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Differential Effects of Clonidine, B-HT 933 and B-HT 920 in Immature Rat Pups

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GOODLETT, C. R. AND M. L. VALENTINO. *Differential effects of clonidine, B-HT 933 and B-HT 920 in immature rat pups.* PHARMACOL BIOCHEM BEHAV 27(2) 283–290, 1987.—The effects of the α-adrenergic agonist clonidine were compared with two experimental hypotensive drugs, B-HT 920 and B-HT 933, in 10-day-old rat pups. Clonidine induced the expected dose-dependent (0.1–1.0 mg/kg) motor activation and wall-climbing syndrome typical at this age. B-HT 933, thought to be a more selective α_2-agonist than clonidine, elicited locomotor activity and wall-climbing only at the highest dose used (50 mg/kg). The high dose of B-HT 933 necessary to begin to mimic the effects of clonidine, a finding consistent with some studies using B-HT 933 in adults, suggests that the wall-climbing syndrome is mediated by receptors which have a low affinity for B-HT 933. In striking contrast, B-HT 920, a presynaptic dopamine agonist in mature rats, produced a very different behavioral profile. B-HT 920 induced long periods of sniffing accompanied by locomotion at low doses (peak at 0.12 mg/kg) and ataxic locomotion and poorly coordinated wall-climbing at high doses (30–50 mg/kg). Experiment 2 demonstrated that the active sniffing evoked by low doses of B-HT 920 was dose-dependently blocked by haloperidol (0.035–1.0 mg/kg). These findings of behavioral effects in 10-day-old rats suggest that B-HT 920 stimulates dopaminergic receptors in immature rats, presumably located on postsynaptic neurons. We propose that B-HT 920 and B-HT 933 also may be differentiated in terms of the time of onset of functional development of dopaminergic and noradrenergic autoreceptors, respectively.

Clonidine B-HT 933 B-HT 920 α-Receptors Dopamine receptors Developmental psychopharmacology

THOUGH clonidine is generally considered an α_2-agonist, it also may stimulate α_1-receptors as determined from preparations involving peripheral (cardiovascular) tissue [16,24]. In mature rats clonidine produces hypotension and behavioral sedation, and both effects are thought to be mediated by α_2-receptors in the central nervous system [10]. However, the specific receptor populations, sites of action and mechanisms responsible for these effects are especially difficult to ascertain, and may include both pre- and postsynaptic locations at many levels of the nervous system [7, 8, 13, 23, 25, 27, 33, 37]. Certainly, receptors for clonidine in the central nervous system cannot completely be ascribed to presynaptic norepinephrine (NE) neurons, as neurotoxic destruction of NE-containing neurons increases rather than reduces the number of clonidine binding sites [38].

Two azepine derivatives, B-HT 920 and B-HT 933 (Boehringer Ingelheim Ltd.), initially were identified as more selective agonists of α_2-receptors than clonidine. Pharmacological studies of B-HT 920 and B-HT 933 in peripheral tissue indicated that they selectively activated presynaptic α_2-adrenoceptors [17, 18, 26, 34]. However, more recent investigations by Anden and colleagues indicated that B-HT 920 and B-HT 933 have important differences in their action on the central nervous system of adult rats. They have shown that in mature animals B-HT 920 appears preferentially to activate presynaptic autoreceptors on dopamine neurons that regulate dopamine metabolism, while B-HT 933 seems to act on noradrenergic autoreceptors [2]. In particular, they have shown that in mature animals B-HT 920 decreases motor activity, and reduces the disappearance of dopamine from striatal and mesolimbic structures following synthesis inhibition [3]. B-HT 920 also antagonizes the increased synthesis of dopamine in limbic structures, and to a lesser extent in the striatum, following treatment with gammabutyrolactone [4]. Both the behavioral hypomotility and the decreased dopamine synthesis are reversed by haloperidol but not by yohimbine. In contrast, B-HT 933 had limited effects on dopamine turnover but did significantly alter measures of norepinephrine turnover [5]. They have also shown that yohimbine blocks the sedative effects of B-HT 933 but not those of B-HT 920 in mature animals [2]. These data provide good support that in adult rats B-HT 920 is a potent agonist of dopamine autoreceptors that regulate dopamine turnover, while B-HT 933 may be more selective for presynaptic norepinephrine receptors.

