BIOCHEMICAL AND BEHAVIOURAL ALTERATIONS FOLLOWING 6-HYDROXYDOPAMINE ADMINISTRATION INTO BRAIN*

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SINCE the discovery that 6-hydroxydopamine (6-OHDA) destroys peripheral adrenergic fibers (THOENEN and TRANZER, 1968), several laboratories have investigated the possibility that 6-OHDA might produce selective destruction of central catecholamine neurons when introduced into brain (UNGERSTEDT, 1968; BREESE and TRAYLOR, 1970; URETSKY and IVERSEN, 1970). Following such treatment with 6-OHDA, BLOOM *et al.*, (1969) described degenerating fibers in areas rich in adrenergic neurons. The reduced brain tyrosine hydroxylase activity and reduced synthesis of catecholamines from tyrosine after 6-OHDA administration provided further evidence for the view that 6-OHDA causes a "central sympathectomy" (BREESE and TRAYLOR, 1970; URETSKY and IVERSEN, 1970).

In initial studies, intracisternal injection of 6-OHDA was found to cause a greater depletion of brain norepinephrine (NE) than dopamine (DA) (BREESE and TRAYLOR, 1970). Subsequent work indicated that administration of multiple small doses of 6-OHDA provided a method to reduce brain NE which had little effect on DA. In contrast, treatment with desipramine prior to administration of 6-OHDA protected noradrenergic fibers while permitting relatively selective destruction of dopaminergic neurons (BREESE and TRAYLOR, 1971). Equivalent depletions of both NE and DA could be produced by 6-OHDA in animals pretreated with pargyline (BREESE and TRAYLOR, 1970). These procedures to produce chronic reduction of brain NE, DA, or both catecholamines have been used by our laboratory to investigate physiological, behavioural and pharmacological responses postulated to be dependent upon central sympathetic neural systems (BREESE *et al.*, 1971; BREESE *et al.*, 1972; BREESE and TRAYLOR, 1972; COOPER *et al.*, 1972; COOPER *et al.*, 1973a,b; SMITH *et al.*, 1973a,b; BREESE *et al.*, 1973; HOLLISTER *et al.*, 1973; Table 1).

EFFECTS OF 6-OHDA TREATMENT ON OPERANT BEHAVIOUR

Early studies with 6-OHDA indicated that depletions of brain catecholamines were not accompanied by a chronic behavioural effect (TAYLOR and LAVERTY, 1972; COOPER *et al.*, 1972). In general, following an initial period of behavioural depression, performance of 6-OHDA treated animals returned to control levels. This apparent dissociation of behavioural and biochemical effects may be similar to that described after treatment with reserpine. In 1967, PIRCH *et al.* found that reserpine produced depression of avoidance responding followed by recovery within a few days, although at the time of recovery biogenic amine concentrations remained severely reduced.

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Subsequently, RECH *et al.* (1968) demonstrated that such animals showed an enhanced sensitivity to a small dose of α-methyltyrosine. Recently, 6-OHDA treated rats, which showed no chronic behavioural deficits, were also found to display enhanced sensitivity to doses of α-methyltyrosine and reserpine which did not affect active avoidance or bar-press responding in control animals (COOPER *et al.*, 1972).

Several possibilities may account for the absence of permanent behavioural deficits following the destruction of brain catecholamine fibres by 6-OHDA treatment (COOPER *et al.*, 1972). These include considerations of increased activity of surviving fibres and denervation supersensitivity. It is also possible that anatomical location of certain catecholamine-containing fibres make them more resistant to destruction by 6-OHDA. Such proposals suggest that greater depletion of catecholamines with 6-OHDA would produce chronic behavioural deficits, as remaining adrenergic fibres became so sparse that catecholamines could no longer be released in sufficient quantity to maintain function. In support of this view, 6-OHDA in combination with pargyline was found to cause drastic depletion of brain catecholamines which chronically reduced acquisition and performance of an active avoidance task (COOPER *et al.*, 1973a).

In an effort to examine the relative role of each of the brain catecholamines in shuttle-box avoidance responding, animals were employed in which either brain NE or DA was preferentially reduced. In initial experiments, no behavioural deficits were observed following these treatments. In fact, animals depleted of NE were more active than controls and displayed a significant increase of avoidance responding during acquisition of this task (COOPER *et al.*, 1973a), such as obtained following treatment with *p*-chlorophenylalanine (TENEN, 1967). Therefore, any interpretation of these findings must consider the significant reduction of serotonin following this treatment (Table 1).

