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Relationship of Brain Levels of Norepinephrine and Dopamine to Avoidance Behavior in Rats after Intraventricular Administration of 6-Hydoxydopamine[1]

LANE G. LENARD[2] AND BERNARD BEER

The Squibb Institute for Medical Research
Princeton, New Jersey 08540

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LENARD, L. G. AND B. BEER. *Relationship of brain levels of norepinephrine and dopamine to avoidance behavior in rats after intraventricular administration of 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 3(5) 895–899, 1975. – Rats trained on a discriminated avoidance procedure showed long-term decrements in performance after intraventricular administration of 6-hydroxydopamine. Biochemical assay showed that DA levels were correlated with avoidance performance no matter when behavior was measured, but NE levels were most highly correlated with avoidance behavior at those times when suppression of responding appeared to be the major influence on responding. The data are discussed in terms of possible separate roles for NE and DA in avoidance behavior.

6-Hydroxydopamine Avoidance Norepinephrine Response suppression Dopamine Catecholamines

THE long-lasting decrease in avoidance performance in rats after the central administration of 6-hydroxydopamine (6-HD) [5,11] appears to involve two distinct factors. Shortly after treatment with 6-HD, there is a transient decrease in responding in general, which results in an early decrement of moderate severity. These rats also exhibit an overreaction to aversive stimuli [10, 12, 15] that results in freezing, a response that is usually incompatible with avoidance. This second factor seems to be responsible for the longevity and severity of the avoidance decrement [2].

That both dopaminergic and noradrenergic neurons are involved in avoidance has been demonstrated in experiments in which 6-HD-induced response decrements were reversed by the dopaminergic agonists DA, apomorphine, or L-DOPA, as well as by the noradrenergic agonists L-NE and clonidine [10]. The present experiment was a further attempt to determine the relationship, in rats treated with 6-HD, between NE and DA levels and avoidance behavior by correlating the degree of depletion of these amines with the extent of the avoidance decrement. Avoidance behavior was measured in each of 4 different ways: (1) the median percent avoidance of all post-6-HD-treatment sessions, a measure of overall behavior; (2) the percent avoidance for the first session after 6-HD treatment, because avoidance performance during this session should be least influenced by response suppression [2]; (3) the lowest percent avoidance during the first 4 sessions after 6-HD-treatment, because performance at this time appears to be maximally influenced by response suppression [2]; and (4) the highest percent avoidance after the fourth session, a point representing the maximum recovery of avoidance after treatment with 6-HD.

METHOD

Animals

Twenty male Sprague-Dawley (Holtzman) rats, 200–250 g, were housed in individual cages and were maintained on a 12 hr light-dark cycle. Food and water were available ad libitum.

The rats came from 2 groups. Eleven rats were tested 2 or 3 times per week for about 10 weeks. Nine rats were tested daily for 5 or 6 weeks. Some rats received occasional injections of noradrenergic or dopaminergic agonists, but

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[2] Much of these data were used by L. G. Lenard to partially fulfill the requirements for the Ph.D. degree at Rutgers, The State University.

data from these sessions were not included in the present analysis.

Procedure

Surgical procedure. Each rat was implanted with a permanent cannula (Plastic Products, Co.) in either the left or right lateral ventricle. The details of the procedure have been described elsewhere [10]. Briefly, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal® – Abbott), 25 mg/kg, and chloral hydrate, 150 mg/kg. They were placed in the stereotaxic instrument (David Kopf Instruments), using earbars designed to avoid puncture of the eardrum. The coordinates for implantation into the lateral ventricle were AP = +7.0, Lat. = ±2.0, DV = +7.0, according to the atlas of Albe-Fessard *et al.* [1]. Training did not begin until at least 7 days after surgery.

Avoidance procedure. Avoidance behavior was measured in shelf-jump avoidance chambers. Shock (2.0 mA, provided by a BRS/Foringer SG-901 shock generator) was delivered to the grids at appropriate times via a shock-scrambler circuit.

Each test session consisted of 50 trials. A trial was initiated by the presentation of the CS (withdrawal of a wall and consequent exposure of a shelf, with accompanying noise and vibration). If the rat jumped onto the shelf within 10 sec after the start of the CS, thus tripping a microswitch under the shelf, shock was avoided and that trial was terminated. A 20 sec intertrial interval (ITI) followed. For the first 10 sec of the ITI, the rat was permitted to remain on the shelf, but was then gently pushed off the shelf by the moving wall, back onto the grids, where it was allowed 10 sec more before the start of the next trial. If the rat failed to jump onto the shelf within 10 sec after start of the CS, a series of shock pulses (0.5 sec in length, separated by a 2 sec shock-shock interval) was initiated. If the rat then jumped onto the shelf, it escaped from the shock, ending the trial and initiating an ITI, as described above. A maximum of 10 shocks was presented if no escape response occurred.

