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Noradrenergic and Serotonergic Mediation of the Locomotor and Antinociceptive Effects of Clonidine in Infant and Adult Rats

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SMYTHE, J. W. AND B. A. PAPPAS. *Noradrenergic and serotonergic mediation of the locomotor and antinociceptive effects of clonidine in infant and adult rats.* PHARMACOL BIOCHEM BEHAV **34**(2) 413–418, 1989.—This experiment examined the necessity for intact noradrenergic and serotonergic function for the locomotor and nociceptive effects of clonidine in 10- and 100-day-old rats. Newborn rats were administered systemically 6-hydroxydopamine (100 μg/g; 12 and 24 hours after birth) to deplete norepinephrine (NE), and at 10 or 100 days they were injected with para-chlorophenylalanine (300 mg/kg PCPA; 5 and 24 hours before testing) to deplete serotonin (5-HT). They were then tested for the locomotor and analgesic effects of one of various clonidine doses (0, 10, 100 or 1000 μg/kg). Clonidine enhanced locomotion at 10 days. This effect was potentiated by NE depletion and reduced by 5-HT depletion. Clonidine reduced locomotion at 100 days, and again this was augmented by NE depletion but reduced by 5-HT depletion. NE depletion did not have an enduring effect on clonidine antinociception whereas 5-HT depletion reduced it at both ages. It is concluded that the locomotor effects of clonidine in both infant and adult rats, despite reversing with maturation, reflect its agonist action at postsynaptic alpha$_2$ adrenoceptors. The results also add to the accumulating evidence for an early maturing and behaviorally relevant serotonergic system(s).

Clonidine Norepinephrine Serotonin Maturation 6-Hydroxydopamine Locomotor Analgesia

RECENTLY, evidence has accumulated which suggests that complex physiological and behavioral interactions occur between norepinephrine (NE) and 5-hydroxytryptamine (5-HT) brain systems. Although no less important, the developmental aspects of these interactions have largely been neglected, and research has generally been conducted on adult animals. We report here on the behavioral effects of the alpha$_2$ agonist clonidine (CLON) and its dependence on NE and 5-HT systems in infant and adult rats.

CLON is analgesic in adult rats (3,4). Its effect on nociception in infant rats is to our knowledge unknown. Conversely, the locomotor effects of CLON are well established in infant and adult rats. They dramatically reverse with maturation (20,24). In rats younger than 21 days CLON elicits hyperactivity while in rats older than 28 days it causes hypoactivity. Both of these results probably reflect CLON's agonist action at brain alpha$_2$ adrenoceptors (2,14). These receptors are most likely postsynaptic since brain NE depletion induced by lesion of the locus coeruleus or by intraventricular or neonatal systemic 6-hydroxydopamine (6-OHDA) does not eliminate CLON's effects on activity (6, 13, 24). Furthermore, specific binding of clonidine to cortical tissue is not reduced by 6-OHDA lesion of NE terminals (16,27).

The behavioral effects of CLON, at least in some instances, require intact brain 5-HT function in the adult rat. For example, CLON inhibition of pentylenetetrazol-induced seizures is prevented by the 5-HT receptor agonists methysergide and metergoline (9). Both the CLON-induced reduction in locomotor activity and in conditioned avoidance are prevented by prior 5,6-dihydroxytryptamine lesion of the medial raphe area (8). Whether or not the effects of CLON in the infant rat are also 5-HT dependent is unknown. This question has additional significance in view of the fact that at least with respect to locomotor activity, manipulation of brain 5-HT has been reported to have no effect until the rat reaches about 15 days of age (11). This latter finding would suggest that brain 5-HT depletion should not alter CLON's effects on locomotion (and nociception) until the pup is least 15 days of age. Indeed, it could be that the reversal of CLON's effect from causing hyper- to causing hypoactivity around 20–30 days of age may depend upon the maturation of brain 5-HT systems.

This experiment compared the effects of neonatal NE depletion by systemic 6-OHDA on the locomotor and analgesic effects of

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CLON in 10- and 100-day-old rats. Additional rats were also depleted of 5-HT by administration of the tryptophan hydroxylase inhibitor para-chlorophenylalanine (PCPA). This permitted the assessment of the effects of 5-HT-only depletion, and as well, conjoint 5-HT and NE depletion.

