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Neurochemical Basis of the Dorsal Bundle Extinction Effect

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MASON, S. T. AND H. C. FIBIGER. *Neurochemical basis of the dorsal bundle extinction effect.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 373–380, 1979.—Injection of 6-hydroxydopamine into the mesencephalon of the rat has been found to cause resistance to extinction on continuously reinforced schedules. The neurochemical basis of this effect was investigated by using another concentration of 6-hydroxydopamine and by another position of injection. Severe depletion of forebrain noradrenaline was found after these injections with no change in dopamine, serotonin, cholinergic or GABAergic parameters in any brain area measured. The noradrenergic nature of the effect was further shown by the reversal of the usual behavioural effect following pretreatment with a noradrenaline uptake inhibitor (desimipramine, 25 mg/kg 30 min prior to intracerebral injection of 6-hydroxydopamine). This rules out non-specific damage caused by the 6-hydroxydopamine as the neurochemical basis of the dorsal bundle extinction effect. Failure to find resistance to extinction after either kainic acid or 5–7 dihydroxytryptamine injection seems also to exclude respectively cell body loss at the injection site or damage to serotonergic systems. It is concluded that the dorsal bundle extinction effect is noradrenergic in nature.

Locus coeruleus Dorsal noradrenergic bundle Noradrenaline Extinction CRF Kainic acid 5–7 DHT Serotonin

MASON and Iversen [25] reported in 1975 that injection of small quantities of the catecholamine neurotoxin 6-hyroxydopamine [34] (6-OHDA) into the mesencephalic course of the ascending dorsal noradrenaline (NA) fibres [33] in the rat leads to resistance to extinction on a continuously reinforced (CRF) runway response. This dorsal bundle extinction effect (DBEE) has subsequently been seen in other paradigms such as extinction of CRF lever pressing [16,31], extinction of a complex motor manipulative task [17], of a go/no-go alternation task [32], of a fixed interval schedule [18], of one way [8], two way [19] and passive avoidance [20], in extinction of a conditioned emotional response [21] and in extinction of the exploratory response [22].

Although 6-OHDA in the appropriate dose and concentration is now accepted as a highly specific manipulation for noradrenergic systems [10, 29, 35], other authors initially claimed that it was no more specific than injection of copper sulphate [27] or an electrolytic lesion [3]. It thus appeared necessary to demonstrate conclusively that the DBEE is due to loss of forebrain NA and not due to non-specific effects, damage to cell bodies at the injection site or interference with other neurotransmitter systems such as dopaminergic, serotonergic, cholinergic or GABAergic as a result of the intracerebral injection.

To achieve this aim two experiments were performed. In the first the concentration of 6-OHDA was halved, that of the ascorbic acid antioxidant reduced fivefold and a new injection site, still on the trajectory of the dorsal bundle but anterior to that used by Mason and Iversen [25], utilised. It would be surprising if any non-noradrenergic system damaged by the 6-OHDA was also present at this more anterior level of the dorsal NA bundle system. In addition, it has been reported that the NA uptake inhibitor, desipramine (DMI) blocks the destructive effects of 6-OHDA on NA neurones [7], which depend for their action on uptake of 6-OHDA into the NA neurone [11], but does not prevent any non-specific effects of the 6-OHDA which are believed to involve extraneuronal superoxide radicals or hydrogen peroxide [30]. Thus, if DMI pretreatment is effective in preventing the usual DBEE, this would indicate a noradrenergic basis, but if the DBEE still occurred, non-specific damage would be implicated. Measurement of a variety of neurochemical parameters of other transmitter systems, such as dopamine, serotonin, acetylcholine and GABA, were carried out to determine the specificity of action of the 6-OHDA injections.

In a second experiment, more direct controls for incidental loss of cell perikarya at the injection site or damage to another amine system, the serotonergic pathways, were carried out by injecting at the same coordinates as the 6-OHDA either kainic acid, which has a preferential action on cell bodies while allowing a considerable degree of sparing of fibres of passage [5,14], or 5–7 dihydroxytryptamine (5–7 DHT) which is much more destructive of serotonin systems than is 6-OHDA [1,6]. If either manipulation produced resistance to extinction, it would indicate a non-noradrenergic basis of the DBEE.