Since developmental psychopharmacological studies

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TABLE 1

MEAN BODY WEIGHT (IN GRAMS ± SEM) AND NUMBER OF SUBJECTS PER GROUP

	N	Body Weight
Saline	13	27.7 ± 1.1
Clonidine		
0.1 mg/kg	10	26.9 ± 1.3
0.5 mg/kg	11	26.4 ± 1.4
1.0 mg/kg	10	26.5 ± 1.2
B-HT 920		
0.012 mg/kg	8	25.1 ± 1.2
0.12 mg/kg	14	25.2 ± 1.1
0.6 mg/kg	11	26.1 ± 1.2
1.2 mg/kg	10	26.1 ± 1.5
12.0 mg/kg	8	24.4 ± 1.2
30.0 mg/kg	8	27.6 ± 1.2
50.0 mg/kg	8	27.5 ± 1.2
B-HT 933		
0.1 mg/kg	9	23.6 ± 1.5
0.5 mg/kg	10	24.8 ± 1.2
1.0 mg/kg	9	25.1 ± 1.5
5.0 mg/kg	8	23.1 ± 1.0
7.5 mg/kg	8	22.9 ± 1.0
10.0 mg/kg	10	24.5 ± 1.6
30.0 mg/kg	8	28.2 ± 1.8
50.0 mg/kg	8	23.8 ± 2.6

using these drugs have not been reported, the present study was designed to compare their effects with those of clonidine in 10-day-old pups. This comparison in immature rats provides additional data concerning these two compounds, since both α_2-adrenergic and presynaptic dopaminergic agonists showed marked changes in their effects over development. For example, the dramatic change in the behavioral effects of clonidine in developing rats is an important feature of the psychopharmacology of clonidine which may help elucidate its action on the CNS in producing its effects in adults. Administration of clonidine in doses greater than 0.1 mg/kg to rat pups younger than 15 days induces a motor activation syndrome in which the primary behavioral characteristic is long bouts of wall-climbing [11, 21, 28, 32]. By the end of the third week of life the effect of clonidine is changed from motor activation to a near-cataleptic sedation interspersed with short bursts of forward crawling [11,28]. Both the behavioral activation and the later behavioral sedation can be blocked by yohimbine and other α_2-antagonists [10, 21, 22]. Thus, both the behavioral activation stimulated by clonidine early in life and the behavioral sedation later in life apparently involve stimulation of α_2-receptors. However, the additional involvement of α_1 stimulation in the neonatal activation cannot be ruled out, given the relatively high doses of clonidine (0.5–1.0 mg/kg) necessary to elicit wall-climbing. In order to specify further the pharmacological differences among clonidine, B-HT 933 and B-HT 920 and to characterize their effects on immature rats, we examined their effects on motor behavior in 10-day-old pups.

TABLE 2

BEHAVIORAL CATEGORIES

(Experiment 1)

1.	Lying still—on ventrum or side
2.	Lying still—on back
3.	Locomoting or turning
4.	Rolling or kicking—on back
5.	Angled in wall—climbing position—no treading
6.	Wall climbing—at 45° angle to wall, front paws treading
7.	Paddling—front paws treading in smooth continuous movements
8.	Paddling—back paws
9.	Twitching
10.	Grooming
11.	Rearing
12.	Sniffing: in air
13.	Sniffing: along ground
14.	Yawning
15.	Chewing
16.	Crouching, not moving

EXPERIMENT 1: COMPARISON OF CLONIDINE, B-HT 920 AND B-HT 933 IN IMMATURE RATS

In comparing the effects of clonidine and B-HT 933 in 10-day-olds, we can determine whether similar behavioral effects are elicited by the presumably more selective α_2-agonist. In examining B-HT 920, we can determine whether the effects in immature rats are different from the two α_2-agonists at an age well before the age (28 days) believed to mark the functional development of dopamine autoreceptors as demonstrated by Shalaby and Spear [30].

METHOD

Subjects

The subjects in this study were 181 Sprague-Dawley albino rat pups obtained from 23 litters born at the Worcester Foundation. Upon arrival from Charles River Breeding Laboratories, the dams were provided a 25% casein diet (Teklad Mills, Madison, WI), which we standardly use as our full-protein laboratory diet. The dams were maintained on this diet throughout the experiment. On the day of birth (day 0) the live litter weight and number of live pups were recorded for each litter. All pups born on a particular day were then placed together and 8–10 pups were randomly assigned to each dam, with (when possible) an equal number of males and females per litter. The dam and her pups were then left undisturbed until the day of testing, ten days later. All rats were given food and water ad lib and maintained on a 12 hr light:12 hr dark cycle (lights on from 0700 to 1900).

Drugs

Clonidine-HCl was obtained from Sigma, Inc. and was dissolved in saline. B-HT 920-2HCl (6-allyl-2-amino-5,7,8-tetrahydro-4H-thiazolo-[4,5-diazepin-dihydrochloride) and B-HT 933-2HCl (2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxa-

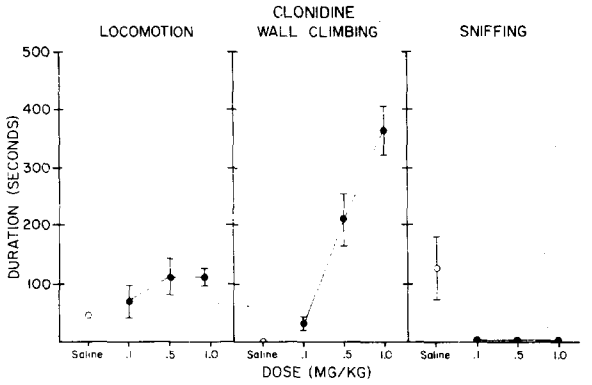

FIG. 1. Mean (±SEM) total duration (seconds) of locomotion, wall-climbing and sniffing during the 20 minute observation period as a function of dose of clonidine, with data being collected continuously beginning five minutes after injection.