METHOD

Subjects

The subjects were the 550 offspring of Wistar dams (Woodlyn Farms, Guelph, Ontario) bred in our laboratory. Dams and their litters were housed in polycarbonate maternity cages with ad lib Purina chow and water. The day of birth was designated day one of life. Litters were cross fostered and culled to 10 pups (equal males and females where possible). They were weaned at 21 days of age and then same-sex housed 5 per cage in wire colony cages. A reverse light cycle was maintained (lights off at 0800 hr and on at 2000 hr). All testing was conducted between 0900 and 1700 hr.

Procedure and Apparatus

Within 12 hr of birth and again 24 and 48 hr later, newborn rats were administered 6-OHDA hydrobromide (Sigma), 100 μg/g, or its vehicle (saline plus 0.1% ascorbic acid). All injections were delivered via 30-gauge needle SC in the nape of the neck. The volume was 5 ml/kg.

At their respective test ages (10 and about 100 days), the rats were weighed and randomly assigned to one of four doses (0, 10, 100 and 1000 μg/kg) of CLON hydrochloride (Boehringer Ingelheim) administered SC in the nape. Injection volume was 5 ml/kg for the 10-day-old rats and 1 ml/kg for the adults. Some of these rats had been pretreated with PCPA methyl ester (Sigma) 24 and 5 hr beforehand. The dose was 300 mg/kg administered intraperitoneally. For the purpose of experimental economy, the 10 μg/kg dose of CLON was not administered to the PCPA-treated rats since the early data indicated that this dose of CLON was behaviorally inactive.

The activity test was carried out in a circular polystyrene chamber (20 cm diameter, 30 cm high). After a 10-min adaptation period, the rats were injected with CLON or vehicle and returned to the chamber. They were videotaped for 40 min using a time lapse recorder set to scan at 12 frames/sec.

Upon completion of the activity test, they were tested for pain sensitivity using a hot-plate procedure. The rat was placed on a 30-cm diameter stainless steel plate which was electrically heated to 55°C.

Activity was scored by measuring the total duration during which locomotor movement was evident (24). This included motion of one or more limbs but did not include movement of the tail only. The tapes were scored blind to the animals treatment.

For the hot-plate test the rats were removed from the plate at the first sign of distress (vocalization, hopping, running, paw-licking, twitching, writhing) (1). The latency to display any of these signs was recorded to the nearest 0.1 sec by activation of a foot pedal linked to a timing device.

Neurochemical Assay

Immediately following testing, randomly selected rats from each treatment group were decapitated. After their rapid removal, the brains were dissected on saline-rinsed, ice-cooled plates into whole cortex-hippocampus, hypothalamus, and brainstem. These and the spinal cords were placed in liquid nitrogen and subsequently stored at −70°C. A previously described fluorometric assay (23) was used to estimate NE and 5-HT levels. Because of the small tissue size of the 10-day-old rats, tissue from 2 rats was pooled for assay.

Statistical Analysis

Univariate analyses of variance (ANOVAs) were used to assess the effects of pretreatment (VEH or 6-OHDA), dose of CLON, and any interactions that occurred between pretreatment and dose. The alpha level was set at 0.01 to avoid Type 1 errors that might have resulted from using univariate ANOVAs for 2 dependent measures. Post hoc comparisons (all possible pairs) were done using Newman-Keul's procedure (alpha = 0.05). Assay results were analyzed using Dunnett's *t*-test, with alpha set at 0.05.

RESULTS

Assay

As shown in Table 1, the 6-OHDA injections significantly reduced NE concentration in the spinal cord, $F(3,24) = 110.42$, $p<0.001$, and the cortex plus hippocampus sample, $F(3,24) = 16.75$, $p<0.01$, of the 10-day-old rats. The depletion (95%) was greatest in the spinal cord although the actual values should be viewed with caution as the levels in the infant rat are near the lower limit of sensitivity for our assay. PCPA significantly depleted spinal, $F(3,26) = 141.96$, $p<0.001$, cortical/hippocampal, $F(3,26) = 127.80$, $p<0.05$, and hypothalamic 5-HT, $F(3,26) = 127.80$, $p<0.001$. Again, the greatest depletion was in the spinal cord (79%). PCPA did not affect NE levels at this age.

