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The Ontogeny of Spinal Cord Monoamines and the Post-Decapitation Reflex

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PAPPAS, B. A. AND R. INGS. *The ontogeny of spinal cord monoamines and the post-decapitation reflex.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 615–619, 1982.—The ontogeny of the post-decapitation reflex (PDR) was examined in neonatal systemic 6-hydroxydopamine (6-OHDA) treated rats. The PDR was absent in the youngest (5 day) and oldest (60 day) 6-OHDA treated rats but present with attenuated characteristics at around 15 and 20 days. The reappearance of the PDR in these rats closely paralleled age-related fluctuations in spinal cord noradrenaline (NE) which increased until 15 days, then precipitously declined, but did not correlate with spinal dopamine (DA) or serotonin (5-HT). The failure of the alpha noradrenergic blocking agents chlorpromazine, phenoxybenzamine, prazosin, or yohimbine to eliminate the vestigial PDR in the 15–20-day-old, 6-OHDA-treated rats indicates that this is probably not mediated by remaining, undamaged spinal NE neurons at this age. Neither is it likely to be mediated by spinal 5-HT neurons since inhibition of 5-HT synthesis had no effect upon the PDR latency in normal or neonatal 6-OHDA-treated, 17-day-old rats. In contrast to NE levels which increased with age in normal rats, spinal cord 5-HT and DA levels were at least as high at 5 days of age as at adulthood.

Spinal cord monoamines Ontogeny Post-decapitation reflex Locus coeruleus 6-Hydroxydopamine

WITHIN one to two seconds of decapitation the rat displays vigorous tail slapping followed by violent, co-ordinated flexions and extensions of the hindlimbs. This so-called post-decapitation reflex (PDR) lasts for about 20 seconds. The reflex was first described by Friedman [7] who suggested a monoaminergic mechanism on the basis of the observation that reserpine and chlorpromazine extended its latency, reduced its duration and disrupted the co-ordination of the hindlimb thrusts. Kamat and Sheth [12] further suggested that decapitation releases spinal interneurons which are normally receiving tonic, inhibitory input from descending, monoaminergic neurons.

Recent research has clearly shown that for the rat the trigger mechanism for the PDR requires intact spinal noradrenergic function [18, 19, 20, 21]. This may originate at least partly from the locus coeruleus (LC) and is blocked by alpha noradrenergic antagonists [19].

While the latency of the PDR is the same for rats from five days to adulthood, its duration shows a distinct maturational increase, with asymptote reached at about 20 days. At this age, spinal norepinephrine (NE) also reaches asymptote [22]. Surprisingly while neonatal 6-hydroxydopamine (6-OHDA) treatment initially reduces the PDR at five through 15 days of age, paradoxically the PDR is very much in evidence at 20 days in these rats but disappears as the rat approaches adulthood. The present experiment re-examined this unusual phenomenon and also determined whether the PDR shown by rats around 15–20 days is in fact dependent upon alpha-NE function. To determine the latter, the PDR was measured after administration of various alpha-adrenergic receptor blocking drugs since such drugs have been reported to block the PDR in adult rats [19]. The effects of the 5-HT synthesis inhibitor, parachlorophenylalanine (PCPA) on the PDR of rats of this age was also assessed because research on the adult mouse has indicated that 5-HT may be involved in at least the modulation of its duration [17,24]. Since our earlier work had indicated that the PDR is not eliminated at 15–20 days in the rat by neonatal 6-OHDA, it seemed important to determine whether this refractiveness to NE depletion reflects mediation by 5-HT neurons at this age.

METHOD

Animals

The subjects were the offspring of Wistar dams delivered 15–16 days pregnant from Canadian Breeding Farms. Dams and offspring were housed in plastic maternity cages on a reversed day-night cycle (lights on at 2000, off at 0800). Within 12 hr of birth, runts were discarded and all litters were randomly cross-fostered and culled to five males and females. At that time and again 24 hr later, each pup received either 0.05 ml saline vehicle plus ascorbic acid (0.5 mg/ml) or vehicle plus 6-OHDA hydrobromide, (100 mg/kg based on the weight of the whole litter). The injections were subcutaneous and delivered dorsally between the shoulder blades.

