Pharmacology Biochemistry & Behavior, Vol. 21, pp. 321–323, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

BRIEF COMMUNICATION

Differential Effects of d-Amphetamine and Scopolamine on the Ontogeny of Rearing[1]

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Received 23 September 1983

BAUER, R. H. *Differential effects of d-amphetamine and scopolamine on the ontogeny of rearing.* PHARMACOL BIOCHEM BEHAV 21(2) 321–323, 1984.—Although rearing is ontogenetically an important behavior, very little is known about the neural bases of rearing. The role development of catecholaminergic and cholinergic neurons play in the ontogeny of rearing was investigated by examining rearing in infant, adolescent, and adult rats following various doses of d-amphetamine (an indirectly acting catecholaminergic agonist) and scopolamine (a cholinergic muscarinic receptor antagonist). d-Amphetamine increased rearing in infants but not in adolescents and adults. These findings suggest that activation of catecholaminergic neurons increases rearing in infants but not in adolescents or adults. Scopolamine increased rearing in adolescents and adults but not in infants, indicating that blocking transmission of cholinergic neurons increases rearing in only older rats.

Scopolamine d-Amphetamine Development Acetylcholine Catecholamines Norepinephrine

IN altricial mammals, rearing appears quite late in development. For example, in the rat rearing first occurs about 15 days after forward locomotion begins [6] and in human infants standing alone occurs about 180 days after the start of crawling [13]. In mammals rearing on the hind legs increases the likelihood of visually detecting distant objects and frees the forepaws for manipulation of objects. In addition, rearing is thought to be an evolutionary forerunner of bipedal locomotion [1]. Although rearing is an ontogenetically and a phylogenetically important behavior, very little is known about the neural bases of rearing.

In the rat, rearing first appears at about 15 days of age, reaches a peak at about 35 days of age, and declines slightly in adults [2, 4, 6]. At least in rats, histochemical and biochemical evidence indicates that the development of neurotransmitter systems and rearing occur at concomitant rates. Axonal growth, enzymes, synaptic vesicles, and other processes associated with catecholaminergic and cholinergic neurons exhibit a caudal-rostral developmental pattern. In the lower brain stem of the rat, cell bodies of catecholaminergic neurons appear to be developed at birth and as the animal grows older, axons from these cell bodies grow in a rostral direction and innervate successively higher structures. Brain stem cholinergic neurons develop about 2 weeks after birth. In the midbrain, maturation of noradrenergic, dopaminergic, and cholinergic neurons is thought to occur at about 15-, 20-, and 40-days of age, respectively, and maturation of cortical noradrenergic, dopaminergic, and cholinergic neurons occurs at about 30-, 40-, and 50-days of age, respectively [7, 9, 10, 11].

In adults, cholinergic and catecholaminergic neurons are known to be involved in a wide variety of behaviors, such as locomotor activity, arousal, and avoidance behavior [2, 3, 4, 5, 12, 14]. Furthermore, drugs, such as scopolamine (a muscarinic receptor antagonist) and amphetamine (an indirectly acting catecholamine agonist), which act on these neurons are known to have differential effects on locomotor activity, arousal, and avoidance in immature and mature rats [2, 3, 4, 5, 14]. In accordance with the more rapid development of catecholaminergic neurons than cholinergic neurons, amphetamine alters behavior at a younger age than scopolamine but after about 20 days of age the behavioral effects produced by these drugs are comparable [2, 3, 4, 5].

Since cholinergic and catecholaminergic neurons and rearing in the rat develop during a comparable age, and these neurons are involved in a wide variety of behaviors, cholinergic and catecholaminergic neurons may be involved in the development of rearing. Unlike other behaviors, however, it appears that scopolamine and amphetamine have opposite developmental effects on rearing in rats. That is, scopolamine does not alter rearing of 15- and 17-day-old rats but gradually increases rearing from 17 to 90 days of age [4]. At least with low doses, amphetamine increases rearing maximally in 15- and 17-day-old rats, but the effects of amphetamine on rearing gradually decrease with development, until at 90 days of age amphetamine does not alter rearing

[1]This research was supported by Mental Health Grants AG-00162 ahd HD-05615.