In the adult rats, 6-OHDA depleted spinal, $F(3,21) = 12.10$, $p<0.05$, hypothalamic, $F(3,36) = 78.96$, $p<0.01$, and cortical, $F(3,28) = 80.06$, $p<0.01$, NE. Conversely, brainstem NE was increased, $F(3,36) = 51.94$, $p<0.01$, as expected (22). PCPA depleted spinal, $F(3,32) = 63.33$, $p<0.01$, brainstem, $F(3,36) = 41.72$, $p<0.01$, hypothalamic, $F(3,36) = 151.99$, $p>0.001$, and cortical 5-HT, $F(3,27) = 79.37$, $p<0.01$. These depletions fell in the 70–80% range. As has been reported for adult rats, PCPA also caused significant (p's<0.05) but much smaller depletions of NE in all four brain regions. These ranged from 22% in cortex to 49% in hypothalamus.

Activity

Ten-day-old rats. As shown in Fig. 1A, CLON caused a dose-dependent increase in activity in 10-day-old rats. This increase was greater for 6-OHDA-treated than for vehicle-treated rats. Two-way ANOVA comparing neonatal treatments (factor one) and CLON dose (factor two) showed significant main effects for both the neonatal treatment, $F(1,72) = 9.84$, $p<0.01$, and CLON dose, $F(3,72) = 42.12$, $p<0.001$. Multiple comparison tests showed that at both the 100 and 1000 μg/kg doses, the 6-OHDA-pretreated rats were more active than their neonatal vehicle controls who also received these doses. The 6-OHDA and control rats did not differ after the 0 or 10 μg/kg injections. Thus, the 6-OHDA-induced lesion did not affect baseline activity but did augment the activating effects of higher doses of CLON.

PCPA (see Fig. 1B) reduced the locomotor activating effects of CLON on 10-day-old rats. A three-way ANOVA was carried out incorporating the groups in Fig. 1B (all of which were pretreated with PCPA) and as well, their corresponding but non-PCPA-treated groups shown in Fig. 1A. Significant main effects were found for 6-OHDA pretreatment, $F(2,108) = 22.42$, $p<0.001$, which potentiated the effects of CLON; PCPA pretreatment, $F(1,108) = 42.23$, $p<0.001$, which attenuated the effects of CLON;

TABLE 1

REGIONAL BRAIN NE AND 5-HT LEVELS (ng/g) TISSUE AFTER NEONATAL VEHICLE OR 6-OHDA INJECTIONS FOLLOWED BY PRETEST ADMINISTRATION OF PCPA

Brain Part		Group: Vehicle	6-OHDA	6-OHDA + PCPA	PCPA
			10-Day-Old-Rats		
Spinal Cord:	NE	95 ± 10	5 ± 1	104 ± 10	14 ± 1
	5-HT	294 ± 20	318 ± 37	62 ± 1	72 ± 1
Brainstem:	NE	69 ± 12	85 ± 10	86 ± 1	82 ± 1
	5-HT	150 ± 1	171 ± 9	181 ± 5	168 ± 4
Hypothalamus:	NE	106 ± 14	101 ± 13	115 ± 14	117 ± 23
	5-HT	275 ± 12	303 ± 14	84 ± 15	91 ± 11
Cortex:	NE	13 ± 0	7 ± 0	22 ± 0	9 ± 0
	5-HT	86 ± 1	94 ± 1	43 ± 1	49 ± 1
			100-Day-Old Rats		
Spinal Cord:	NE	135 ± 1	34 ± 1	92 ± 23	43 ± 21
	5-HT	415 ± 23	398 ± 21	116 ± 13	145 ± 30
Brainstem:	NE	332 ± 14	537 ± 23	221 ± 12	373 ± 22
	5-HT	658 ± 23	651 ± 22	139 ± 11	128 ± 10
Hypothalamus:	NE	1382 ± 64	1021 ± 34	701 ± 44	614 ± 23
	5-HT	1250 ± 71	1155 ± 43	361 ± 32	350 ± 30
Cortex:	NE	174 ± 12	60 ± 1	137 ± 11	43 ± 1
	5-HT	365 ± 22	368 ± 23	98 ± 2	98 ± 1

The data are for rats tested at 10 or 100 days of age and are the means and standard errors of 10 animals except for cells where tissue values were unavailable due to technical difficulty. In no case, however, was the cell n less than 8.

and CLON, $F(2,108) = 48.62$, $p<0.001$, which increased activity. There was also an interaction between PCPA treatment and CLON, $F(2,108) = 28.40$, $p<0.001$. As the figures show, PCPA had no effect on activity of pups who received vehicle but reduced the activating effects of CLON, particularly the 1000 μg/kg dose.