PDR Ontogeny

At 5, 10, 15, 20, 30 or 60 days of age, 30 rats from either of

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these two treatments were weighed and decapitated with a small animal guillotine (Harvard Apparatus) and placed into a 4000 ml beaker. The latency from decapitation to the first bilateral hindlimb kick as well as the total number of kicks were recorded.

When muscle relaxation was apparent in the carcass a uniform sample of the thoracic cord (T1 to T10 since other research in our laboratory has shown that monoamine depletions here accurately reflect changes throughout the entire cord) was removed and stored in liquid nitrogen until assayed. The sample of cord tissue from 10 rats from either treatment group at each age up to 30 days of age were separately assayed within two weeks for either NE, DA or 5-HT using minor modifications of previously described fluorometric procedures [3,15]. These three amines were assayed simultaneously in tissue samples from 10 6-OHDA and 10 vehicle-treated rats at 60 days of age.

Drug effects on the PDR of 17-day-old rats. Rats who had been neonatally pretreated with either vehicle or 6-OHDA as described above were injected with the following drugs and their PDR's recorded at 17 days of age: chlorpromazine (10 mg/kg, IP, 30 min before decapitation) which very effectively blocks the PDR in adults presumably due to its antagonism of alpha adrenoreceptors [19], yohimbine (2.0 mg/kg, IP, 30 min beforehand) a predominantly presynaptic alpha blocker [1], phenoxybenzamine (20 mg/kg, IP, 45 min beforehand) which exerts mainly postsynaptic alpha receptor antagonism [13], and prazosin (5.0 mg/kg IP, 105 min beforehand). Prazosin is a selective postsynaptic alpha antagonist [2]. While only single doses of each drug were used, each dose was selected from the literature to exceed by several fold that required for effective receptor block.

Additional groups of rats were injected with PCPA (methylester, 300 mg/kg IP) at 48 and 24 hr prior to decapitation. Whole brains and spinal cords of these rats were assayed for NE and 5-HT.

RESULTS

6-OHDA Effects on Spinal Monoamines

Figure 1 shows the levels of spinal cord NE, DA and 5-HT across age for vehicle control and 6-OHDA groups. Analysis of variance showed no effect of 6-OHDA on either DA or 5-HT levels although consistent with Saari *et al.* [22], the levels of these amines were slightly and transiently elevated after this treatment. Spinal DA levels significantly declined overall with age ($p<0.001$), with the levels at 30 and 60 days lower than those at 5 and 10 days ($p<0.05$, Tukey's test). Spinal 5-HT levels also changed with age ($p<0.01$), although rather than a simple decline, only the levels at 30 days were lower than those at 10 days ($p<0.05$, Tukey's test). Analyses of the NE data showed a significant reduction after 6-OHDA ($p<0.001$) and an interaction between age and drug treatment ($p<0.01$). As can be seen from Fig. 1, this interaction occurred because the 6-OHDA treatment did not significantly lower NE levels until 20 days of age and beyond. Thus, the 6-OHDA rats, like the controls, showed an increase in spinal NE until 15 days but unlike the controls, demonstrated a precipitous decline thereafter.

6-OHDA Effects on the PDR

Analysis of the PDR latency scores showed a significant increase due to 6-OHDA ($p<0.001$) and also an interaction between age and treatment ($p<0.001$). As shown in Fig. 2, latencies were constant across age in the control rats while for the 6-OHDA rats they were a u-shaped function of age. At five and 60 days of age none of these rats showed a PDR while between 15 and 30 days their latencies averaged around 13–14 sec.