EXPERIMENT 1

The original demonstration by Mason and Iversen [25] of the DBEE used 8 micrograms of 6-OHDA dissolved in 2 microlitres of 0.9% saline containing 1 mg/ml ascorbic acid antioxidant injected bilaterally into the dorsal noradrenergic bundle at the level of the dorsal raphe. To determine if the DBEE could be obtained at another site along the course of the dorsal bundle or whether it was due to some other structure near the raphe, we reduced the concentration of 6-OHDA to 4 micrograms in 2 microlitres, reduced the concentration of ascorbic acid to 0.2 mg/ml and moved the injection site anterior to the level of the substantia nigra. A further demonstration of the noradrenergic nature of the effect was attempted by pretreating another group of rats with DMI in order to prevent the selective damage to NA systems [7]. Both groups were then tested on acquisition and extinction of a CRF lever pressing response and post-mortem assays conducted on a number of neurochemical markers in a variety of brain regions.

A group of unilaterally injected rats was also prepared in order to determine if this manipulation would be effective in causing resistance to extinction.

METHOD

Surgical

Dorsal bundle 6-OHDA (DBA). Ten male albino Wistar rats (Woodlyn Farms, Ontario) weighing 300 g were anaesthetised with Nembutal (50 mg/kg IP), positioned in a stereotaxic apparatus (David Kopf Instruments) and two holes drilled in the skull through which a 34 ga cannula was lowered bilaterally to the following coordinates; AP+2.6 mm from interaural line, ML ± 1.1 mm from midline suture at bregma and DV+3.7 mm from interaural line with the animal's head level between lambda and bregma. Four micrograms of 6-OHDA (weight expressed as base of 6-OHDA HBr, Regis Chemicals) dissolved in 2 microlitres of 0.9% saline with 0.2 mg/ml ascorbic acid was infused at the rate of one microlitre per min over 2 min and the cannula left in for an extra minute to permit diffusion of the drug. Ten control rats received similar infusions of saline-ascorbic vehicle. Two weeks were allowed for completion of anterograde degeneration of forebrain NA terminals [28] and then behavioural testing commenced.

DMI pretreated (DMI). Ten rats received intracerebral injections of 6-OHDA as described above except that 30 min prior to the intracerebral injection they also received an intraperitoneal injection of desipramine HCl (DMI, 25 mg/kg IP) [7]. Control rats received DMI IP injection and saline-ascorbic intracerebral injection.

Unilateral DB. Ten male Wistar rats received unilateral injections into the dorsal bundle as described above. Ten control rats received saline-ascorbic injections.

Behavioural

Animals were reduced to 90% of free-feeding weight by a 1 hr a day food access schedule and then given 15 g food per day thereafter. They were lever shaped as described elsewhere [16,23], and trained on a continuously reinforced lever press response for a daily 15 min session in standard operant chambers controlled by BRS Digibit solid state logic for 12 days and placed into extinction on the thirteenth and subsequent days. In extinction a lever press no longer produced either a food pellet or the click of the automatic feeder. Animals remained in the chamber during extinction until no lever press had been emitted for two consecutive minutes. The time to reach this extinction criterion and the number of lever presses emitted were recorded and the animal removed from the chamber for the day. Four extinction days were given in total [24].

Biochemical

Following completion of behavioural testing the animals were sacrificed by cervical fracture and the brain rapidly removed and dissected into regions as described elsewhere [16]. These areas were assayed for NA, dopamine and serotonin by the methods of McGeer and McGeer [15], for a marker of cholinergic systems, choline acetyltransferase (CAT), by the method of McCaman and Dewhurst [13] and for a marker of GABA systems, glutamic acid decarboxylase (GAD), by the method of Chalmers *et al.* [4].