A separate ANOVA of only the PCPA-pretreated groups of Fig. 1B showed both a 6-OHDA pretreatment, $F(1,54) = 16.72$, $p<0.001$, and a CLON dose, $F(2,54) = 6.21$, $p<0.005$, effect. The results of pairwise comparisons showed that CLON at 100 and 1000 μg/kg significantly (p's<0.01) activated the 6-OHDA plus PCPA-pretreated rats but had no effect on the PCPA-only pretreated rats. Thus, PCPA pretreatment eliminated the activating effects of CLON in otherwise normal rats. It attenuated but did not eliminate the hypersensitivity of 6-OHDA-injected rats to CLON.

Adult rats. While ANOVA of the data in Fig. 1C showed only a main effect for CLON dose, $F(3,72) = 28.15$, $p<0.001$, multiple comparisons showed that all doses of CLON reduced activity in control rats ($p<0.05$), although in a biphasic manner. Neonatal treatment with 6-OHDA exaggerated the hypoactivating effect of the 100 μg/kg dose. Neonatal 6-OHDA-treated rats were significantly less active than their vehicle controls after receiving the 100 μg/kg dose of CLON ($p<0.01$).

Pretreatment with PCPA reduced activity in control and 6-OHDA-treated rats. It also abolished the hypoactivating effect of 100 μg/kg CLON in control rats and the hypoactivating effect of both 100 and 1000 μg/kg CLON in the 6-OHDA-treated rats. ANOVA incorporating the data of Figs. 1C and D showed a main effect of CLON dose, $F(2,108) = 44.40$, $p<0.001$, an interaction between neonatal treatment and CLON doses, $F(2,108) = 5.89$, $p<0.01$, and an interaction between PCPA pretreatment and CLON dose, $F(2,108) = 41.31$, $p<0.005$.

Hot-Plate Test

Ten-day-old rats. As Fig. 2A shows, CLON increased distress latencies in a dose-related manner in neonatal vehicle-injected rats. It had no statistically significant effect in 6-OHDA-injected rats. ANOVA of these data indicated a main effect for CLON, $F(3,72) = 5.87$, $p<0.025$, and a CLON by neonatal treatment interaction, $F(3,72) = 4.25$, $p<0.01$. Multiple comparisons indicated that while the 1000 μg/kg dose increased the latencies of the vehicle rats (up 35% over controls) ($p<0.05$), it had no effect on the 6-OHDA-treated rats.

The effect of PCPA was to eliminate CLON analgesia in 10-day-old rats (see Fig. 2B). ANOVA incorporating the data of Fig. 2B and as well the corresponding non-PCPA-treated groups from Fig. 2A indicated a main effect for CLON, $F(2,108) = 16.40$, $p<0.05$, a neonatal treatment × CLON, $F(2,108) = 5.01$, $p<0.01$, interaction and a CLON × PCPA interaction, $F(2,108) = 4.75$, $p<0.025$. Multiple comparisons indicated that while 1000 μg/kg CLON increased distress latencies in neonatal vehicle rats ($p<0.05$), this effect was eliminated by PCPA.

Adults. As shown in Fig. 2C, CLON caused a dose-dependent increase in distress latencies. Neonatal 6-OHDA injections had no

