Pharmacology Biochemistry & Behavior, Vol. 22, pp. 1075–1078, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

BRIEF COMMUNICATION

Neonatal 6-Hydroxydopamine Potentiates Clonidine's Locomotor Effects Throughout Maturation in the Rat

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Received 31 May 1984

SMYTHE, J. W. AND B. A. PAPPAS. *Neonatal 6-hydroxydopamine potentiates clonidine's locomotor effects throughout maturation in the rat.* PHARMACOL BIOCHEM BEHAV 22(6) 1075–1078, 1985.—Newborn rats were administered 6-hydroxydopamine (6-OHDA) systemically (2×100 μg/g) or vehicle and subsequently tested for their locomotor response to one of three doses of clonidine (10, 100 or 1000 μg/kg) at either 10, 20, 30 or 50 days of age. Clonidine caused a dose-related increase in activity in 10-day-old rats and this effect was potentiated in the 6-OHDA-treated rats. At 20 days, clonidine did not affect activity in the vehicle rats but at the highest dose, increased that of the 6-OHDA rats. At 30 days, clonidine again did not affect activity in the vehicle rats but the 1000 μg/kg (when compared to the 100 μg/kg) dose, significantly increased activity of the 6-OHDA rats. A biphasic effect of clonidine was apparent at 50 days. At this age the 6-OHDA treatment exaggerated the depressant effect of the 100 μg/kg dose. Thus, neonatal-OHDA generally potentiated the locomotor response to clonidine despite the fact that the effect of the drug changed with age. It is concluded that the locomotor effects of clonidine are mediated through alpha 2 adrenoceptors which are not located on noradrenergic terminals but rather are postsynaptic to them. With maturation, the "functional wiring" of these terminals appears to be altered.

Neonatal 6-Hydroxydopamine Clonidine Denervation Supersensitivity Alpha 2 adrenoceptor

THE overt behavioral effects of the alpha adrenergic receptor agonist clonidine strikingly reverse during rat ontogeny. Rat pups 14 days of age or younger show an intense behavioral activation characterized by forward crawling and when this is impeded, by climbing of the wall which blocks forward progress [2,6]. At around 20 days the drug begins to elicit motor depression rather than activation and apparently this effect persists into adulthood since sedation and catalepsy are the usual observations of its effect in the mature rat [6]. The reason for this reversal of the drug effect is unresolved. One possibility is that it reflects a change with maturation of the adrenergic receptor subtype which is most predominantly activated. Clonidine has been shown to act as an agonist at both alpha 1 and alpha 2 receptors [3] although it has greater affinity for the latter [9]. That these two receptors are differentially activated by clonidine as the rat matures is not supported, however, by the study of differential receptor blockers on the drug's effect in infant and adult animals, since both the activating effect in the young and the sedating effect in the mature rat are eliminated by pharmacological block of the alpha 2 receptor [4,7].

Another alternative is that the neuronal location of the alpha 2 adrenoceptor with which clonidine interacts, changes with age. Current evidence favours a location different from the norepinephrine (NE) terminal for the adult rat since the 6-OHDA-induced degeneration of the NE terminals does not eliminate but rather augments the sedative effect of clonidine in the adult [8]. Conceivably, in the infant rat, the drug may interact primarily with the presynaptic alpha 2 adrenoceptor. Thus, a presynaptic alpha 2 adrenoceptor may mediate clonidine-induced activation in the infant rat while a postsynaptic site mediates its effect in the adult. The present experiment addresses this possibility by tracing through ontogeny the effect of neonatal 6-OHDA (NS-6-OHDA) on the behavioral response to clonidine. If this response is mediated by presynaptic alpha 2 adrenoceptors in the infant rat then it should be absent in those infants who have suffered extensive lesion of the NE terminals. As the rat matures and the behavioral response changes, reflecting the drug's action on receptors located elsewhere than on the NE terminal, then the response to clonidine should emerge and, in fact, should be augmented in the NE-lesioned rats due to denervation supersensitivity.