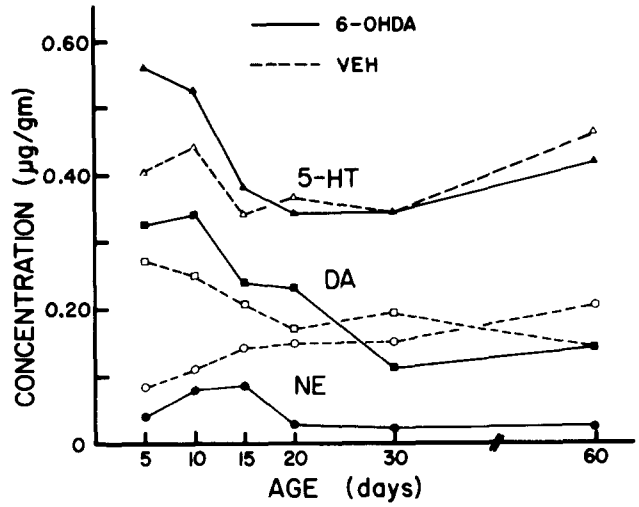

FIG. 1. Spinal cord concentrations of NE, DA and 5-HT as a function of age for neonatal 6-OHDA and vehicle-treated rats. Each point represents the mean of ten rats. Separate groups of rats were assayed for each amine, except at 60 days when the tissue was assayed for all three amines.

The 6-OHDA treatment reduced the number of bilateral kicks during the PDR ($p<0.001$). There was also an interaction ($p<0.001$) between treatment and age. As Fig. 2 shows, this was due to the fact that while kick frequency increased with age until 20 days for both groups, there was only a slight decline after this age for the controls and a dramatic decline to zero frequency by 60 days in the 6-OHDA injected rats.

Drug Effects on the PDR of 17 Day Old Rats

As shown in Table 1, the PDR latency was increased and kick frequency was decreased significantly by chlorpromazine, phenoxybenzamine, prazosin and yohimbine (p's<0.05, t-test) in control rats. In 6-OHDA treated rats, the PDR was further attenuated but not eliminated by chlorpromazine (kick frequency reduced), phenoxybenzamine (latency further extended) and yohimbine (kick frequency further reduced). Surprisingly, while prazosin totally eliminated the PDR in the controls, it did not do so in the 6-OHDA rats although kick frequency was further reduced in these animals.

PCPA had no effect on the PDR in vehicle rats. Furthermore, no effect of this treatment on PDR latency was found for the 6-OHDA rats although it did reduce their number of kicks. PCPA reduced (p's<0.001, t-test) whole brain 5-HT and spinal cord 5-HT in both the vehicle and 6-OHDA groups but did not affect brain or spinal cord NE (see Table 2).

DISCUSSION

The latency of the PDR in normal rats was found here to be constant across age while the bilateral kick frequency