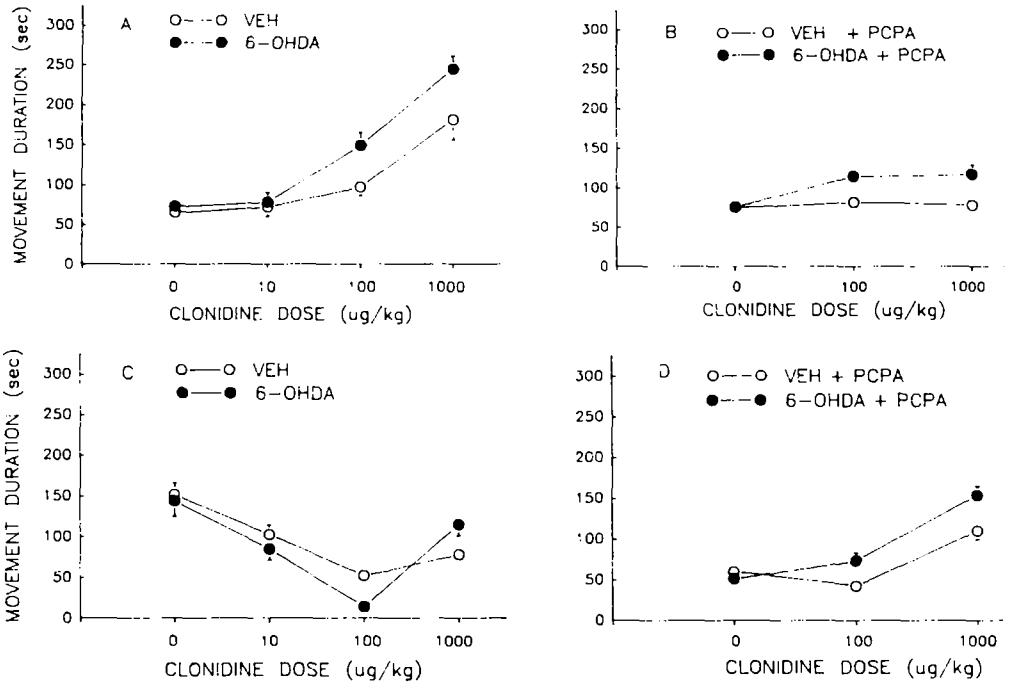

FIG. 1. The two top and bottom panels show the duration of movement scores (explained in text) for 10- and 100-day-old rats respectively after the indicated doses of CLON. The left panels present data for neonatal vehicle- and 6-OHDA-treated groups while the right panels show data for similar groups but who also received PCPA. Each point represents the average for 10 rats ± SEM.

effect by themselves or on the CLON effect. ANOVA showed only a main effect for CLON, $F(3,72) = 25.84$, $p<0.001$. Multiple comparisons showed that the 1000 μg/kg doses significantly elevated distress latencies in both vehicle- and 6-OHDA-treated rats ($p<0.05$). 6-OHDA tended to augment distress latencies over those of vehicle rats but this effect was not significant.

PCPA pretreatment by itself lowered hot-plate latencies, and as well eliminated the analgesic effects of CLON (see Fig. 2D). ANOVA incorporating these data and the corresponding groups from Fig. 2C showed main effects for CLON, $F(2,108) = 28.34$, $p<0.001$, and PCPA pretreatment, $F(1,108) = 15.55$, $p<0.01$, as well as an interaction between CLON and PCPA, $F(2,108) = 7.91$, $p<0.01$. Multiple comparisons showed that 1000 μg/kg CLON significantly ($p<0.05$) increased distress latencies in neonatal vehicle- and 6-OHDA-injected rats ($p<0.05$) but not when they had been pretreated with PCPA.

DISCUSSION

It was found here that CLON increased activity of otherwise normal 10-day-old rats but decreased activity of normal adult rats. This replicates previous findings from this and other laboratories (20,24). CLON also caused a dose-dependent increase in distress latencies at both ages. This probably reflected its analgesic effect in both infant and adult rats rather than its elicitation of behaviors which competed with the distress response. This interpretation is indicated by the fact that whereas the effects of CLON on activity were opposite for infant and adult rats, at both of these ages it increased distress latencies. Hence, it is difficult to argue that the increase in distress latencies reflects a general behavioral depression. While there are numerous publications which show CLON's analgesic effect in adult rats (3,4), we are unaware of any other demonstration of its analgesic effect in infant rats. Separation from the dam will, by itself, produce analgesia (7). It is conceivable that the effect of CLON reported here might have been even more apparent if no prior separation had occurred, or if the period of separation had been decreased. Even with a presumably higher baseline, we still found that CLON produced a significant increase in hot-plate response latencies in infant rats. Parenthetically, CLON has been reported to suppress isolation-induced distress vocalisation in 1-day-old domestic chicks (22).

Neonatal 6-OHDA had different and age-related consequences for the effects of CLON on the activity and hot-plate tests. By itself, neonatal 6-OHDA had no effect on activity. This agrees with earlier results (23,24). The 6-OHDA treatment significantly augmented the hyperactivating effects of CLON in 10-day-old rats and augmented the hypoactivating effect of 100 μg/kg CLON in adult rats. These results replicate previous observations from this laboratory (24). The mechanisms whereby CLON excites and inhibits activity in infant and adult rats respectively is unclear. It has been suggested by Hartley and Seeman (5) that CLON acts at postsynaptic alpha$_1$ receptors in neonatal rats and presynaptic alpha$_2$ receptors in adult rats. They based this on their observation that the maturation of mesolimbic alpha$_2$ receptors lags behind that of the alpha$_1$ receptor. While it is noteworthy that Morris *et al.* (12) reported concurrent ontogeny of these two receptors, their assays were carried out on whole brains (minus cerebella), so these results do not necessarily conflict with the regional analysis of receptor density of Hartley and Seeman.