RESULTS

Biochemical

The results of the post-mortem assays are shown in Table 1 and serve to confirm that severe and permanent loss of forebrain NA occurred to less than 5% of control values in cortex-hippocampus with no change in brain dopamine or serotonin and no alteration in cholinergic (CAT) or GABA (GAD) markers in any of the brain areas examined. Table 2 shows the results of pretreating with the NA uptake inhibitor, DMI. Considerable but subtotal protection of NA systems in cortex-hippocampus was obtained with complete protection of NA in the hypothalamus. The results of the unilateral 6-OHDA injection are also shown in Table 2. On the side of the 6-OHDA injection, NA concentrations in cortex-hippocampus were reduced to 6% of control while on the contralateral side a loss of 20% was seen relative to control values. It has previously been indicated that the dorsal NA bundle has approximately a 20% crossed component [9].

Behavioural

The extinction data are shown in Fig. 1 and indicate that despite the change in concentration of 6-OHDA and ascorbic acid and the new, more anterior injection site, resistance to extinction still occurred when reward was withdrawn in extinction (Fig. 1, left). Pretreatment with DMI was indeed effective in preventing the occurrence of the usual DBEE (Fig. 1, middle). Unilateral lesion failed to cause resistance to extinction (Fig. 1, right).

DISCUSSION

Despite significant change in the concentration of 6-OHDA and ascorbic acid, designed to enhance the specificity of the lesion [26], and a complete change in the injection site, the DBEE was still seen. That resistance to extinction occurs after 6-OHDA injection at either the level of the dorsal raphe or at the level of the substantia nigra suggests that the common element between these two placements, namely the dorsal noradrenergic bundle, is indeed the substrate for the resistance to extinction. Post-mortem assays revealed marked loss of NA in forebrain areas but no change in dopamine, serotonin, cholinergic or GABAergic markers. This strongly suggests a causative role of NA in the DBEE.

TABLE 1

POST-MORTEM ASSAYS ON CONTROL AND DORSAL BUNDLE LESION RATS

Dorsal Bundle 6-OHDA	Control (n=10)	Lesion (n=10)	%	p
Noradrenaline				
Cortex	289 ± 14	9 ± 6	3	0.001
Hippocampus	305 ± 18	15 ± 3	5	0.001
Hypothalamus	2380 ± 110	860 ± 90	36	0.001
Amygdala	408 ± 5	61 ± 4	15	0.001
Septum	954 ± 12	435 ± 34	46	0.01
Cerebellum	219 ± 12	271 ± 8	124	NS
Spinal cord	255 ± 6	307 ± 12	120	NS
Dopamine				
Striatum	13570 ± 660	1284 ± 1230	95	NS
Amygdala	88 ± 9	66 ± 27	75	NS
Septum	650 ± 70	500 ± 20	77	NS
Hypothalamus	452 ± 19	421 ± 26	93	NS
Serotonin				
Cortex	377 ± 21	382 ± 19	101	NS
Hippocampus	493 ± 38	485 ± 41	98	NS
Hypothalamus	1206 ± 110	1156 ± 96	96	NS
Choline Acetyltransferase				
Cortex	13.4 ± 0.7	13.1 ± 0.6	98	NS
Hippocampus	15.0 ± 0.5	14.9 ± 0.6	99	NS
Hypothalamus	11.9 ± 1.68	10.2 ± 1.14	86	NS
Septum	15.1 ± 0.8	13.7 ± 0.4	91	NS
Amygdala	18.4 ± 1.5	19.6 ± 1.2	106	NS
Glutamic Acid Decarboxylase				
Cortex	12.1 ± 0.4	11.3 ± 0.4	93	NS
Hippocampus	10.8 ± 0.4	11.0 ± 0.5	101	NS
Hypothalamus	25.0 ± 1.2	23.6 ± 1.5	91	NS
Septum	16.7 ± 0.9	16.3 ± 1.3	98	NS
Amygdala	10.2 ± 0.2	11.0 ± 0.8	108	NS

Values are means with standard error of the mean. Noradrenaline, dopamine and serotonin values are in nanograms of amine per gram wet weight of tissue and CAT (choline acetyltransferase) and GAD (glutamic acid decarboxylase) are micromoles per 100 mg protein per hour. % column represents values of lesion groups expressed as percent relative to control values. Protein was determined by the method of Lowry *et al.* [12]. *p* column is the two-tailed significance of the difference between control and lesion groups using Student's *t* test. NS, not significant.