Since 6-OHDA treated rats were found to be more sensitive to the behavioural depressant effects of α-methyltyrosine, this treatment was applied to animals preferentially depleted of NE or DA. In addition, the dopamine-β-hydroxylase inhibitor, 1-phenyl-3-[2-thiazolyl]-2-thiourea [U-14,642], was also administered to reduce only brain NE. It was reasoned that the additional catecholamine depletion produced by these drugs might reveal deficits related to prior reduction of NE or DA in the groups depleted of either brain amine. Doses of α-methyltyrosine which did not alter performance of control animals resulted in decreased responding from rats depleted of brain DA (Fig. 1a). Rats depleted of NE also showed reduced avoidance responding following α-methyltyrosine, but their performance remained above the group in which brain DA was depleted. Administration of U-14,642, which further reduced brain NE by approximately 75 per cent in all animals, produced no behavioural depression (Fig. 1a). These findings support the view that DA plays a critical role in the maintenance of avoidance behaviour (SEIDEN and CARLSSON, 1963).

Since multiple injections of 6-OHDA have been shown to produce greater depletion of brain catecholamines, two doses of 6-OHDA (240 μg) were given to desipramine-treated rats to increase the depletion of brain DA. This procedure antagonised acquisition of the shuttle-box avoidance response as well as acquisition of a one-way avoidance task (Table 1). In other experiments, 6-OHDA was injected into various brain sites to destroy specific NE and DA fibre tracts. In contrast to results after depletion of NE with intracisternal injections, destruction of dorsal and ventral NE

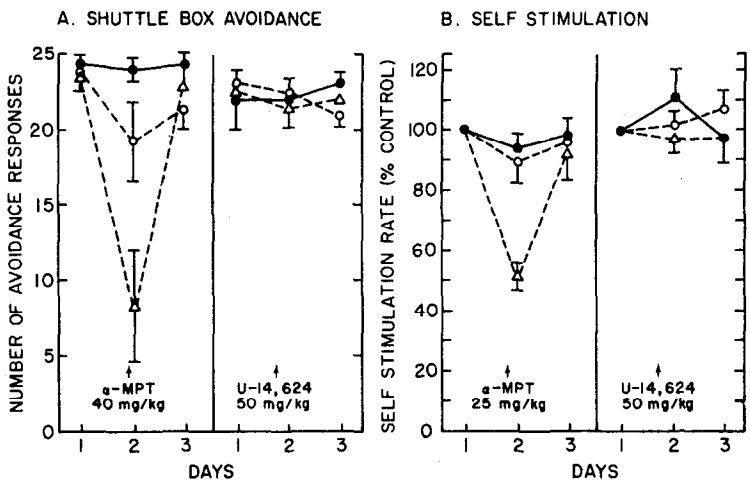

FIG. 1.—Shuttle box avoidance (a) and self-stimulation (b) responding from control animals (●——●) or from rats depleted of DA (Δ- - - -Δ) or of NE (○- - - -○) after α-methyltyrosine (α-MPT) or U-14,624. Doses of α-MPT and U-14,624 were administered 4 hr and 6 hr, respectively, before behavioural testing. Methods to deplete NE or DA are described by BREESE *et al.* (1972).

Brain catecholamines 4 hr after α-MPT (40 mg/kg).

Control rats:	NE = 51 ± 2 and DA =	40 ± 2% control
Rats depleted of NE:	NE = 23 ± 2 and DA =	39 ± 7% control
Rats depleted of DA:	NE = 38 ± 4 and DA =	11 ± 3% control

Brain catecholamines 4 hr after α-MPT (25 mg/kg).

Control rats:	NE = 62 ± 7 and DA =	55 ± 7% control
Rats depleted of NE:	NE = 21 ± 1 and DA =	53 ± 6% control
Rats depleted of DA:	NE = 52 ± 4 and DA =	18 ± 4% control

Brain catecholamines 6 hr after U-14,624 (50 mg/kg).

Control rats:	NE = 27 ± 6 and DA =	117 ± 19% control
Rats depleted of NE:	NE = 8 ± 2 and DA =	91 ± 11% control
Rats depleted of DA:	NE = 27 ± 3 and DA =	24 ± 3% control

pathways with 6-OHDA did not facilitate acquisition of the avoidance task, even though NE was reduced by 85 per cent in the forebrain. Administration of 6-OHDA into the nigro-striatal pathway as well as into the caudate was found to block acquisition of the shuttle-box avoidance response providing further evidence for the proposal that dopaminergic neurons are essential for maintaining avoidance responding.

Evidence suggests that central catecholamine-containing fibres also play a crucial role in self-stimulation of brain (POSCHEL and NINTEMAN, 1963). In accord with this view, 6-OHDA in combination with pargyline was found to cause a chronic depression of responding in this task (BREESE *et al.*, 1971). Whereas preferential depletion of NE or DA alone did not alter self-stimulation, administration of α-methyltyrosine to these groups caused drastic reduction of self-stimulation rates in rats depleted of brain DA, but did not significantly alter responding of rats depleted of brain NE (Fig. 1b). Furthermore, depression of self-stimulation was not observed in any group following inhibition of dopamine-β-hydroxylase to reduce brain NE (Fig. 1b). Thus, brain DA may play a role in maintaining electrical self-stimulation of brain.