Nomura and Segawa (14) have reported that phentolamine, phenoxybenzamine, yohimbine and piperoxan (but not the histamine receptor antagonist metiamide) all bocked CLON-induced hyperactivity in 7-day-old rats in a dose-related manner. All of these drugs can act at both alpha$_1$ and alpha$_2$ receptors. The first two are more potent at the alpha$_1$ while the latter two are more potent at the alpha$_2$ receptor. The relative potencies of these drugs for blockade of CLON's effect in neonates suggested to Nomura and Segawa that this blockade was mediated via alpha$_2$ receptors. They did point out, however, that the characterization of the receptor interactions of these drugs in neonates was (and remains)

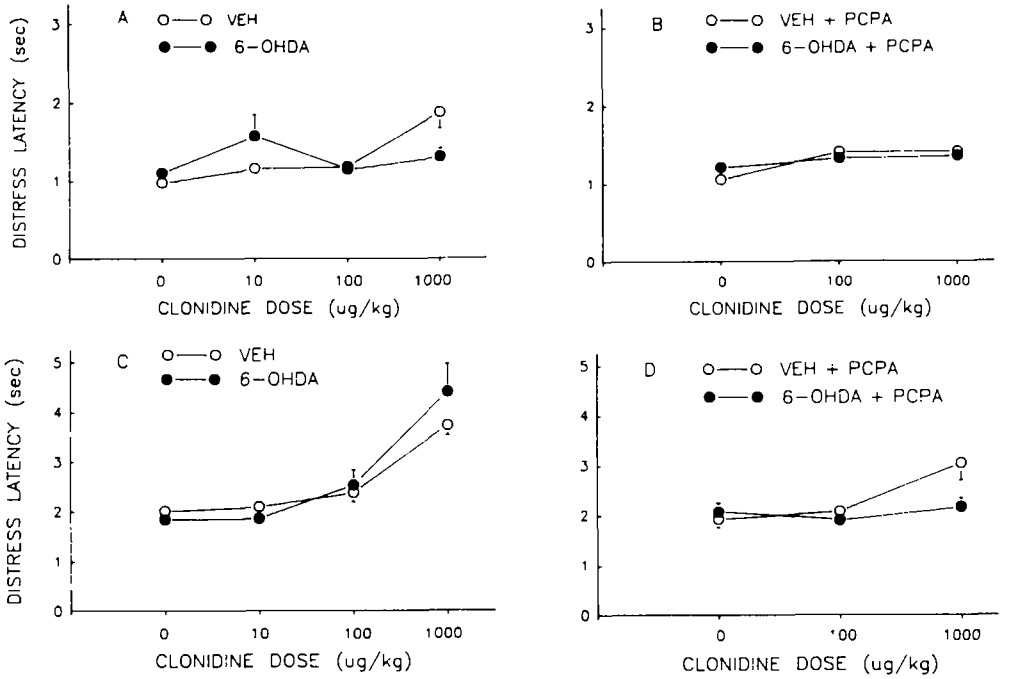

FIG. 2. The two top and bottom panels show the hot-plate distress latencies (explained in text) for 10- and 100-day-old rats respectively after the indicated doses of CLON. The left panels present data for neonatal vehicle- and 6-OHDA-treated groups while the right panels show data for similar groups but who also received PCPA. Each point represents the average for 10 rats ± SEM.

indeterminate so the possibility that CLON acts via alpha$_1$ receptors in the neonate could not be ruled out. In a subsequent experiment, Nomura *et al.* (15) reported that phentolamine, yohimbine and piperoxan antagonized CLON-induced hypoactivity in 20-day-old rats. Their potencies did not differ, but yohimbine and piperoxan were effective at relatively low doses, suggesting, but not compellingly so, that the blocking effect was via action at presynaptic alpha$_2$ receptors.