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TABLE 1

NE CONCENTRATIONS (MEAN + SE μg/g) IN NEONATAL SYSTEMIC 6-OHDA (NS-6-OHDA) TREATED RATS AND THEIR VEHICLE CONTROLS (NS-VEH)

	Tissue NE Concentration (μg/g)		
Group	Cortex/Hippocampus	Brainstem	Spinal Cord
NS-VEH	0.154 ± 0.016	0.291 ± 0.043	0.111 ± 0.074
NS-6-OHDA	0.035 ± 0.019*	0.574 ± 0.069*	0.014 ± 0.009*
Percent of NS-VEH	23%	197%	13%

*Significantly different from NS-VEH, $p<0.01$, analysis of variance.

METHOD

Subjects

The subjects were 489 offspring of Wistar dams (Woodlyn Farms, Guelph) that were bred in the laboratory or occasionally obtained 14 days pregnant from the breeder. On the day of birth, which was designated as day one for the pups, the litters were cross-fostered and culled to 10 with equal numbers of males and females where possible. The litters and dams were housed in polystyrene maternity cages until 21 days when the pups were weaned and housed 10 per wire colony cage. The colony room was on a reverse light cycle (off at 0800, on at 2000) and testing took place between 0900 and 1700 hr.

Procedure and Apparatus

On the day of birth, whole litters were assigned to receive either vehicle (saline plus ascorbic acid, 0.2 mg/ml) or 6-OHDA hydrobromide (Sigma, 100 mg/kg of the salt) dissolved in the vehicle. The injections were administered SC at the nape of the neck through a 30 gauge needle at 12 hr after birth and again 24 hr later. Injection volume was 0.05 ml.

At 10, 20, 30 or 50 days of age, four or five rats per litter were sexed, weighed and randomly assigned to one of five experimental chambers which had been previously designated to contain a rat injected with one of three doses (10, 100 or 1000 μg/kg) of clonidine or the vehicle (saline). These injections were administered SC at the nape of the neck through 26 gauge needles at 5 μl/g for the three youngest age groups and 1 μl/g for the 50-day-old rats. The rats were first placed in their respective chamber, and injected 5 min later. They were then returned to the chambers for a 1 hr test session. Each rat was tested only once.

The three younger age groups were tested in circular polystyrene chambers 17 cm in diameter, 15 cm high. The floors were marked with intersecting orthogonal lines so as to define 16 zones of approximately equal area. The air temperature was maintained at about 35°C with two 250 W infrared lamps controlled by a proportional temperature control (YSI model 72) activated by a thermistor hanging between the chambers, for the two youngest ages. The 30-day-old rats were tested at room temperature. The 50-day-old rats were tested at room temperature in larger circular chambers 20 cm diameter and 30 cm high with the floor divided into quadrants. The behavior of the rats was recorded on videotape using an RCA time lapse video recorder (model BW004) set to scan 12 times per second. These tapes were later scored by an experimenter who was blind to whether the rats were 6-OHDA or vehicle pretreated and to the dose of clonidine administered.

It had been intended that the basic datum for all age groups would consist of the number of zones entered (defined as the movement of both front paws into a zone). Because the 10-day-old rats showed too few zone entries for meaningful analyses, however, they were instead scored for the total amount of time that their limbs were in motion. This was accomplished with a manually operable microswitch which controlled a timer accurate to 0.1 sec. The timer was activated for the duration of single or multiple limb movements but not for tail movements.

Catecholamine Assay

To verify the extent of the NE depletions, 16 rats (eight of each sex) from the vehicle and 6-OHDA groups were decapitated at 90–100 days of age. Their brains were rapidly dissected on ice-cooled plates into cortex plus hippocampus, brainstems (pons plus medulla). These and their spinal cords were frozen in liquid nitrogen and then transferred to a deep freeze for storage until assayed within a few weeks by a fluorescence technique [5].