[2]. Thus, when the effects of amphetamine on rearing are declining with age the effects of scopolamine on rearing are increasing with age. However, rats in these rearing studies were obtained from different sources and the studies were conducted a number of years apart. Therefore, these factors could be responsible for the differential developmental effects of scopolamine and amphetamine on rearing. The finding that developmental effects of scopolamine and amphetamine on a number of behaviors are comparable but the developmental effect of these drugs on rearing differ suggests that the differential effects of scopolamine and amphetamine on rearing during development may be spurious.

The major purpose of the present study was to further examine the effects of various doses of d-amphetamine and scopolamine on rearing in rats of different ages. Since d-amphetamine increases the release and reduces the reuptake of catecholamines [12,14] and scopolamine blocks cholinergic receptors [8], results of the present study are expected to provide information concerning the role of catecholaminergic and cholinergic neurons in the development of rearing.

METHOD

Subjects, Apparatus, and Procedure

The subjects were 15-, 17-, 21–22-, 36–37-, and 90–100-day-old experimentally naive, male, Sprague-Dawley rats. Rats younger than 15 days of age were not tested because prior to this age their eyes are closed. The rats had free access to Purina Rat Chow and water throughout the experiment. Light onset and offset in the colony room were at 06.00 and 20.00, respectively.

The apparatus has been described in greater detail elsewhere [2,4]. Briefly, the apparatus consisted of five Plexiglas chambers with dimensions that varied according to the approximate spine length of each age. The reasons for equating the apparatus size with the size of the animals have been discussed previously [2,3]. The oldest age was tested in a 45×45×45-cm box; the floor was aluminum and aluminum sheets were attached to the inside walls, 13.0 cm above the floor. The apparatus dimensions for younger rats were reduced as follows: 15-day-olds, 61%; 17-day-olds, 57%; 21-day-olds, 48%; 36-day-olds, 33%. With these dimensions, the animals were required to lift both front feet off the floor to complete the circuit between the metal floor and metal walls. Rearing (completing the circuit between the metal floor and walls) was recorded on counters. Each activity chamber was placed inside a sound resistant box with a fan mounted on the outside wall to provide ventilation and mask extraneous sounds.

The rats were weighed and given an intraperitoneal injection of either physiological saline or 0.5-, 1.0-, 4.0-, 8.0-, or 16.0-mg/kg of scopolamine hydrobromide or d-amphetamine sulfate (n=11 per each drug, dose, and age group). The drug solutions were coded, so the experimenter did not know the drug or dose being injected until the experiment was complete. Rearing was recorded for 1 hour, starting immediately after the injection. After each animal was tested the apparatus was cleaned with a damp paper towel. Other procedures have been described previously [3,4].

RESULTS AND DISCUSSION

The effects of d-amphetamine on rearing in rats of different ages are shown in the upper panel of Fig. 1. As can be seen, low doses of d-amphetamine increased rearing in the three youngest ages but had little effect in older rats. A 5 (age) × 6 (doses of d-amphetamine) complete factorial analysis of variance on the number of rearing responses showed that rearing changed with age, $F(4,300)=19.72$, $p<0.001$ and dose, $F(5,300)=22.49$, $p<0.001$ (for all statistics reported, the criterion for significance was $p<0.05$). A significant age × dose interaction, $F(20,300)=3.79$, $p<0.001$ and inspection of the upper panel of Fig. 1 shows that the dose-response curves differed as a function of development. Separate one-way analysis of variance on the number of rearing responses of each age revealed that d-amphetamine significantly increased rearing in 15-, 17-, and 21-day-old rats, $Fs(5,60)=4.02$, 4.37, and 4.92, respectively, $p<0.001$ but decreased rearing in 36- and 90-day-olds, $Fs(5,60)=3.62$ and 3.59, respectively, $p<0.01$.