These rats had been trained to a criterion of at least 90 percent avoidance during two consecutive sessions. When the criterion had been met, they were injected intraperitoneally with pargyline (Eutonyl® – Abbott), 50 mg/kg, 30 min prior to receiving an intraventricular injection of 6-HD (Regis), 250 μg. A second intraventricular injection of 6-HD, 250 μg, was given 24 hr later, without pargyline pretreatment. The intraventricular injection procedure has been described in detail elsewhere [10].

NE and DA levels (as percent of control) found in these animals were correlated with the four measures of avoidance performance noted above by use of the Pearson Product-Moment correlation procedure [7]. Correlation coefficients were tested for significance according to the procedure described by Edwards [6].

Biochemical assay procedure. For the purposes of the biochemical assay, 4 rats of the same age and weight as those receiving 6-HD received no drug treatment and were designated as controls. Two of these rats, each with an implanted cannula, were tested in the shelf-jump procedure in the standard manner. The other 2 rats were housed in individual cages for the duration of the experiment, but were never tested. Since no significant differences in CA levels were found among these control rats, the data from the assays of their brains were pooled.

About 3 weeks after the conclusion of the experiment, all the rats were decapitated and their brains were excised and immediately frozen on dry ice. NE and DA were measured fluorometrically by the trihydroxyindole procedure. Whole brains were homogenized in 4 volumes of fresh 0.4 N perchloric acid at 0–4°C, as described by Brodie *et al.* [3]. The homogenized samples were centrifuged for 20 min at 9000 rpm. The CA's were adsorbed onto alumina at pH 8.5, then eluted onto 0.1 N acetic acid and oxidized according to the method described by Chang [4]. After oxidation, the samples were read for NE on a spectrophotofluorometer (Aminco-Bowman) at excitation wavelength 385 mμ/emission wavelength 485 mμ. The samples were stored overnight under a fluorescent light and read the next day for DA at 320/380 mμ.

RESULTS AND DISCUSSION

CA levels for the 20 rats treated with 6-HD and pargyline are shown in Table 1. It was assumed that the CA levels at the time of the assay, several weeks after treatment with 6-HD, had not changed substantially since the period of avoidance testing. It has been shown that no recovery of brain CA levels occurs for at least 142 days after treatment with 6-HD [15].

The 20 rats treated with 6-HD and pargyline in the present experiment showed the typical progressive decline in avoidance responding that has been described elsewhere [2,10]. The correlation coefficients obtained are shown in Table 2. Table 2A shows that brain levels of NE and DA were correlated only slightly with the median percent avoidance for the entire test period after treatment with 6-HD, a measure of overall behavior. It is important to note, though, that these correlations were nearly the same for both monoamines. The correlation between DA and median percent avoidance was significant, but only marginally so. The correlation of this behavioral measure with the combination of NE and DA levels was slightly higher than the correlation of this measure with either CA alone. These results suggest that both NE and DA levels were about equally related to overall performance, as measured over a long period, but the summary of the behavior in this way makes these relationships appear to be minor ones.

It has been shown [2], however, that the probability of an avoidance response occurring after treatment with 6-HD tends to vary with the time of measurement. This variability might have accounted for the poor and apparently equal correlations of NE and DA with median percent avoidance. It seemed more appropriate, then, to correlate CA levels with avoidance performance at various, apparently crucial, stages of testing. These relationships are seen in the next 3 sets of correlations. When the measure of avoidance behavior was the percent avoidance during the first session after 6-HD treatment (Table 2B), NE levels were completely unrelated to performance, whereas the correlation of DA levels with performance was 0.40, which was statistically significant. Beer and Lenard [2] proposed that the decrease in avoidance during this first session after treatment with 6-HD was primarily a function of a decrease in conditioned responding and that the influence of hyper-responsiveness to aversive stimuli would be minimal at this time. This set of correlations suggests that brain levels of NE may be unrelated to this general decrease in

TABLE 1

LEVELS OF NE AND DA IN WHOLE BRAIN OF RATS AFTER ADMINISTRATION OF 6-HD + PARGYLINE

	NE		DA	
Rat	ng/g	Percent of Control	ng/g	Percent of Control
485	13.6	3	89.2	12
486	142.8	32	89.2	12
487	176.7	40	175.5	24
489	23.8	5	75.9	10
490	34.0	8	89.2	12
491	57.8	13	138.5	19
492	40.1	9	38.0	5
493	23.8	5	75.9	10
494	57.8	13	127.2	17
495	328.0	73	100.6	14
496	17.0	4	n.d.*	0
415	168.2	38	465.0	32
418	69.7	16	n.d.	0
420	10.2	2	n.d.	0
425	3.4	1	n.d.	0
426	139.4	31	1328.5	92
427	69.7	16	637.7	44
429	64.6	14	578.8	40
433	91.8	21	607.3	42
434	69.7	16	235.3	16

*Not detectable; below the level of sensitivity of the assay

behavior, but that brain levels of DA are, at least partially, related to it.