RESULTS

The results of this assay are shown in Table 1 and are similar to those typically observed after this neonatal 6-OHDA treatment, namely significantly reduced cortical/hippocampal and spinal cord NE and significantly elevated NE in the brainstem [5]. No sex differences were observed for the result of the 6-OHDA treatment.

Figure 1 shows the effects of clonidine plotted in separate panels for the four ages. These data were analysed by separate analyses of variance (ANOVA) for each age with clonidine dose and neonatal treatment as main effects. Multiple comparisons were subsequently carried out using the Neuman-Keuls test. No sex differences for the neonatal 6-OHDA or clonidine effects had been observed in preliminary analyses at any age so the data were collapsed across this variable.

As the figure shows, the effect of the NS-6-OHDA treatment was consistent across age insofar as it generally exaggerated any effects of clonidine that were apparent for the neonatal vehicle treated rats. At 10 days of age, the ANOVA showed significant main effects for NS-6-OHDA, $F(1,120)=5.40$, $p<0.025$, and clonidine dose, $F(3,120)=64.90$, $p<0.001$. In the NS-VEH rats, only the 1000 μg/kg dose significantly ($p<0.05$) elevated activity in

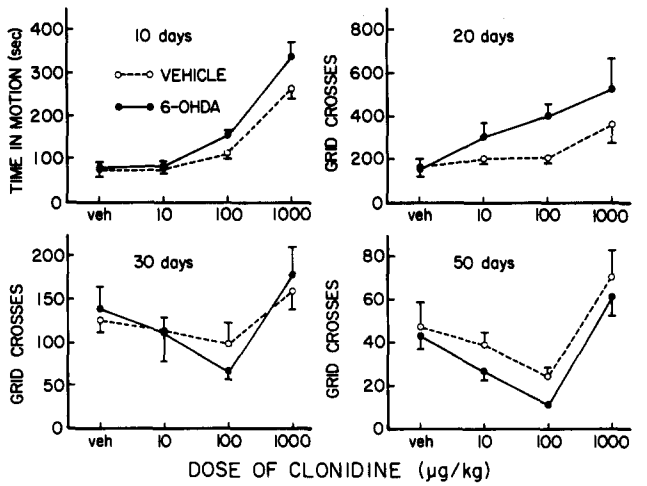

FIG. 1. The effects of clonidine (0 (VEH), 10, 100 or 1000 μg/kg) on the locomotor activity of 10-, 20-, 30- or 50-day-old neonatal vehicle or 6-OHDA treated rats. Activity for 10-day-olds was scored as time spent in limb motion while for the other rats, it was scored as grid crosses (see text). Note that the ordinal scales vary for the different ages. Filled asterisks denote significant differences between the neonatal vehicle and 6-OHDA-treated groups at each dose of clonidine.

comparison to its vehicle whereas for the NS-6-OHDA rats both the 100 and the 1000 μg/kg doses elevated activity (p's<0.05) when compared to the vehicle or 10 μg/kg groups. Furthermore, at the 1000 μg/kg dose, the activity of the NS-6-OHDA rats was higher than that of the NS-VEH group treated with this dose. Similarly, at 20 days of age both the 6-OHDA, $F(1,120)=5.03$, $p<0.05$, and the clonidine, $F(3,120)=5.66$, $p=0.001$, effects were significant. Comparisons among the NS-VEH rats showed that there was no significant effect of clonidine at any dose although there was a tendency for increased activity at the highest dose. For the NS-6-OHDA rats, however, the 1000 μg/kg dose significantly ($p<0.05$) increased activity when compared to the 0 mg/kg group and to the NS-VEH rats that received 10 and 100 μg/kg (p's<0.05).

