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Effects of Serotonergic Agonists and Antagonists on the Locomotor Activity of Neonatal Rats[1]

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LUCOT, J B AND L S SEIDEN *Effects of serotonergic agonists and antagonists on the locomotor activity of neonatal rats* PHARMACOL BIOCHEM BEHAV **24**(3) 537–541, 1986 —The locomotor activity of neonatal rats was measured after treatment with serotonin agonists or antagonists Treatment with the serotonin agonists 5-hydroxytryptophan or quipazine resulted in the elimination of the peak in activity which normally results from increases in activity from days 10 to 15 of life followed by decreases from days 15 to 20 of life The drug-induced decreases in activity occurred at doses that did not alter locomotor activity after day 17, when most of the peak in activity had passed The dose of 5 mg/kg of the serotonergic antagonist methysergide eliminated the peak in activity without changing locomotor activity after the peak had passed The antagonists methiothepin and cinanserin only produced decreases in locomotor activity which did not appear to be related to the peak in activity The serotonergic agonist data are compatible with the hypothesis that the development of the serotonin system contributed to the inhibition of locomotor activity The methiothepin and cinanserin data neither confirm nor dispute the hypothesis, as their effects may have been either nonserotonergic or on serotonin receptors that were different than those acted on by the agonists

Serotonergic drugs Neonatal rats Locomotor activity Neonatal development

NEONATAL rats removed from the nest display increases in levels of locomotor activity from day 10 to about day 15 of life, after which the activity declines until about day 20 [8, 9, 26] One factor which may contribute to the decrease in locomotor activity after the peak is the development of the serotonin system [24] The binding of LSD and serotonin to serotonin receptors and of spiroperidol to 5-HT_2 receptors increases rapidly between days one and 14 after birth and plateaus by day 21 [3,7], while the response of cAMP to serotonergic stimulation rises rapidly between days one and seven before declining to day 14 [11, 30, 31] These observations suggest that some developmental factor diminishes receptor supersensitivity as measured by cAMP response even as the number of receptors increases The developmental factor is probably the formation of active synapses, since there is both a rapid proliferation of serotonin terminals, which starts between days seven and 14 and continues to day 21 [20], and an increase in the serotonin terminal functions of uptake and accumulation, which rises between days seven and 21 [29] These correspond with the rapid formation of synapses as seen by electron microscopy [1] Serotonin levels increase linearly from day seven to day 21 [2,25], slightly in advance to the rise in tryptophan hydroxylase from day 10 to day 30 [12], suggesting that increased storage capacity for serotonin also develops rapidly. Functionally, regulatory feedback inhibition of serotonin synthesis is not evident until day 14 [5] Thus, there is a development of functional serotonin synapses between days 14 and 21 and this development corresponds with the decline in locomotor activity of isolated neonatal rats

The role of serotonin in the decline in activity was inferred from the ontogeny of the locomotor increasing effects of the tryptophan hydroxylase inhibitor, p-chlorophenylalanine [24] Support for the inhibitory role of serotonin was provided when permanent depletions of serotonin in rat pups neonatally treated with the serotonin neurotoxin, 5,7-dihydroxytryptamine, produced increases in locomotor activity when measured on day 14 [6] and shifted the peak in locomotor activity to after day 15 [23] However, daily measures of locomotor activity in rat pups with consistent but reversible alterations in serotonin functioning, to avoid the development of denervation supersensitivity, are necessary to ascertain the role of serotonin in regulating locomotor activity in the neonatal rat

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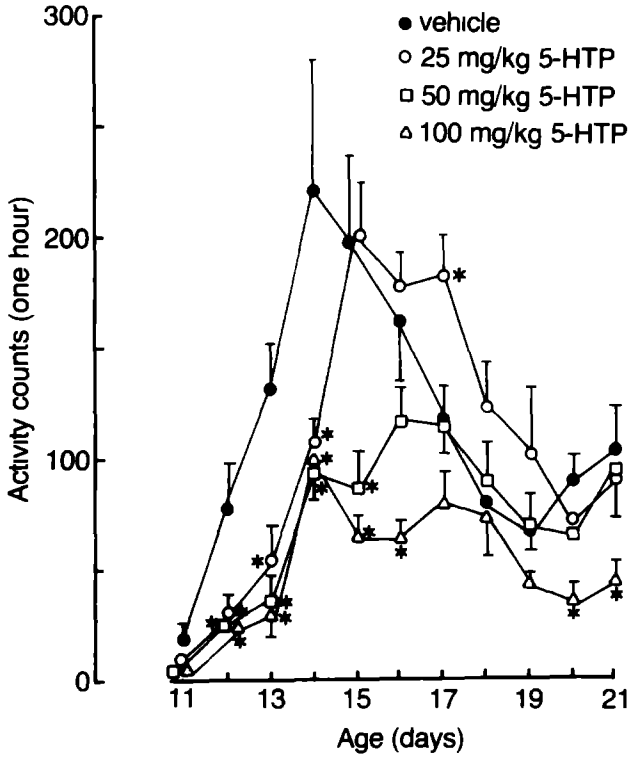