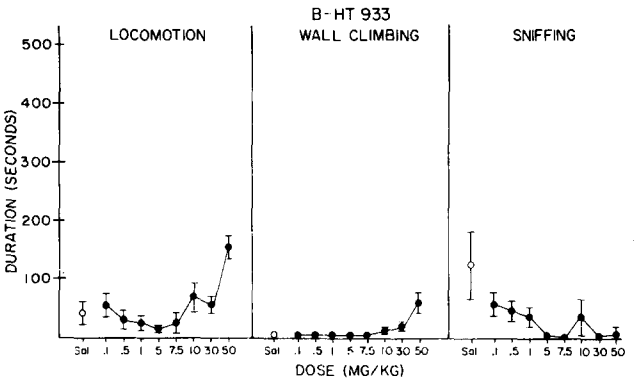

FIG. 2. Mean (±SEM) total duration (seconds) of locomotion, wall-climbing and sniffing during the 20 minute observation period as a function of dose of B-HT 933.

zolo-[5,4-d] azepin-dihydrochloride) were obtained as a gift from Boehringer Ingelheim, Ltd. These azepin derivatives were dissolved in saline for administration [5]. All drug solutions were prepared fresh each day by an experimenter other than the observer.

Procedure

All testing was done during the light phase of the light: dark cycle in a room separate from the vivarium. All pups from a litter were tested on the same day (10 days old) and each pup was given a single injection either of saline or a specific dose of B-HT 920, B-HT 933 or clonidine and tested only once. Dose-response relationships were determined for each drug using the saline vehicle and the following different drug doses:

B-HT 920: 0.012, 0.12, 0.6, 1.2, 12, 30, or 50 mg/kg;
B-HT 933: 0.1, 0.5, 1.0, 5.0, 7.5, 10.0, 30, or 50 mg/kg;
Clonidine: 0.1, 0.5, or 1.0 mg/kg.

Pups were randomly assigned to one of the drug conditions within each litter, and no more than two pups within a litter received the same dose of a particular drug. At least eight litters were represented at each dose for each of the three drugs, except for the two highest doses of B-HT 920 and B-HT 933 where only four litters were represented (see Table 1 for group n's). The drug solutions were prepared by an experimenter other than the observer and coded so that the observer was not informed as to the drug or dose given.

The pups were observed individually in an 18×18×12.5 cm (high) Plexiglas chamber with a wire mesh floor, which was placed inside a clear plastic incubator (Marsh Manufacturing, Inc.) which maintained the ambient temperature at 33°C. Each pup was weighed prior to testing and placed in the chamber for a five minute adaptation period. Following the adaptation period the pup was injected subcutaneously with the appropriate coded dose of either saline, B-HT 920, B-HT 933, or clonidine in a volume of 10 μl/g body weight. The 20 minute period of behavioral recording began five minutes after the injection. Behavior was recorded continuously for the entire 20 minutes using an Apple IIe computer-aided data collection procedure (Micro World, Inc., Binghamton, NY) in which duration (in tenths of seconds), frequency and average duration for each of 15 behavioral categories (see Table 2) were recorded for each minute of the observation period and summed over the 20 minute testing period. Following testing the pups were kept separate from the litter until all pups in the litter were tested.

Data Analysis

Since locomotion, wall-climbing and sniffing proved to be the most relevant measures of the drug effects, the total duration scores over the 20 minute test interval were analyzed statistically for these measures. The saline-treated pups served as controls for all drug-treated groups, and are included in the analysis of each drug. Due to the markedly different dose-response patterns of the drugs on these measures and the fact that they were clearly not equipotent (on a mol/kg basis) on any measure, statistical comparisons were made only among the groups treated with the same drug (plus the saline controls). A one-way analysis of variance was performed on each measure within each drug, with dose as the between-group factor. Newman-Keuls tests were used to compare experimental group means in all *post hoc* multiple comparisons. Since the grooming, rearing, mouthing and twitching measures occurred at such low frequencies, no further analyses were performed on these individual measures.

RESULTS

The significance of this study rests in the characterization of dose-response curves of the behavioral effects of the experimental compounds relative to that of clonidine. Thus, we first will present data for the three primary behavioral measures (locomotion, wall-climbing and sniffing) for clonidine, then for the experimental compounds.