At 30 days, the ANOVA showed no neonatal treatment effect but a main effect of drug dose, $F(3,120)=5.67$, $p<0.001$. For the NS-VEH rats, none of the comparisons between the clonidine doses and the vehicle were significant but activity was slightly suppressed at the 100 μg/kg and slightly elevated at the 1000 μg/kg doses. For the NS-6-OHDA rats, the 100 μg/kg dose tended to depress activity when compared to the vehicle although this effect fell just short of statistical significance ($p<0.10$). The 1000 μg/kg dose, however, significantly elevated activity in comparison to the 100 μg/kg dose ($p<0.05$). At 50 days of age, there were both significant neonatal treatment $F(1,97)=5.36$, $p<0.025$, and clonidine dose, $F(3,97)=11.29$, $p<0.001$, effects. For the NS-VEH rats, the 100 μg/kg dose tended to depress activity in comparison to the vehicle treatment while the 1000 μg/kg dose tended to increase it. These comparisons fell short of statistical significance ($p<0.10$). The rats that received the 1000 μg/kg dose were significantly ($p<0.05$) more active, however, than those that had received 100 μg/kg. For the NS-6-OHDA rats, the 100 μg/kg doses significantly reduced activity ($p<0.05$) in comparison to both the 0 mg/kg and to the 1000 μg/kg groups. Thus, the principal effect of the 6-OHDA treatment at this age was to amplify the depressant response to the 100 μg/kg clonidine dose.

DISCUSSION

The locomotor effect of clonidine in the control rats was found here to change with age. Thus at 10 days the drug caused a dose-dependent increase in activity. At 20 and 30 days, no significant responses to the drug were observed. A tendency towards a biphasic dose-related effect was apparent at 50 days. It should be noted that our results for the 20-day-old rat differ from those previously published which show a clear locomotor depressant effect by 20 days within the range of drug dose that we used here [6]. It is not clear what accounts for this difference. The rat strain is unlikely to be a factor since this depressant effect has been reported for three different strains including the Wistar used here. One possibility is that our control rats were subjected to some degree of stress perinatally due to the vehicle injection procedure and this may have modified their later response to clonidine.

The neonatal loss of NE was found to generally amplify the response to clonidine. Thus, the drug's excitatory effect in 10-day-old rats was enhanced by neonatal 6-OHDA. Additionally, the 1000 μg/kg dose caused an excitatory effect at 20 days but only in the NE-depleted rats. The tendency toward a biphasic dose-related response in the 50-day-old rats was exaggerated by NE loss. At this age, the 100 μg/kg clonidine dose more effectively depressed activity in the NE-depleted rats. From these results, we infer that clonidine's effect is not on presynaptic receptors located on the NE terminals since the assays confirmed the destruction of these terminals after the 6-OHDA treatment, but rather on receptors which are located either post synaptic to these terminals or elsewhere. This is consistent with other studies which show that both the adult [12] and the neonatal [8] lesion of NE terminals do not eliminate but rather augment the effects of clonidine when measured in adulthood. Furthermore, they are consistent with radioligand binding studies which show that augmented binding of clonidine occurs after either the adult [9,10] or neonatal [1] 6-OHDA-lesion of the NE terminals. Thus both behavioral and neurochemical criteria support the idea that both neonatal and adult lesion of NE terminals induce denervation supersensitivity of the alpha 2 adrenoceptor. The unique feature of the present study is its demonstration that as the behavioral response to clonidine changes with brain maturation, amplification of this response due to denervation supersensitivity tends to be maintained.

We conclude that as the rat matures, its response to clonidine changes. This response at all ages is mediated through the drug's action on alpha 2 adrenoceptors which are located elsewhere than at the NE terminal. With maturation the "functional wiring" of these adrenoceptors changes leading to an altered behavioral expression of their interaction with clonidine. The further significance of this maturation for brain and behavioral development in the normal animal remains to be elucidated.

ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada grant to B.A.P. We thank Boehringer Ingelheim of Canada for their generous donation of the clonidine.

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