FIG 1 The effects of 5-HTP on the locomotor activity of rat pups Activity was counted for one hour on each day from 11 to 21 days of age All points are the mean of six pups Pups were injected days 12 to 21 Brackets extend 1 S E $*p<0.05$

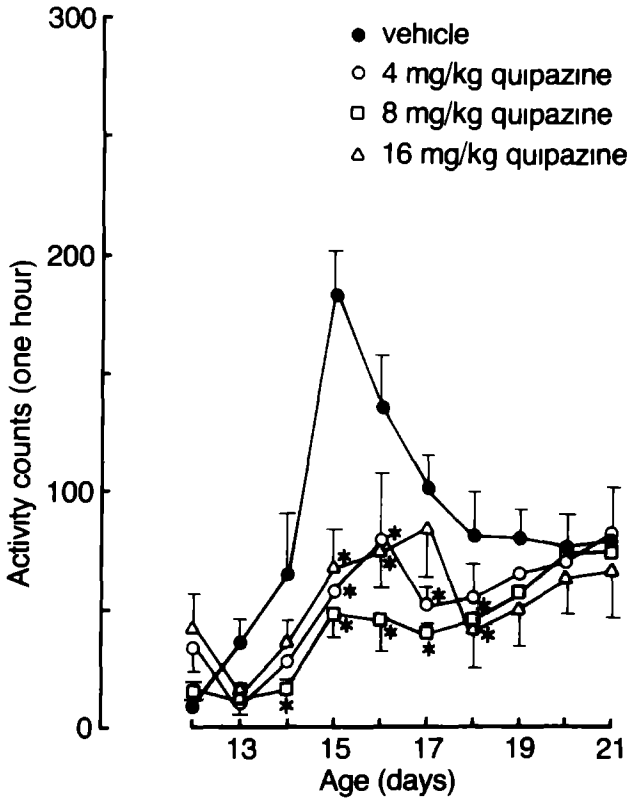

FIG 2 The effects of quipazine on the locomotor activity of rat pups Activity was counted for one hour on each day from 12 to 21 days of age All points are the mean of six pups Pups were injected days 13 to 21 Brackets extend 1 S E $*p<0.05$

The serotonergic regulatory mechanism involved may be the same as the ascending serotonergic system that inhibits locomotor activity in an unfamiliar environment [16] since a novel environment is necessary to elicit the hyperactivity in neonatal rats [9,26] The ascending inhibitory serotonin system is to be differentiated from the descending excitatory system that underlies the serotonin-stereotypy syndrome [16] The serotonin-stereotypy syndrome is produced at doses far higher than used to affect locomotor activity [10,17] and relies exclusively on midbrain and spinal serotonergic function [18]

In the present study, the role of serotonin in the regulation of locomotor activity during the peak in locomotor activity was examined using the 5-HT_1 and 5-HT_2 agonist, quipazine [4], the synthesis precursor of 5-HT (the ligand for 5-HT_1 receptors [19]), 5-hydroxytryptophan (5-HTP), the mixed 5-HT_1 and 5-HT_2 antagonist methysergide and the 5-HT_2 antagonists cinanserin and methiothepin [19]

METHOD

Sprague-Dawley rats 14 days pregnant were obtained from the Holtzman Company (Madison, WI) Three days after birth, the pups were sexed and the males were randomly assigned to mothers in litters of eight Litters were housed individually in cages with Sanicel® animal bedding The quarters were illuminated between 0700 and 1900 hr and maintained at 21±1°C

Animals were tested in 12 wire mesh stabilimeters which were 20 5×10×10 5 cm and which tilted on a central axle The stabilimeters were enclosed in a sound attenuating chamber equipped with a fan and a 28 V houselight The illuminated one hour sessions were conducted between 0700 and 0900 hr The sessions were timed and the data collected by a PDP/8e computer [13] Each drug was tested in a separate series of locomotor activity measurements Thus, 24 pups were tested in two groups of 12 for each drug, with each drug tested separately Litters were randomly assigned to receive either vehicle injections or injections of one dose of one drug