The particular NA system involved has, on the basis of previous work [17, 25, 32] been localised to the dorsal systems rather than the ventral bundle. A further demonstration of the noradrenergic basis of the DBEE was obtained from the reversal of the behavioural effect by pretreatment with DMI, a procedure which prevented the NA-depleting, but not any non-specific effects of intracerebral 6-OHDA [7].

Despite DMI pretreatment, a 30% decrease in cortical-hippocampal NA occurred. That this was not effective in causing resistance to extinction allows us to place a lower limit on the depletion required in order to be behaviourally effective. In the case of 6-OHDA injection without DMI pretreatment this was not possible because of the uniformly better than 95% depletion obtained. The unilateral lesion group also further delineates the neurochemical basis of the DBEE by indicating that it requires a bilateral lesion.

EXPERIMENT 2

The converse strategy of that reported in Experiment 1 would be to seek to emulate any non-specific effects of 6-OHDA without causing a NA depletion and determine whether these non-specific effects are adequate on their own to cause a DBEE. One technique which has recently become available is kainic acid. This substance, if injected into a brain region, appears to destroy cell perikarya without directly affecting fibres of passage [5,14]. It would thus be possible with this neurotoxin to mimic the possible damage to cell bodies around the dorsal bundle but without damaging the noradrenergic fibres themselves. A further control procedure for any effects that 6-OHDA may have on serotonergic neurones would be intracerebral injection of 5–7 DHT, a neurotoxin with considerably greater specificity towards serotonin systems than 6-OHDA [1,6]. If 5–7 DHT injected

TABLE 2
POST-MORTEM AMINE ASSAYS

	Control (n=10)	Lesion (n=10)	%	p
DMI Pretreated				
Noradrenaline				
Cortex-hippocampus	302 ± 15	210 ± 18	70	0.05
Hypothalamus	2250 ± 220	2100 ± 180	93	NS
Dopamine				
Striatum	15700 ± 590	13200 ± 1100	84	NS
Unilateral DB Injected Side				
Noradrenaline				
Cortex-hippocampus	348 ± 38	24 ± 3	6	0.001
Hypothalamus	2320 ± 110	1020 ± 120	44	0.001
Dopamine				
Striatum	14100 ± 540	12150 ± 1130	86	NS
Uninjected Side				
Noradrenaline				
Cortex-hippocampus	390 ± 25	325 ± 41	82	0.05
Hypothalamus	2360 ± 130	1960 ± 100	83	0.05
Dopamine				
Striatum	14850 ± 600	14540 ± 530	98	NS

Values are means with SEM's in nanograms of amine per gram wet weight of tissue. % column represents values of lesion groups as percent relative to control values and p column is the two-tailed significance comparing control and lesioned groups using Student's t test. NS, not significant.

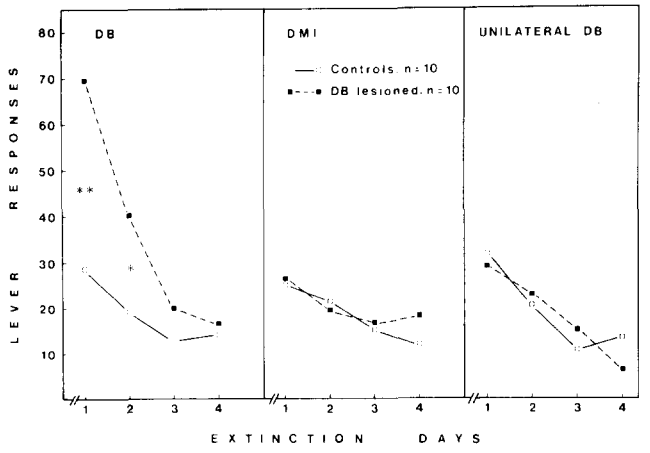

FIG. 1. Extinction behaviour of dorsal bundle 6-OHDA injected (DB), desimipramine pretreated 6-OHDA injected (DMI) and unilateral 6-OHDA injected rats. Lever responses emitted prior to reaching extinction criterion are shown for the four days of extinction testing. Stars indicate that the control and lesion rats were significantly different (Mann-Whitney U test, two-tailed) at the following levels; $^{**}p<0.01$; $^{*}p<0.05$.

at the same coordinates as 6-OHDA fails to induce a DBEE, the resistance to extinction seen after 6-OHDA would be unlikely to be due to any effect on serotonin systems.