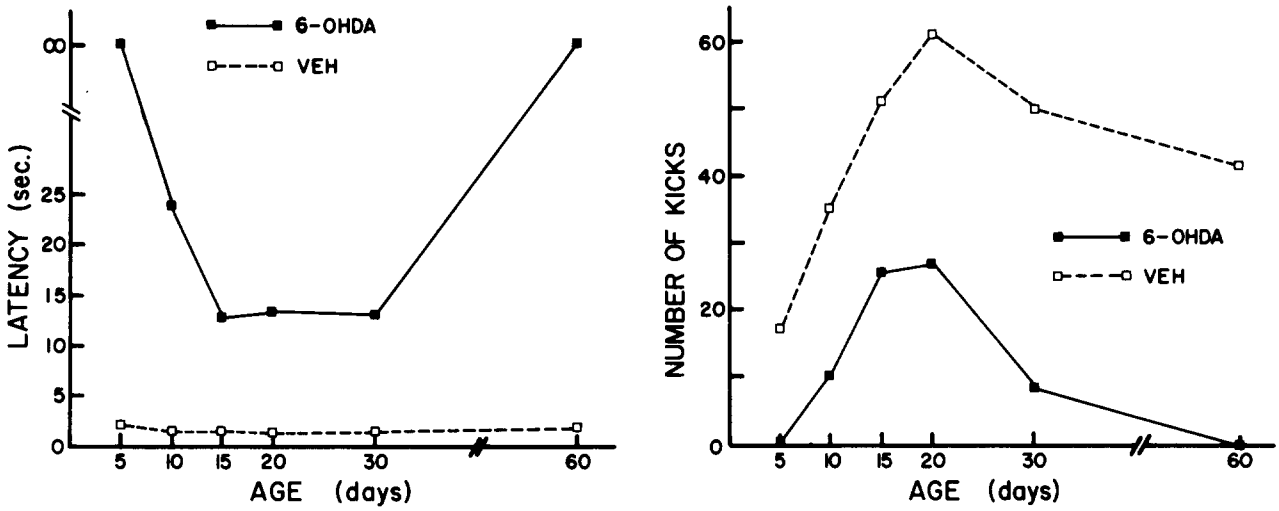

FIG. 2. The PDR latency (left panel) and kick frequency (right panel) as a function of age for neonatal 6-OHDA and vehicle-treated rats. Each point is the mean of 30 rats.

increased until around 20 days. It subsequently declined slightly. The PDR was eliminated by neonatal systemic 6-OHDA in the youngest (five days) and oldest (60 days) rats tested, whereas it was only attenuated at other ages, particularly around 15 and 20 days. The reappearance of the PDR in the 6-OHDA rats closely mirrored the increase in spinal NE in these rats while the subsequent disappearance paralleled the precipitous decline in this amine beyond 20 days. While these correlations between the PDR and age-related changes in spinal NE are consistent with a noradrenergic trigger mechanism, they do not explain the reappearance of the PDR around 15 days. Thus, despite the obvious attenuation of the PDR by the alpha one adrenoreceptor blocking drugs chlorpromazine, phenoxybenzamine and prazosin and the alpha two blocking agent yohimbine in control rats at 17 days, these drugs further attenuated but did not eliminate the residual PDR in 6-OHDA-treated rats. In fact, prazosin, which totally eliminated the PDR in control vehicle rats did not do so in the 6-OHDA rats. Conceivably this could reflect proliferation of spinal alpha one receptors after 6-OHDA, this effect causing a net increase in the dose of prazosin required for effective receptor block. The dose of prazosin used here was very large, however, and unlikely to be insufficient to block an increased receptor complement which typically increases about 50% in denervated noradrenergic systems such as the cerebral cortex [8]. Furthermore, results from this laboratory indicate that neonatal systemic 6-OHDA causes a modest reduction rather than an increase in spinal alpha and beta noradrenergic receptors (Peters, Pappas and Ings, unpublished observations). Thus the reappearance of the PDR, albeit attenuated, in 6-OHDA rats of this age does not depend upon a temporary reinstatement of spinal alpha noradrenergic function. Neither is it likely to be mediated by spinal 5-HT neurons as earlier work might suggest since PCPA was ineffective towards eliminating the onset of the reflex of control and 6-OHDA rats at this age. There was, however, a modest reduction of bilateral kick frequency in the PCPA-treated, 6-OHDA rats. In adult rats, serotonin reduction reduces the vigor of the reflex but has no effect upon latency or duration [19]. Since 6-OHDA itself drastically reduced the reflex vigor in our young rats, a further reduction with PCPA may have rendered some bilateral kicks too indistinct for accurate counting in this experiment. We conclude that the PDR of weanling rats is cooperatively mediated by at least two neurochemical systems, of which one is alpha noradrenergic possibly originating in the LC [19] and exerting a tonic, inhibitory input upon spinal motor interneurons [12]. The identity of the second non-noradrenergic mediator does not seem to be serotonin and remains to be elucidated.