Pups were tested daily beginning on day 11 or 12 of life Six individuals from each litter were randomly assigned to different test chambers and times of day for the duration of each experiment to minimize differences between test chambers and between different times of the day Beginning on day 12 for 5-HTP, day 13 for quipazine and day 14 for the antagonists, drug or saline was injected before the start of the test session Injections of the antagonists were ended on day 20, while the agonists were injected until the end of the experiment

Drugs used were 5-HTP, methysergide maleate, methiothepin maleate, cinanserin HCl and quipazine maleate All drugs were dissolved in physiological saline such that the injection volume was 1 ml/100 g Injections were intraperitoneal, 20 min prior to the start of the activity session All doses are expressed as the salt

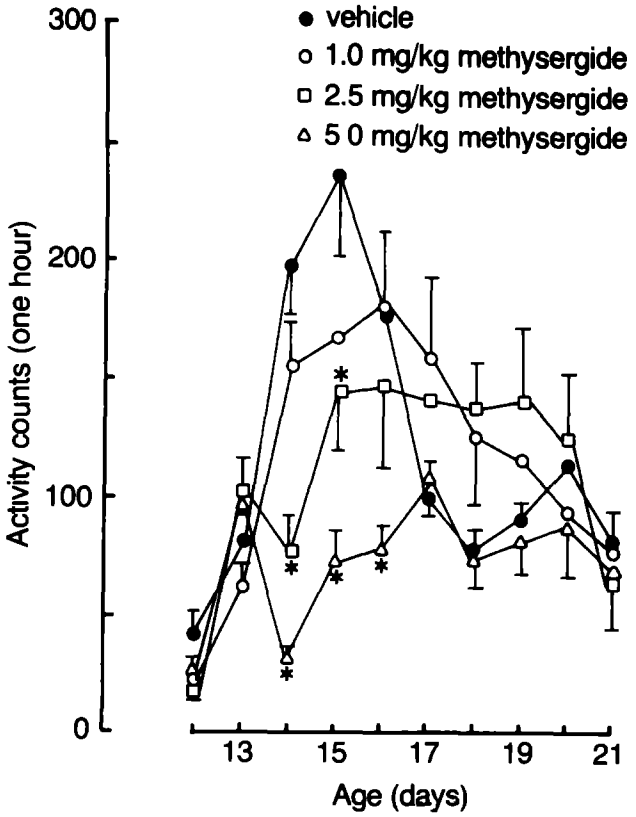

FIG 3 The effects of methysergide on the locomotor activity of rat pups Activity was counted for one hour on each day from 12 to 21 days of age All points are the mean of six pups Pups were injected days 14 to 20 Brackets extend 1 S E *$p<0$ 05

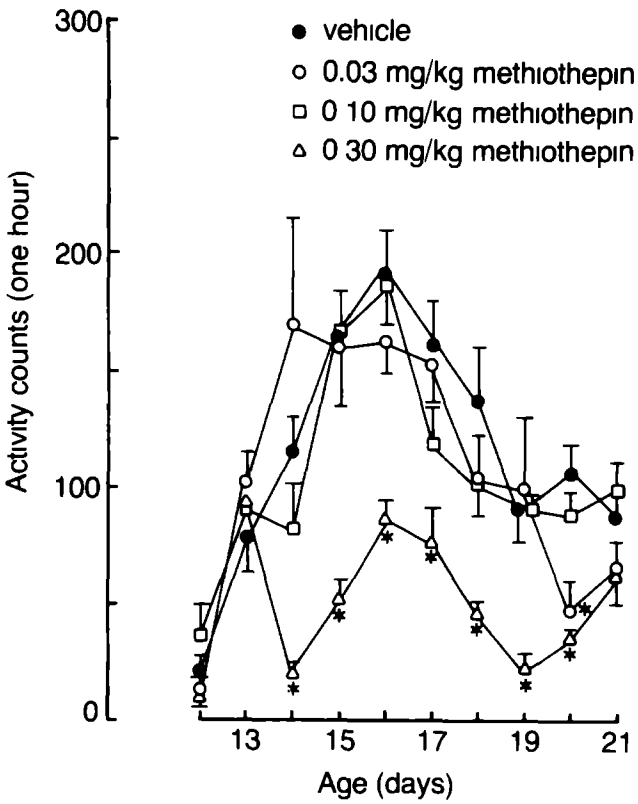

FIG 4 The effects of methiothepin on the locomotor activity of rat pups Activity was counted for one hour on each day from 12 to 21 days of age All points are the mean of six pups Pups were injected days 14 to 20 Brackets extend 1 S E *$p<0$ 05