METHOD

Surgical

Ten male albino Wistar rats weighing 300 g were injected as described in Experiment 1 but with either 0.2 nanomoles of kainic acid dissolved in 0.1 microlitres of phosphate buffer, pH 7.2, or with 4 micrograms of 5–7 DHT creatine sulphate (weight expressed as free base, Regis Chemicals), dissolved in 2 microlitres of 0.9% saline with 0.2 mg/ml ascorbic acid antioxidant. To prevent a direct action on NA fibres animals were pretreated with DMI (25 mg/kg IP 30 min prior to intracerebral injection).

Behavioural

Operant testing, food deprivation and post-operative recovery time were similar to those described in Experiment 1.

Biochemical

Post-mortem assay procedures were similar to those described in Experiment 1.

Histological

The mesencephalic injection site was saved in 10% For-

TABLE 3

POST-MORTEM
ASSAYS AFTER KAINIC ACID, 5-7 DIHYDROXYTRYPTAMINE (5-7 DHT) OR VEHICLE INJECTIONS INTO THE DORSAL BUNDLE

	Control (n=10)	Lesion (n=10)	%	p
Kainic DB				
Noradrenaline				
Hippocampus-cortex	340 ± 15	242 ± 24	71	0.001
Hypothalamus	2360 ± 110	2440 ± 140	103	NS
5-7 DHT DB				
Noradrenaline				
Cortex	578 ± 28	582 ± 19	101	NS
Hippocampus	653 ± 30	588 ± 26	90	NS
Hypothalamus	3740 ± 210	3400 ± 140	91	NS
Serotonin				
Cortex	400 ± 16	372 ± 26	93	NS
Hippocampus	508 ± 24	417 ± 19	82	NS
Hypothalamus	1170 ± 110	1038 ± 95	89	NS

Values are means with standard error of the mean in nanograms of amine per gram wet weight of tissue. % column represents values of lesion groups expressed as percent relative to control values and p column is the two-tailed significance comparing control and lesion groups using Student's t test. NS, not significant.

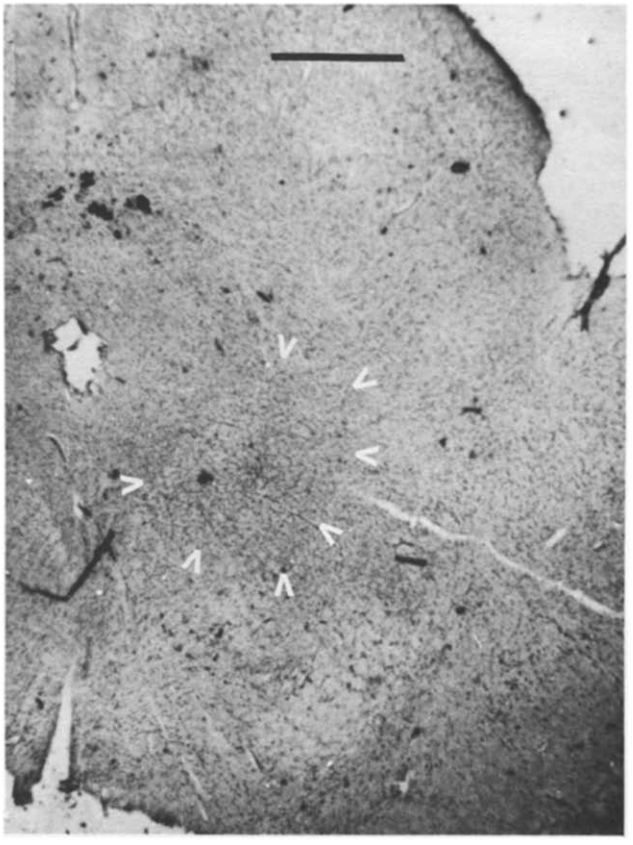

FIG. 2. Section through the dorsal bundle injection site after injection of 0.2 nanomoles of kainic acid dissolved in 0.1 microlitres of phosphate buffer, pH 7.2. Calibration bar is 100 μ. Arrows delineate region of cell body loss and glial proliferation.