Clonidine (see Fig. 1)

As expected, these doses of clonidine resulted in bouts of wall-climbing activity which increased linearly with dose in our 20 minute continuous collection procedure (see middle panel). The increase in wall-climbing duration with the dose of clonidine was statistically significant, $F(3,40)=36.20$, $p<0.001$, and specific comparisons using the Newman-Keuls test indicated that with the exception of the saline to 0.1

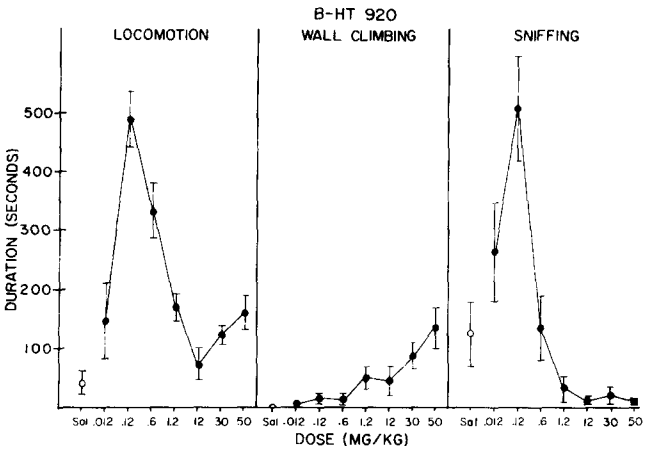

FIG. 3. Mean (±SEM) total duration (seconds) of locomotion, wall-climbing and sniffing during the 20 minute observation period as a function of dose of B-HT 920.

mg/kg comparison, all doses were significantly different from each other ($p<0.01$). The increased locomotor activity produced by clonidine did not reach statistical significance, as most of the motor activity was in the form of wall-climbing in these chambers. Finally, as shown in the right panel of Fig. 1, clonidine almost completely suppressed observable bouts of sniffing, $F(3,40)=3.25$, $p<0.05$, and all three doses produced significant reductions relative to saline controls ($p<0.05$).

The overall picture of the effects of clonidine in 10-day-old rat pups was a dose-dependent motor activation of the animal marked by extended bouts of persistent wall-climbing and an absence of active sniffing. This was entirely consistent with our previous report using a 90 minute time-sampling procedure [11] and suggests that the predominant effect of the drug was a facilitation of locomotor systems in which patterns or programs of movement were activated and not altered even in the face of obstacles. The fact that wall-climbing was the predominant behavior observed in clonidine-treated pups likely depended to some extent on the high probability of encountering a vertical surface in our relatively small arenas. However, it was the persistence of the individual wall-climbing bouts which was the signature effect of clonidine (mean bout lengths=6.7±1.2 sec, 10.7±1.5 sec and 11.5±0.9 sec for 0.1, 0.5 and 1.0 mg/kg, respectively).

B-HT 933 (see Fig. 2)

In contrast to clonidine, over the range of doses used, the only behavioral differences induced by B-HT 933 relative to the saline controls were seen at the highest dose. The effect of drug dose was significant for locomotion, $F(8,74)=5.67$, $p<0.001$, and for wall-climbing, $F(8,74)=15.27$, $p<0.01$. However, as can be seen in Fig. 2, only the 50 mg/kg dose resulted in significant increases in these two measures relative to saline controls ($p<0.01$). There were no significant group differences in sniffing, $F(8,74)=1.54$, $p>0.10$. It appears that the 50 mg/kg dose of B-HT 933 was the initial effective dose in eliciting clonidine-like effects. Perhaps B-HT 933 would elicit greater wall-climbing in 10-day-olds if we had included even higher doses.

B-HT 920 (see Fig. 3)

The forms of the dose-response curves of the behavioral effects of B-HT 920 in 10-day-olds were remarkably different from those of clonidine and B-HT 933. Locomotor activation and sniffing were the predominant effects of B-HT 920, especially at the lower doses. Wall-climbing was seen only at the highest doses, and then reached only 15% of that elicited by clonidine. As seen in the left and right panels of Fig. 3, low doses of B-HT 920 induced a pronounced and correlated increase in locomotion and sniffing, $F(7,72)=15.31$, $p<0.001$, for locomotion; $F(7,72)=7.50$, $p<0.001$, for sniffing. The peak duration of active sniffing was reached with the 0.12 mg/kg dose, and both the locomotion and sniffing were significantly higher for this dose than for all other doses, including the higher doses ($p<0.01$ for locomotion; $p<0.01$ for sniffing).