Data were analyzed by a two-way analysis of variance on repeated measures Significant between group results were analyzed by Duncan's test and differences from control levels of activity were extracted for each day Significance was defined as $p<0$ 05

RESULTS

A two-way analysis of variance with repeated measures on one factor demonstrated a significant drug by day interaction for 5-HTP, $F(20,180)=4$ 35, $p<0$ 001, quipazine, $F(20,160)=3$ 16, $p<0$ 001, methysergide, $F(20,160)=3$ 26, $p<0$ 001, and methiothepin, $F(20,120)=1$ 92, $p<0$ 05, but not cinanserin, $F(20,120)=1$ 46, $p=0$ 1168 However, cinanserin did produce a strong drug effect, $F(3,20)=13$ 67, $p<0$ 001, and the absence of a significant drug by day interaction may have been due to the absence of an obvious graded dose response function

The dose of 25 mg/kg of 5-HTP decreased locomotor activity on days 12 to 14, then produced an increase on day 17 (Fig 1) The dose of 50 mg/kg decreased activity through day 15 The dose of 100 mg/kg decreased activity on all days except days 16 to 19 Thus, the low dose of 5-HTP delayed the rise in activity while the higher doses eliminated the peak altogether Quipazine produced decreases in locomotor activity from days 13 to 17, but not thereafter, thus also eliminating the peak in activity (Fig 2)

Methysergide at the dose of 2 5 mg/kg decreased activity on days 14 and 15, then produced high levels of activity that did not vary between days 15 and 19 (Fig 3). The dose of 5 mg/kg simply decreased activity from days 14 to 16 Methiothepin produced no effects at the doses of 0.03 and 0 1 mg/kg and decreased activity at all days tested (14 to 20) at the dose of 0 3 mg/kg (Fig 4) Cinanserin produced no effects at the dose of 0 3 mg/kg and decreased activity on days 14 to 19 at the dose of 1 mg/kg and decreased activity on days 15 to 20 (except day 18) at the dose of 3 mg/kg (Fig 5)

DISCUSSION

Both serotonin agonists produced decreases in locomotor activity during the peak that occurs when locomotor activity is measured in an unfamiliar environment The agonists did so at doses that did not change activity after the peak had passed and activity had ceased its rapid decline The decrease in activity only during the peak was especially notable with quipazine, which did not decrease activity at any dose tested after day 17 Other developmental differences in the response to quipazine have been recorded Components of the 5-HT syndrome can be elicited in 3–4 day old rat pups at the dose of 5 mg/kg [27], while 50 mg/kg is necessary to elicit the same response in adults [17] These findings are consistent with the reports that serotonin receptors are more sensitive in immature rats [30,31] and that activation of serotonin receptors can decrease locomotor activity [24] It is likely that the serotonin receptors are highly sensitive until

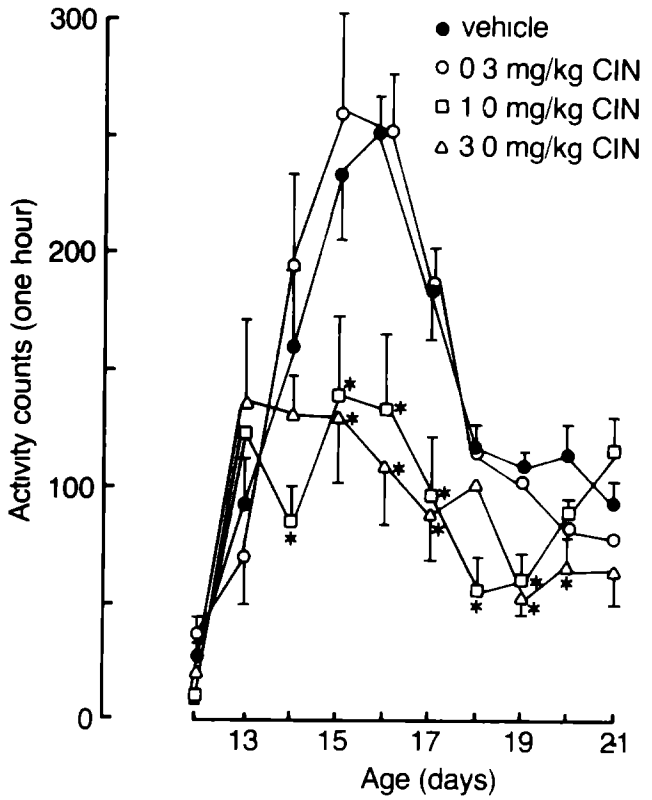

FIG 5 The effects of cinanserin on the locomotor activity of rat pups Activity was counted for one hour each day from 12 to 21 days of age All points are the mean of six pups Pups were injected days 14 to 20 Brackets extend 1 S E $*p<0$ 05

days 15 to 17, when increased serotonin transmission may both decrease the sensitivity of serotonin receptors and begin to decrease locomotor activity The observation that 5-HTP and quipazine share an action on 5-HT_1 receptors and that 5-HT_1 receptors predominate in all brain regions except frontal cortex [4] suggests that this is the receptor type involved