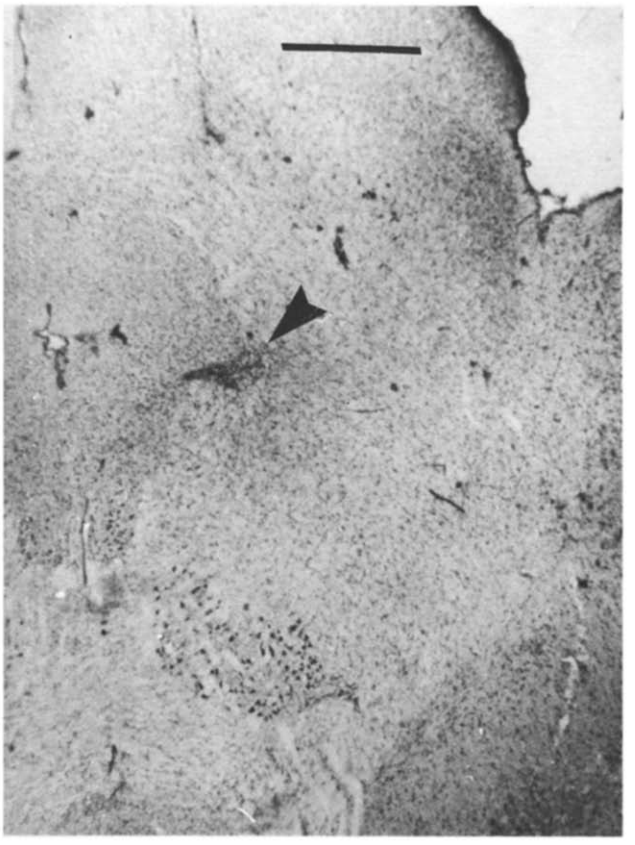

FIG. 3. Section through the dorsal bundle injection site after injection of 4 micrograms of 5-7 dihydroxytryptamine (5-7 DHT, weight expressed as free base) dissolved in 2 microlitres of 0.9% saline with 0.2 mg/ml ascorbic acid. Calibration bar is 100 μ. Non-specific gliosis is indicated by arrow.