There was a significant effect of dose of B-HT 920 on the duration of wall-climbing observed (see middle panel, Fig. 3), $F(7,72)=11.5$, $p<0.001$. Only the two highest doses (30 and 50 mg/kg) were significantly elevated relative to saline controls ($p<0.01$). More importantly, the locomotion and wall-climbing elicited by B-HT 920 was not the coordinated, persistent activity seen with clonidine or B-HT 933. Rather, the movement was stumbling, uncoordinated and ataxic, and bouts of wall-climbing were generally short. Therefore, the increase in wall-climbing in pups treated with B-HT 920 was neither as dramatic as that seen following clonidine treatment (compare middle panels of Figs. 1 and 3), nor was it comparable in terms of the quality of movement.

Thus, unlike the other two drugs, the types of behavior elicited in 10-day-olds by B-HT 920 varied markedly with dose level. For low doses, the signature effect was potent induction of active sniffing. At higher doses, as the (ataxic) wall-climbing began to appear (reaching statistical significance), active sniffing was reduced.

DISCUSSION

It is clear that the three drugs had different dose-response effects on 10-day-old rat pups. Only clonidine elicited dose-dependent wall-climbing in doses less than 1 mg/kg, whereas B-HT 933 elicited some coordinated locomotion and wall-climbing only at 50 mg/kg. The effects of high doses of B-HT 920 were classified as locomotion and wall-climbing, but the ataxic quality was clearly distinguished from the motor activity induced by clonidine or B-HT 933.

The relatively high doses of clonidine (compared to effective doses in adults, albeit with opposite effects) and the extremely high doses of B-HT 933 needed to elicit wall-climbing suggest that α_2-receptors may not be the primary receptors involved in clonidine's effects on immature rats, and that α_1-receptors may be producing the wall-climbing behavior. However, others have found that clonidine-induced wall-climbing can be blocked by yohimbine or other α_2-antagonists [21] which we have confirmed in unpublished observations. In addition, others have found that these azepine derivatives may be 30–500 times less potent than clonidine in producing some of their pharmacological effects in mature animals [9,36]. Consequently, despite any greater selectivity of the compounds for α_2-receptors, these compounds are not comparable to clonidine in terms of potency in producing some of their psychopharmacological effects. While it is possible that differences in potency in producing motor behavior between clonidine and B-HT 933 may be a result of differences in distribution of these vasoactive drugs

TABLE 3
BEHAVIORAL CATEGORIES

	(Experiment 2)
1.	Head weaving
2.	Locomoting/turning
3.	Rolling
4.	Angled on the wall—no treading
5.	Twitching
6.	Wall climbing
7.	Grooming
8.	Rearing
9.	Lying on back—not moving
10.	Passive sniffing: sniffing the ground or air, not locomoting
11.	Active sniffing: sniffing the ground or air while locomoting
12.	Yawning
13.	Chewing/mouthing
14.	Paddling
15.	Lying still

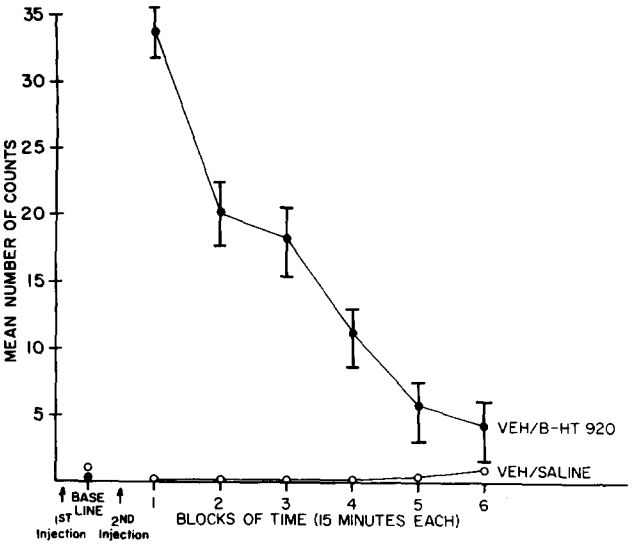

FIG. 4. Mean number of counts of active sniffing during baseline (after vehicle in the first injection) and during each 15 minute block of time after injection of either 0.1 mg/kg B-HT 920 or saline (second injection).

following the subcutaneous injection or in differences in penetration of the brain, the same cannot be said for the structurally similar B-HT 920 compound, since very low doses quite effectively elicited the active sniffing. Nevertheless, it is necessary to determine whether the less potent B-HT 933 can produce a stronger wall-climbing syndrome at higher doses that can be blocked by yohimbine. Likewise, the effects of clonidine in immature rats could be better specified by psychopharmacological studies comparing the effectiveness of specific α_1- and α_2-antagonists in blocking wall-climbing.