Tukey's test was used to compare the mean number of rearing responses of different dose groups within each age. The two youngest ages given 0.5 and 1.0 mg/kg made more rearing responses than their same aged counterparts given other doses. The 21-day-old rats given 0.05-, 1.0-, and 4.0-mg/kg made more responses than animals of this age given

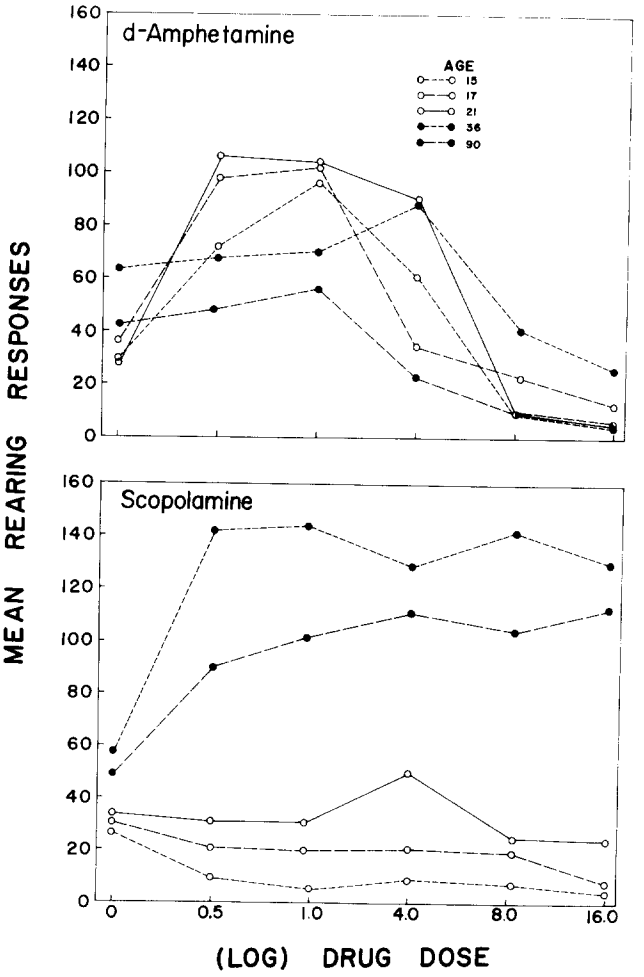

FIG. 1. Mean number of rearing responses for five different ages as a function of d-amphetamine and scopolamine dosage.

other doses. The 36 and 90-day-old rats given the highest dose made significantly fewer rearing responses than the same ages given saline and the two lowest amphetamine doses.

The age-dependent effects of drugs can best be understood in terms of the development of central neurons and the action of drugs on these neurons [2, 3, 4, 5]. As indicated above, catecholaminergic cell bodies in the lower brain stem appear to be fully developed at birth and, as the animal grows older, axons from these cell bodies grow in a rostral direction and innervate successively higher structures [11]. Lower d-amphetamine doses (<4.0 mg/kg) are thought to produce behavioral changes by increasing the release and reducing the re-uptake of norepinephrine at synapses [12,14]. Since low d-amphetamine doses increase rearing in only rats of 21 days of age and younger and at this age norepinephrine neurons have not yet innervated the cortex, amphetamine may increase rearing in young rats by acting in noncortical structures, possibly the brain stem or midbrain. Amphetamine no longer increases rearing about the age that norepinephrine neurons innervate the cortex, i.e., 36-days of age, suggesting that the release of norepinephrine in cortical neurons inhibits lower centers which are involved in rearing. Higher d-amphetamine doses produce a variety of stereotyped behaviors that are incompatible with rearing and these behaviors are thought to be due to the increased release and reduced re-uptake of dopamine [12,14]. Therefore, higher d-amphetamine doses may reduce rearing in all ages by acting on dopaminergic neurons.

Figure 1 shows that scopolamine produced virtually no change on rearing in the three youngest ages but increased rearing in the two oldest groups. A 5 (age) × 6 (dose) analysis of variance showed that the number of rearing responses increased with age, $F(4,300)=31.31$, $p<0.001$ and dose, $F(5,300)=7.29$, $p<0.001$. The age × dose interaction was significant, $F(20,300)=2.49$, $p<0.01$, indicating that the effect of scopolamine depends on the age of the animals. Furthermore, separate one-way analysis of variance on each group showed that scopolamine increased rearing in 36- and 90-day-olds, $Fs(5,60)=2.49$ and 2.83, respectively, $p<0.03$ but did not alter rearing in the three youngest groups. Tukey's test showed that 36- and 90-day-old rats given scopolamine made more rearings than the same age saline controls.

Scopolamine produces behavioral changes by blocking transmission across cholinergic synapses [8]. Since scopolamine does not alter rearing in rats until about the age that cholinergic neurons develop in the midbrain, reduced transmission across midbrain cholinergic neurons may be involved in the ontogeny of rearing.

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