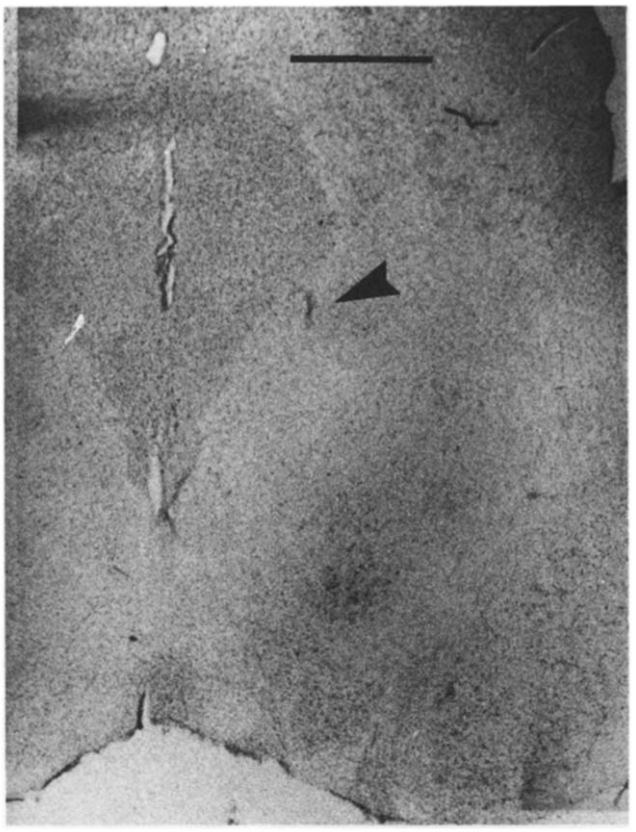

FIG. 4. Section through dorsal bundle injection site after injection of 4 micrograms of 6-OHDA dissolved in 2 microlitres of 0.9% saline with 0.2 mg/ml ascorbic acid. Compare extent of non-specific gliosis (arrow) in this with that in Figs. 2 and 3. Calibration bar is 100 μ.

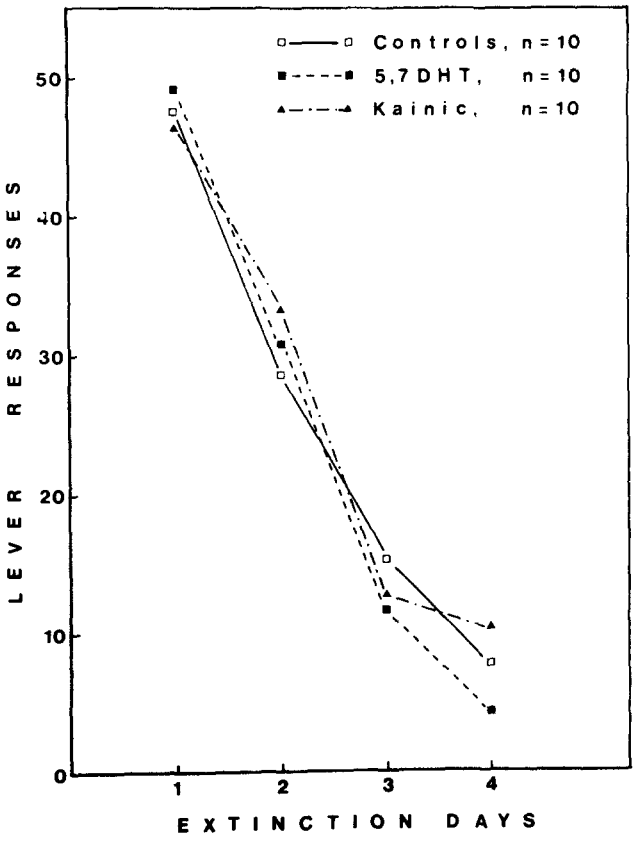

FIG. 5. Extinction behaviour of kainic injected, 5–7 dihydroxytryptamine (5–7 DHT) injected and vehicle-injected control rats. Lever responses emitted prior to reaching extinction criterion are shown for four days of extinction testing.

malin for at least two weeks, sectioned at 50 μ on a freezing microtome and stained with Cresyl Violet.

RESULTS

Biochemical

The results of the post-mortem amine assays are shown in Table 3. Injection of kainic acid produced a 30% loss of forebrain NA, large gliosis (Fig. 2) and severe cell loss (Fig. 6) in a large area surrounding the dorsal bundle in the mesencephalon. Injection of 5–7 DHT after DMI pretreatment failed to alter NA in any region measured and had only a small effect on serotonin, the largest effect being on hippocampal serotonin which was reduced by an average of 20% and in some individual cases by 40%. Histological section through the 5–7 DHT injection site showing gliotic zone is to be seen in Fig. 3 and through the 6-OHDA injection site showing lesser gliosis in Fig. 4.

Behavioural

The extinction behaviour of the kainic acid, 5–7 DHT and control groups is shown in Fig. 5 and no resistance to extinction was seen in either experimental group relative to controls.

DISCUSSION

Kainic acid injection into the dorsal bundle induced widespread cell body loss and a small loss of NA fibres. This latter effect on NA fibres of passage will be reported in more detail elsewhere. That resistance to extinction did not occur after the marked cell body loss appears to exclude incidental damage to cell perikarya surrounding the dorsal bundle as the neurochemical basis of the DBEE. The failure of 5–7 DHT injected into the dorsal bundle to affect serotonin concentrations to any great degree makes it virtually certain that the less toxic 6-OHDA would not directly affect serotonin systems, in agreement with the unchanged concentrations of serotonin found in Experiment 1. Our results, of course, do not indicate that serotonin is not involved in extinction, merely that the 6-OHDA-induced resistance to extinction does not involve serotonin. Indeed, other workers have claimed serotonergic influences on extinction processes following parachlorophenylalanine treatment [2].