Perhaps the most important results from these studies are the effects of low doses of B-HT 920. The locomotor and sniffing activity that occurred in the 0.012–1.2 mg/kg dose range resemble developmental effects reported for dopamine agonists rather than α_2-agonists. For example, (+)3-PPP, pergolide and apomorphine all greatly increased motility (infrared beam interrupts) in 11-day-old rats [1]. While sniffing behavior was not recorded in that report, others have shown that apomorphine stimulates sniffing activity in addition to locomotor activity in the first two weeks of life [14, 29, 30]. Our finding that low doses of B-HT 920 stimulate locomotion accompanied by active sniffing in 10-day-olds is consistent with stimulation of dopaminergic receptors in the developing rat, an effect generally interpreted to involve postsynaptic receptor activation.

EXPERIMENT 2: EFFECT OF HALOPERIDOL ON ACTIVE SNIFFING INDUCED BY B-HT 920

The stimulation of locomotion and sniffing in 10-day-old rats in Experiment 1 apparently resulted from stimulation of dopaminergic receptors. Since functional effects of presynaptic autoreceptor stimulation may not be expressed until the end of the fourth week of life [30], the drug may be activating existing postsynaptic dopamine receptors in immature rats. Since the developmental effects of B-HT 920 had not been characterized prior to our work and it seemed to involve dopaminergic receptors, we were interested in whether the dopaminergic antagonist haloperidol would block the active sniffing effect of B-HT 920. In addition, Experiment 1 involved behavioral observations that lasted for only 20 minutes. In the present study we used a time-sampling procedure to examine the time-course of the effects of B-HT 920 over a 90 minute period.

METHOD

Subjects

Fifty-nine Sprague-Dawley albino rat pups obtained from seven litters born at the Worcester Foundation served as subjects in this study. Maintenance of the dams and their litters was similar to that described in Experiment 1. All pups were tested at 10 days of age.

Procedure

The drug solutions were prepared fresh each day by an experimenter other than the observer. Haloperidol (Sigma) was dissolved in a few drops of glacial acetic acid and brought to volume in 5.5% glucose solution; B-HT 920 (Boehringer Ingelheim) was dissolved in 0.9% saline. The pups within each litter were randomly divided into six groups, and each group was given two subcutaneous injections separated by 30 minutes. One group (n=9) received the vehicle in the first injection and 0.1 mg/kg B-HT 920 in the second injection. The remaining four groups (n=10 each) received one of four doses of haloperidol in the first injection (0.035, 0.1, 0.35 or 1.0 mg/kg) and 0.1 mg/kg B-HT 920 in the second injection. All pups from a litter were tested on the same day and each pup was tested only once. No more than two pups within a litter received the same drug treatment and all seven litters were represented in each treatment group.

All testing was done during the light phase of the light: dark cycle in a room separate from the vivarium. The pups were observed in one of four individual Plexiglas 18×18×12.5 cm (high) chambers with wire mesh floors placed inside clear plastic incubators (Marsh Manufacturing, Inc.). Ambient temperature in the incubators was maintained

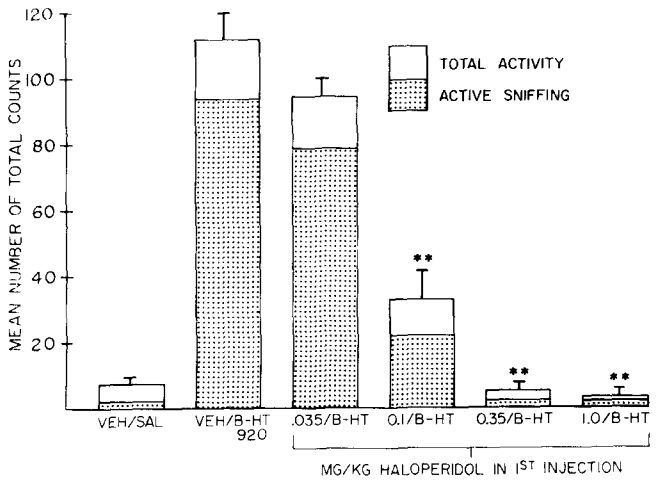

FIG. 5. Histograms of the 90 minute, time-sampling summed scores of total activity (locomotion, wall-climbing, rolling and active sniffing) and active sniffing alone, as a function of treatment groups. Abbreviation to left of slash indicates solution in first injection (veh: vehicle; numbers: dose of haloperidol). Abbreviations to the right of slash indicate solution in second injection (sal: saline; B-HT: 0.1 mg/kg B-HT 920). Asterisks indicate a significant reduction of activity in haloperidol-treated groups relative to vehicle/B-HT 920 group ($p<0.01$).