The effects of the antagonists on locomotor activity were more complex than those of the agonists The dose of 2 5 mg/kg of methysergide produced a decrease in activity on day 14 but produced a steady high level of activity on days 15 to 19, although it did not reach significance This brief effect is consistent with the hypothesis that at least the initial part of the decline in activity after the apex of the peak is partly due to the development of the serotonin system [24] However, the dose of 5 mg/kg produced a decrease in activity on days 14 to 16, but not thereafter, an effect similar to that produced by the agonists Both methiothepin and cinanserin produced only decreases in locomotor activity Unlike methysergide and the agonists, however, the decreases in locomotor activity produced by methiothepin and cinanserin continued beyond day 17, and was present at all days until injections ceased on day 21

The decrease in activity produced by serotonin agonists during the peak in isolation-induced locomotor activity suggests that serotonin receptors for the ascending inhibitory serotonergic system are present and that activation of them can decrease locomotor activity The absence of effect by serotonin synthesis inhibition before day 15 [24] suggests that the serotonin neurons are not functionally active enough to stimulate the serotonin receptor and decrease locomotor activity before that age The formation of serotonergic synapses around day 15 begins to exert an inhibition that progressively decreases locomotor activity However the absence of prolonged or significant locomotor increases with methysergide and the observation that neonatal depletion of serotonin with neurotoxins only delays the decline in locomotor activity [22,23] suggests that the serotonergic inhibition is of transient importance The development of cholinergic inhibition slightly after the development of serotonergic inhibition [15] may exert a more important inhibitory effect

The locomotor decreasing effects of the antagonists may have been a result of their blockade of the 5-HT_1 receptors in the spinal cord and medulla that mediate the stereotypy syndrome elicited by extremely high doses of 5-HT agonists [18, 19, 21] The failure of methysergide to decrease locomotor activity after the peak had passed may have resulted from striking a balance between blockade of 5-HT_1 and 5-HT_2 receptors [4] An alternative is that the decreases in locomotor activity by the serotonin antagonists may be a consequence of blocking dopamine receptors Depending on the measure, methiothepin and cinanserin may be more potent or equipotent at dopaminergic receptors compared to serotonergic receptors, while methysergide binds more avidly to serotonergic than to dopaminergic receptors [14,28] It is possible that the antagonists decreased locomotor activity during the peak in activity by blocking the dopaminergic systems that develop before serotonergic systems and which are proposed to underlie the rise in activity from days 12–15 [24] Methysergide would then have produced less of a decrease in locomotor activity based on its greater potency at serotonergic sites than at dopaminergic sites It is not possible at this time to determine which mechanism was responsible for the effects of the antagonists

It has been shown that the increase in locomotor activity is dependent on the unfamiliarity of the environment and not simply on the mismatched development of stimulatory and inhibitory neural systems [9,26] It is possible that the development of the catecholaminergic systems around day 10 may respond to the novelty of the environment to stimulate locomotor activity The subsequent development of inhibitory neural systems may modify the response to unfamiliar environments as the pup ages and develops a greater capacity to rely on sensory input and memory

One can speculate that such a developmental sequence may have adaptive value Prior to day 10, attachment to the mother's nipple is independent of food deprivation From days 10 to 15, the development of the serotonergic systems causes suckling to become dependent upon deprivation, suggesting that this frees the pups to explore other food sources [32] During this time, nondeprived pups decrease the time attached to the nipple and consequently have a greater chance of leaving the nest The unfamiliarity of the non-nest environment would trigger increased locomotor activity that only diminishes upon reentering the nest where the probability of survival is greater Further development of the serotonin system after day 15 begins to inhibit the increased locomotor activity of the novel environment at the same time that the eyes open and the pups develop alternative strategies for returning to the nest While the link between changes in suckling and increased locomotor activity

in unfamiliar environments is speculative, it is worthy of note that both behavioral phenomena are modified in parallel by the development of the serotonergic system and that both behaviors are eventually inhibited more strongly by the subsequent development of non-serotonergic systems [15, 23, 32]

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