$ or better.

amine synaptic uptake systems represent primarily an increase in number of nerve endings or in the number of uptake sites per ending, while the substrate affinities of the uptake systems are relatively unchanged (Coyle & Axelrod, 1971; Nomura *et al.*, 1976; Kirksey *et al.*, 1978).

The time course of development obtained here for [^{3}H]-dopamine uptake is in close agreement with histofluorescent microscopic studies of the development of dopaminergic innervation in the rat; patches of fluorescent terminals present at birth become progressively intense and approach adult levels by 4 to 5 weeks (Loizou, 1972; Olson, Seigler & Fuxe, 1972). A similar pattern was also found for developmental increases in levels of dopamine and 5-HT in various brain regions measured chemically (Nomura *et al.*, 1976). Furthermore, tyrosine hydroxylase, the rate-limiting enzyme in the synthetic pathway for dopamine, is present in the neonatal striatum with a specific activity about 10% of that of the adult; the activity attains 75% of adult levels by 4 weeks of age (Coyle & Campochiaro, 1976). The correlation between these previous studies and the present results would suggest that the observed increases in tritiated monoamine uptake quantitively reflect alterations in the number of nerve terminals, rather than in uptake per terminal.

In cerebral cortex and corpus striatum, dopaminergic and 5-hydroxytryptaminergic developmental patterns of synaptic uptake were parallel and statistically indistinguishable from each other in terms of percentage of adult value. Both synaptosomal uptakes exhibited a biphasic characteristic with rapid proliferation occurring from 0 to 14 days of age, a developmental stage of the rat brain termed the 'critical period' (McIlwain, 1966) and marked by increased axonal and dendritic growth and active myelin formation. In terms of behavioural development, the rapid phase of synaptic outgrowth appears to be the most significant period in ontogeny, as a number of patterns of behaviour become mature at the end of this period (Jacobs, 1976; London & Buterbaugh, 1978). However, there are some differences between behavioural and biochemical results. Experiments using electroshock-induced convulsions have suggested that catecholaminergic influences may be present early in development, prior to influences of the 5-hydroxytryptaminergic system (London & Buterbaugh, 1978). This is not necessarily inconsistent with the present finding that dopamine and 5-HT presynaptic terminals form at about the same time, since postsynaptic factors (such as receptor number or sensitivity or responsiveness of postsynaptic cells) may also be involved in ontogeny of behavioural responses. It also should not be overlooked that a particular behavioural response may involve development of only a small proportion of the total number of terminals in a given region.

The initial, rapid developmental phase of dopamine and 5-HT uptake was followed in all three regions by a slower rate of increase in the next 3 to 4 weeks postpartum. While there were only small differences between dopamine and 5-HT uptake in cerebral cortex and corpus striatum in this late developmental phase, in midbrain plus brainstem (an area rich in 5-HT cell bodies) a consistent difference was seen, with dopamine uptake tending to mature earlier than 5-HT uptake. This indicates that some regional differences in development may exist.

In conclusion, although there are some regional differences, there is generally a parallel biphasic synaptogenesis of dopamine and 5-HT systems. The develop-

mental increases in neurotransmitter uptake appear to reflect changes in the numbers of terminals, rather than in the affinities of the uptake systems. While these two transmitter systems may not directly influence each other's development, the striking uniformity of their maturational patterns indicates the possibility that a common ontogenetic event or series of events may trigger the outgrowth of both dopaminergic and 5-hydroxytryptaminergic neurones.

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