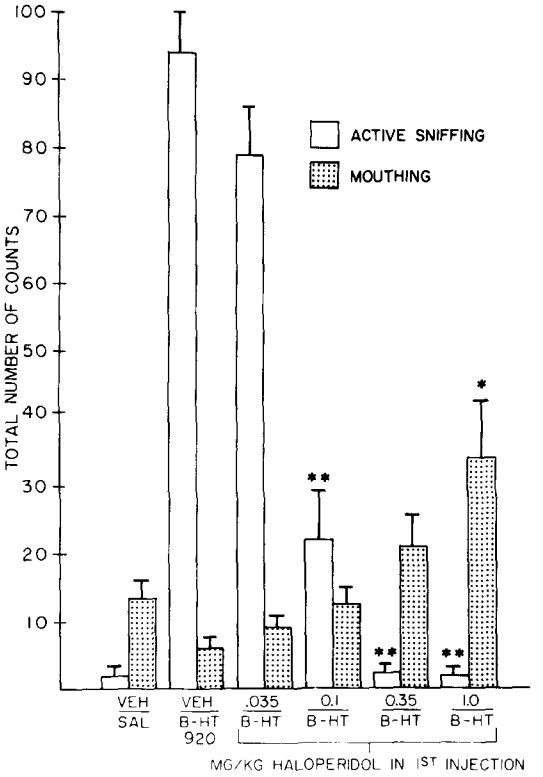

FIG. 6. Comparison of effects of drug treatments on active sniffing and mouthing behaviors. Double asterisks indicate significant reduction of active sniffing relative to the active sniffing of the vehicle/B-HT 920 group ($p<0.01$). Single asterisk indicates significant elevation of mouthing activity relative to that of vehicle/saline group ($p<0.05$).

at 33°C. On the day of testing (10 days of age) the first four pups of a litter were weighed and placed inside their respective chambers for a five minute adaptation period. Following the adaptation period each pup was injected subcutaneously with the appropriate coded dose of haloperidol or vehicle. Twenty-five minutes after the last pup was given the first injection, a five minute time-sampling of behavior was begun with observations recorded every 15 seconds. This period served to establish a baseline of behavior with haloperidol or vehicle alone. At the end of this five minute period each pup received a subcutaneous injection of saline (injection control) or of 0.1 mg/kg B-HT 920. Five minutes after the second injection a 90 minute time-sampling of behavior was begun with observations recorded once every 15 seconds. Each observation was one of 15 mutually exclusive behavioral categories (see Table 3), yielding 360 total observations per pup. Note that the behavioral categories were modified from those used in Experiment 1, since active sniffing was identified as the predominant behavior elicited by B-HT 920. After the testing of the first four pups was completed, the remaining pups of the litter were tested in the same manner.

Data Analysis

In order to determine the time course of activity of B-HT 920 the 90 minute time-sampling data from the vehicle/B-HT 920 group and the vehicle/saline group were examined as a function of six 15 minute blocks of time. Observations were totaled within these six blocks and scores for total activity, which included the locomoting, rolling, wall-climbing and active sniffing measures, for the active sniffing category alone, and for the non-sniffing activity, were examined. Since active sniffing constituted a large majority of total activity, a repeated measures analysis of variance was performed on the active sniffing scores with injection group (B-HT 920 or saline) as the between-group factor and blocks of time as the within-group factor.

For the analysis of the effect of haloperidol on behavior elicited by B-HT 920, a total score for each behavioral category across the entire 90 minute recording period was calculated for each animal. One-way analyses of variance were performed on the total activity, the active sniffing and the non-sniffing activity with haloperidol dose as the between-group factor. Since other behavioral categories occurred with such low frequencies, no analyses were performed on any of the other behavioral categories. Newman-Keuls tests were used for all specific comparisons involving group means.

RESULTS

Time Course of the B-HT 920 Action

As we found previously in Experiment 1, the predominant behavioral effect of 0.1 mg/kg B-HT 920 was a marked increase in motor activity, most of which was active sniffing. The active sniffing was apparent within five minutes after the injection and as shown in Fig. 4, was highest during the first 15 minute block, then declined over time. The analysis confirmed the main effect of B-HT 920, $F(1,17)=143.8$, $p<0.001$, as well as a significant drug × time interaction, $F(5,87)=29.37$, $p<0.001$. The group treated with B-HT 920 remained significantly elevated above vehicle-treated controls for the first four of the six 15 minute blocks over the recording period ($p<0.05$).

Haloperidol Antagonism

During the five minute baseline period recorded 25–30 minutes after the first injection, haloperidol alone did not produce any significant differences in observed behavior relative to vehicle controls, $F(4,54)=1.11$, $p>0.25$. However, during the 90 minute observation period, haloperidol inhibited the behavioral effects of B-HT 920 in a dose-dependent manner, yielding a significant effect of dose for the active sniffing, $F(5,53)=62.59$, $p<0.001$, for total activity, $F(5,53)=72.45$, $p<0.001$, and for non-sniffing activity, $F(5,53)=7.04$, $p<0.05$. As can be seen in Fig. 5 a significant decrease in the active sniffing induced by B-HT 920 occurred with 0.1 mg/kg haloperidol ($p<0.01$) and the blockade of activity was complete with 0.35 and 1.0 mg/kg haloperidol ($p<0.01$ for both). The significant increase in non-sniffing activity induced by B-HT 920 ($p<0.05$) was also significantly reduced by the 0.35 and 1.0 mg/kg doses of haloperidol ($p<0.05$).