It is unlikely that presynaptic alpha$_2$ receptors located on noradrenergic terminals mediate CLON's sedative effect in adult rats, however, since intraventricular or locus coeruleus applications of 6-OHDA fail to eliminate this effect (6,13). Our findings here and in a previous study (24) that neonatal 6-OHDA also did not eliminate CLON's hyperactivating effect on neonates, indicates that like its sedative effects in adults, this effect is mediated via receptors (presumably alpha$_2$) which are not located on NE neurons. How the system changes with maturation is not clear. It could reflect the functional wiring of the receptors changing with age such that their activation in neonates causes hyperactivity while their activation in adults causes hypoactivity. Alternatively, the changes in behavioral response to CLON might reflect the sequential maturation of two independent neural systems. Future research should address this issue. Despite this dramatic reversal, the effect of neonatal 6-OHDA is to render these receptors supersensitive to CLON in both infancy and adulthood.

6-OHDA-induced NE depletions had no effect on baseline nociception, a finding which agrees with an earlier report (17). Neonatal 6-OHDA eliminated CLON analgesia in 10-day-old but had no effect on CLON analgesia for adult rats. Experiments show that intraspinal administration of CLON causes analgesia (1, 16, 19). The spinal cord could also mediate CLON's antinociceptive effect after it is administered systemically, although a supraspinal locus cannot be ruled out. Earlier research from this laboratory found that neonatal intraspinal 6-OHDA treatment of infant male rats had no effect on their sensitivity to CLON in adulthood (18). Our assays confirmed that neonatal systemic 6-OHDA also lesions spinal NE terminals. Hence, the results of the current experiment are consistent with our earlier findings—neonatal spinal NE depletion does not induce a heightened analgesic effect of CLON in the male rat, as assessed by the tail-flick procedure.

PCPA reduced CLON's hyperactivating effects in 10-day-old rats and eliminated its hypoactivating effect in adults. PCPA also eliminated CLON's analgesic effects for 10-day-old and adult rats. It may be argued that for the adult rats the NE depletions that resulted from the PCPA administration eliminated CLON's locomotor and analgesic effects. This logic is flawed in 2 respects. First, the NE depletions were relatively small, compared to the 5-HT depletions; secondly, 6-OHDA-induced NE depletions (which were larger than those produced by PCPA) did not eliminate but enhanced CLON's hypoactivating effect. CLON produced hyperactivity in adult PCPA-treated rats, a finding consistent with those of Herman *et al.* (6), strongly indicating that brain 5-HT systems are necessary for the hypoactivating effect of CLON. Further research with direct or indirect 5-HT agonists would provide unequivocal evidence for the necessity of 5-HT systems for CLON's effects; 5-HT receptor agonists should prevent while 5-HT receptor antagonists should enhance CLON's actions.

These results indicate that serotonin neurons are essential to the effects of CLON on activity and pain sensitivity. Furthermore, this is evident both in infancy and adulthood and occurred despite the reversal of CLON's effects on activity with maturation. Earlier work by Mabry and Campbell (10,11) had shown that PCPA caused hyperactivity and also potentiated amphetamine-induced activity in 15-day-old but not 10-day-old rats. This was interpreted to reflect the delayed maturation of serotonergic inhibition of behavioral arousal between 10 and 15 days. More recently, however, it has become evident that serotonin agonists and antagonists affect behavior in younger pups (21,25) and the results

presented here are consistent with early-maturing, behaviorally relevant serotonergic circuits.

In conclusion, we have shown that the locomotor and analgesic effects of CLON are dependent upon brain serotonin in neonatal as well as adult rats. In both neonates and adults, the locomotor effects are probably mediated through alpha$_2$ adrenoceptors which are not located on NE terminals but are postsynaptic to them. Since PCPA-induced 5-HT depletion reduced the effects of CLON, these postsynaptic alpha$_2$ receptors could reside on serotonergic neurons in both neonates and adults. Neonatal NE depletion exaggerated the locomotor response to CLON in neonatal and adult rats and this was also reduced by serotonin depletion. Thus, as measured by locomotor behavior, denervation supersensitivity of the alpha$_2$ adrenoceptors was evident in both infant and adult rats. Furthermore, it occurred despite the dramatic reversal with maturation of the locomotor consequence of activation of these receptors by CLON and it required intact serotonergic function for its behavioral expression. In contrast to these effects for locomotion, neonatal NE depletion eliminated CLON analgesia in 10-day-old rats, but had no effect on it during adulthood. Denervation supersensitivity of these receptors apparently does not develop after neonatal NE lesion, at least for the male rat (18).

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