GENERAL DISCUSSION AND CONCLUSION

Despite the suggestions that 6-OHDA is no more selective than injection of copper sulphate [27] or electrolytic lesion [3], the behavioural effects of injection of 8 micrograms of

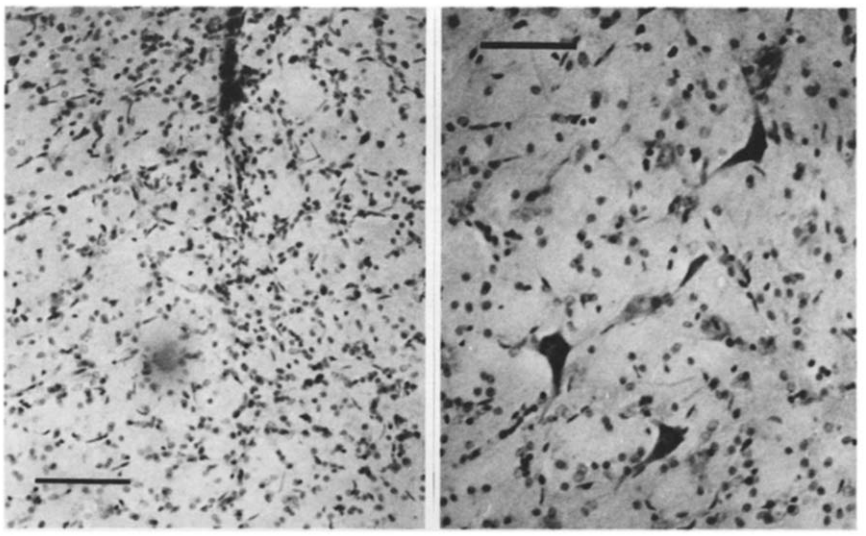

FIG. 6. Section of high power through dorsal bundle injection site after kainic acid injection (left) and at corresponding position in a control (right). Calibration bar is 10 μ

6-OHDA into the dorsal bundle are in fact due to the NA depletion obtained. This is supported by the absence of any change in the markers of other neurochemical systems measured, such as dopamine, serotonin, acetylcholine or GABA. It is supported by the reversal of the behavioural effects of 6-OHDA by prior treatment with DMI, which prevented the NA depletion but not any non-specific effects of 6-OHDA [7]. It is supported by the failure of massive cell body loss around the dorsal bundle, induced by kainic acid injection [5,14], to cause resistance to extinction. It is further supported by the demonstration that 6-OHDA did not affect serotonin concentrations after injection into the dorsal bundle and by the finding that the more potent serotonergic neurotoxin [1,6], 5–7 DHT, injected at the same coordinates failed to induce resistance to extinction. All these lines of evidence allow us to make a very firm statement that the dorsal bundle extinction effect is due solely and specifically to the depletion of forebrain NA.

The 20% increase in cerebellar and spinal NA seen in the present DB lesion rats may be excluded as the basis of the DBEE since, using other 6-OHDA injection coordinates, such increases did not occur yet the DBEE did [16,17]. Further, use of neonatal intraperitoneal administration of 6-OHDA to deplete forebrain NA produced a DBEE but actually *depleted* both cerebellar and spinal NA to 15% of control [17,24]. Previous work in which either electrolytic lesions [32] or neonatal intraperitoneal administration of 6-OHDA [17,25] were used to deplete dorsal bundle projection areas, without affecting the ventral bundle which innervates the hypothalamus, allow us to identify the substrate of the resistance to extinction as the dorsal NA bundle. Which terminal projection area of the dorsal system (cortex, hippocampus, septum, amygdala, or the small hypothalamic projection of the dorsal bundle) is of critical importance remains for future research.

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