An unexpected and significant potentiation of the mouthing/chewing behavioral category was apparent with increasing doses of haloperidol, $F(5,53)=4.71$, $p<0.01$. As can be seen in Fig. 6 this increase was significantly inversely correlated with the decrease in the active sniffing behavior ($r=-.34$, $p<0.01$). The mouthing/chewing was observed most frequently at the highest dose of haloperidol ($p<0.05$ for vehicle/B-HT 920 vs. 1.0 mg/kg haloperidol/B-HT 920), when the active sniffing behavior was completely inhibited.

DISCUSSION

The active sniffing behavior induced by B-HT 920 in this study replicated our initial observations in 10-day-old pups. In Experiment 1, the effect was dose-dependent, occurring between 0.012–1.2 mg/kg, with a peak effect at 0.12 mg/kg. The active sniffing induced by low doses of B-HT 920 in immature rats most resembles developmental effects reported for other dopaminergic agonists (i.e., (+)-3PPP, pergolide and apomorphine). However, these effects are usually interpreted as resulting from postsynaptic stimulation of dopamine receptors [19, 20, 30]. These findings also contrast to the behavioral sedation and dopaminergic synthesis inhibition produced by dopaminergic autoreceptor stimulation by B-HT 920 in mature rats [2]. Thus, it appears that low doses of B-HT 920 act at postsynaptic dopamine receptor sites in the immature brain and at presynaptic autoreceptors that regulate dopamine turnover in the mature brain. The ataxic locomotion and wall-climbing induced at high doses in rat pups may result from activity of B-HT 920 on α_2- (or other) receptors, albeit via less effective stimulation of these receptors than clonidine or even B-HT 933.

It is possible that the proposed postsynaptic receptor site in immature rats and the presynaptic receptor site in mature rats for B-HT 920 are pharmacologically identical, but differ in terms of their functional development. Grabowska-Anden and Anden [12] have shown that under certain pathological states evidence of stimulation of postsynaptic receptors by B-HT 920 can be demonstrated. In rats pretreated with reserpine, these investigators observed behavioral effects consisting mainly of jerks of the head and neck elicited by doses less than 1 mg/kg of B-HT 920. The jerks were blocked by haloperidol, and potentiated by prior 6-hydroxydopamine lesions. Low doses of apomorphine (0.05 mg/kg) elicited similar jerks, but at higher doses apomorphine caused the more typical stereotyped behavior. These authors suggested that two types of postsynaptic dopamine receptors may exist, one stimulated by high doses of apomorphine and mediating stereotyped behavior, and another which is pharmacologically identical to the presynaptic receptor and stimulated by B-HT 920 and low doses of apomorphine. Thus, the receptors stimulated by B-HT 920 in infant rats may be the latter type.

The results of these two experiments support Anden's contention that B-HT 920 and B-HT 933 act on different receptor populations in the central nervous system. Unlike the presynaptic dopaminergic effects of B-HT 920 in mature rats, the active sniffing we observed for B-HT 920 in 10-day-old rats likely is associated with postsynaptic stimulation of dopamine receptors.

In fact, evidence has accumulated suggesting that the presynaptic dopamine receptors regulating turnover do not develop in some brain regions until the end of the fourth week of life in the rat [15, 30, 31]. Thus, if it is the case that the dopamine autoreceptors are identical to the postsynaptic receptor sites in the immature rats, then with the functional development of the autoreceptors, the presynaptic actions of B-HT 920 would mask the effects of stimulating the postsynaptic receptors. Thus, there may be a developmental change in behavioral response to B-HT 920 parallel to that reported for apomorphine [30].

An interesting prediction may be made concerning the developmental effects of B-HT 920 and B-HT 933, derived from their proposed actions on presynaptic catecholamine receptors. From Anden's evidence that B-HT 920 preferentially stimulates mesolimbic dopamine autoreceptors and from Shalaby and Spear's [30] evidence that dopamine autoreceptors become functional around the fourth week of life, we suggest that a developmental onset of the inhibitory effects of B-HT 920 on locomotor activity should become apparent around 28 days of age. This would parallel the existing biochemical and psychopharmacological evidence that dopamine autoreceptors become functional at this age. In contrast, if B-HT 933 acts on presynaptic norepinephrine receptors and mimics the development of clonidine-induced behavioral sedation, then the onset of behavioral sedation should occur at least a week earlier, since the hypoactivity effect of clonidine matures around 20 days of age [28]. While psychopharmacological tests of these predictions would be strongly supportive, more direct biochemical and receptor-binding studies using these drugs would be necessary to establish better the developmental onset of presynaptic catecholaminergic receptor function.

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