TABLE 1

THE EFFECTS OF SALINE, CHLORPROMAZINE (CPZ, 10 MG/KG, IP, 30 MIN PRIOR), PHENOXYBENZAMINE (PBZ, 20 MG/KG, IP, 45 MIN PRIOR), YOHIMBINE (YOH, 2.0 MG/KG, IP, 30 MIN PRIOR), PRAZOSIN (PRA, 5.0 MG/KG, IP, 105 MIN PRIOR), AND PARACHLORAPHENYLALANINE (PCPA, 300 MG/KG, IP, 48 AND 24 HR PRIOR) ON THE PDR IN VEHICLE (V) OR NEONATAL 6-OHDA PRETREATED 17-DAY-OLD RATS

	PDR Attribute	
Treatment	Latency	No. of Kicks
V-saline	1.5 ± 0.1	62.1 ± 1.3
6-OHDA-saline	14.5 ± 0.6*	40.3 ± 2.0*
V-CPZ	17.9 ± 1.0*	30.5 ± 1.9*
6-OHDA-CPZ	16.9 ± 1.4*	29.0 ± 3.1
V-PBZ	10.4 ± 1.2*	39.9 ± 4.3*
6-OHDA-PBZ	17.7 ± 1.1*	31.6 ± 1.8*
V-PRA	30.0*‡	0.0
6-OHDA-PRA	17.5 ± 0.8*†	24.8 ± 1.7*†‡
V-YOH	9.8 ± 1.0*	52.3 ± 3.7*
6-OHDA-YOH	15.5 ± 0.9	25.1 ± 1.8*
V-PCPA	1.2 ± 0.1	63.8 ± 0.9
6-OHDA-PCPA	11.8 ± 1.9*†	27.0 ± 1.4*†‡

The data are presented as mean ± SEM.
*Significantly different from V-saline.
†Significantly different from corresponding V group.
‡Significantly different from 6-OHDA-saline group.

TABLE 2

MEAN (±SEM) WHOLE BRAIN AND SPINAL CORD NE AND 5-HT LEVELS (μg/g) FOR NEONATAL VEHICLE (VEH) OR 6-OHDA (6-OHDA) AFTER TREATMENT AT 17 DAYS WITH EITHER PCPA OR ITS VEHICLE (VEH)

	Brain		Spinal Cord	
Treatment	NE	5-HT	NE	5-HT
Veh-Veh	0.23 ± 0.01	0.45 ± 0.01	0.52 ± 0.01	0.96 ± 0.04
Veh-PCPA	0.23 ± 0.01	0.16 ± 0.01*	0.48 ± 0.01	0.31 ± 0.01*
6-OHDA-Veh	0.07 ± 0.01	0.40 ± 0.01	0.04 ± 0.01	0.94 ± 0.02
6-OHDA-PCPA	0.07 ± 0.01	0.11 ± 0.01*	0.05 ± 0.01	0.30 ± 0.03*

*Indicates significant ($p<0.01$, t-test) effects of PCPA upon 5-HT levels compared to the same neonatally treated group.

One striking result of this and our earlier [22] experiment is the increase in spinal NE until 20 days in the 6-OHDA rats and its precipitous decline thereafter. These NE terminals most likely arise from the LC [19]. The LC also contributes the NE innervation of the cortex and in this region, beta noradrenergic [8] and both alpha one and alpha two [16] noradrenergic receptors peak around 20 days. It would be valuable to quantitate spinal receptor numbers at this age in our 6-OHDA treated rats. It seems that the spinal noradrenergic system may reach a programmed milestone at this age and that the decline in spinal NE in our 6-OHDA rats then reflects a final down-regulation of the number of terminals or the NE content of remaining terminals. Behaviorally, this decline is reflected by a permanent loss of the PDR. Thus, if one interprets the co-emergent rise in spinal NE and reappearance of the PDR as reflecting CNS plasticity or compensatory adaptation to neural damage, then it must be concluded that this plasticity is short lived. The reversal, of unknown mechanism, ultimately renders the newborn rat as no more capable of recovering from spinal monoamine lesion than the adult rat.

Finally, it should be noted that the levels of spinal 5-HT and DA reported here for the developing rat are considerably lower than this laboratory indicated earlier [22] and are more consistent with other published values for 5-HT [5,11] and particularly DA [4,5]. Despite the discrepancy in absolute values, this experiment is consistent with our earlier report insofar as spinal 5-HT and DA levels are at peak levels in the youngest age group (five days). These results for 5-HT are consistent with other indications of highest concentration of this monoamine in the early postnatal period [8,14]. Insofar as we can determine, there are no corresponding reports for DA.

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