The third set of correlations (Table 2C) was between NE and DA brain levels and the minimum level of avoidance performance reached during the first four sessions. This minimum level of responding, which was reached after a mean of 3.2 sessions, represented a point of maximum influence for the response suppression factor. The coefficient for the correlation between the minimum level of avoidance at this time and NE level was 0.83, ($p<0.005$), whereas that between DA and performance was 0.50 ($p<0.025$). Thus, the level of NE in the brain, although unrelated to performance during the first session, when freezing was a small factor, was highly related to performance when freezing appeared to be at a peak. The lower the level of NE in the brain, the less chance there was of an avoidance response occurring.

The coefficient for the correlation between the maximum percent avoidance reached after Session 4 and the level of NE in the brain was 0.85 ($p<0.005$), whereas the corresponding coefficient for the correlation with the level of DA was only 0.32 (n.s.). Thus, the extent of recovery

TABLE 2

CORRELATION OF CA LEVEL AND FOUR MEASURES OF AVOIDANCE PERFORMANCE IN RATS TREATED WITH 6-HD + PARGYLINE

	Measure of Avoidance Behavior After Treatment with 6-HD	Catecholamine*	Correlation Coefficient (r)	Probability
A	Median percent avoidance all control sessions	NE	0.34	n.s.
		DA	0.38	$p<0.05$
		(NE + DA)	0.45	$p<0.025$
B	Percent avoidance Session 1	NE	0.04	n.s.
		DA	0.40	$p<0.05$
		(NE + DA)	0.29	n.s.
C	Minimum percent avoidance Sessions 1–4	NE	0.83	$p<0.005$
		DA	0.50	$p<0.025$
		(NE + DA)	0.43	$p<0.05$
D	Maximum percent avoidance after Session 4	NE	0.85	$p<0.005$
		DA	0.32	n.s.
		(NE + DA)	0.15	n.s.

*NE and DA levels were calculated as percent of control.

after 6-HD treatment also appears to be largely a function of the level of NE remaining in the brain; the greater the level of NE in the brain, the greater the maximum level of avoidance was likely to be.

The results of the present experiment, in which both NE and DA were depleted, suggest strongly that NE and DA play separate roles in the maintenance of avoidance behavior. Moreover, these roles correspond, at least in part, to the two processes that seem to be responsible for the decrease in avoidance behavior after the administration of 6-HD. Levels of DA, but not of NE, in the brain were moderately related to avoidance at each point that performance was assayed, including the first session after treatment, when the influence of response suppression should have been minimal. Therefore, the integrity of DA neurons may be related primarily to the likelihood of responses occurring.

Freezing behavior appeared to compete successfully with avoidance behavior, no matter when the performance was assayed, apparently as a result of both the animals' inability to make an avoidance response and the animals' overreaction to the aversive nature of the CS and the shock [2]. This overreaction was primarily a function of the level of NE in the brain; the less NE remaining in the brain, the less likely the animal was to avoid. Furthermore, the maximum degree of recovery of avoidance behavior after the initial decrease was also related primarily to the level of NE. This finding suggests that the recovery of behavior was proportional to the amount of NE remaining in the brain.

The relationship between brain levels of NE and the overreaction to aversive stimuli observed in the present experiment is similar to other data showing that depletion of NE by 6-hydroxydopa [8, 13, 14] apparently increased emotionality in rats in mildly aversive situations. Such a role for NE might also help to explain the mode of action of clonidine in improving avoidance behavior in rats treated with 6-HD [9]. If interference with NE transmission caused hyperemotionality and a concomitant increase in suppression of responding, and this resulted in decreased avoidance responding in rats treated with 6-HD, then clonidine might have decreased emotionality by stimulating noradrenergic receptors in the brain selectively. This selective stimulation would then have been reflected in less suppression of responding and, consequently, more avoidance.

An hypothesis relating NE and emotionality might also

be helpful in explaining the beneficial effect of diazepam (DZP) on avoidance performance in rats treated with 6-HD [2]. Nakamura and Thoenen [12] noted that the degree of increased irritability after treatment of rats with 6-HD was correlated with the extent of depletion of brain NE. Moreover, the administration of DZP was found to decrease the irritability and to increase the rate of ^{3}H-NE turnover in rats treated with 6-HD. Thus, clonidine and DZP may both have been acting on a common neurochemical substrate when they improved avoidance performance.

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