TABLE 1. BEHAVIOURAL RESPONSES IN RATS FOLLOWING VARIOUS 6-HYDROXYDOPAMINE TREATMENTS

Behaviour	"6-OHDA"*	"NE Down"†	"DA Down"‡
Acquisition: shuttle-box avoidance	Blocked	Enhanced	Blocked
Acquisition: one-way avoidance	Blocked	—	Blocked
Acquisition: passive avoidance	Like control	Reduced	Like control
Habituation of motor activity	Like control	Enhanced	Like control
Self-stimulation of brain	Reduced	Like control	—
Sucrose consumption	Reduced	Like control	Reduced
Saline consumption (DOCA-induced)	Reduced	Like control	Reduced
Motor activity (Dopa-induced)	Enhanced	Like control	Enhanced
Motor activity (amphetamine-induced)	Reduced	Like control	Reduced
Stereotypy (amphetamine-induced)	Reduced	Like control	Reduced
Hypothermia (6-OHDA-induced)	Blocked	Reduced	—

* "6-OHDA" refers to intracisternal administration of 2 × 200 μg of 6-OHDA, one with pargyline (50 mg/kg) pretreatment and the other without. Brain NE concentration has been shown to be 15·2 ± 3·7% of control, DA 7·1 ± 1% of control and serotonin 87·2 ± 3·4% of control after this treatment.

† "NE Down" refers to intracisternal injection of 3 × 25 μg of 6-OHDA. Brain NE has been found to be 41·9 ± 3·7% of control, DA 94·2 ± 6·7% of control and serotonin 81·3 ± 6·1% of control after this treatment.

‡ "DA Down" refers to intracisternal administration of 2 × 240 μg of 6-OHDA to desipramine (30 mg/kg) pretreated rats. Brain NE has been shown to be 80 ± 3·8% of control, DA 12 ± 1·4% of control and serotonin 79 ± 5% of control after this treatment.

EFFECT OF 6-OHDA ON CONSUMMATORY BEHAVIOUR

Following administration of 6-OHDA into brains of rats pretreated with pargyline, an acute period of aphagia and adipsia occurs which lasts several days (ZIGMOND and STRICKER, 1972). Furthermore, UNGERSTEDT (1971) has reported acute depression of food and water consumption after injection of 6-OHDA into the substantia nigra, implicating disruption of dopaminergic systems in this response. Intracisternal treatment with 6-OHDA to reduce brain DA also causes an acute decrease in eating and drinking further supporting this view (BREESE *et al.*, 1973).

Recovery of consummatory function after 6-OHDA treatment has been likened to the syndrome occurring after lesioning of the lateral hypothalamus (UNGERSTEDT, 1971). Similar to animals "recovered" from lateral hypothalamic lesions(TEITELBAUM and EPSTEIN, 1962), rats surviving treatment with 6-OHDA in combination with pargyline failed to increase food intake in response to 2-deoxyglucose (ZIGMOND and STRICKER, 1972) and insulin (BREESE *et al.*, 1973). Enhanced saline preference found in control rats following desoxycorticosterone (DOCA) treatment is also markedly reduced in rats treated with 6-OHDA. In addition, it has been observed that control rats drink large volumes of sucrose solution substituted for water, while 6-OHDA treated rats show little increase in consumption of this solution. Investigation of sucrose consumption in rats preferentially depleted of NE or DA suggest that the failure of 6-OHDA treated rats to increase fluid intake in response to a sucrose solution is related to depletion of brain DA (BREESE *et al.*, 1973).

TREATMENT OF DEVELOPING RATS WITH 6-OHDA

Administration of 6-OHDA to immature rats has been found to produce marked reductions of brain catecholamine levels and tyrosine hydroxylase activity. Accompanying the destruction of central catecholamine-containing fibres in developing

rats is a marked deficiency in growth (BREESE and TRAYLOR, 1972; LYTLE *et al.*, 1972). Furthermore, immature rats treated with 6-OHDA not only fail to increase fluid consumption when a sucrose solution is substituted for water, but also fail to increase preference for saline when treated with desoxycorticosterone (SMITH *et al.*, 1973b). Food and water intake of 6-OHDA treated rats is also reduced when compared with intake of controls. Such evidence would suggest that the growth deficiency may be related to a permanent change in consummatory behaviour. In addition, rats treated with 6-OHDA when immature show a significant deficit in acquisition of the shuttle-box avoidance response consistent with previous findings from adult rats that received 6-OHDA in combination with pargyline.

Treatments developed in adult animals to deplete NE or DA were also applied to the neonate. Rats depleted of brain DA displayed deficits in growth, consummatory behaviour and acquisition of avoidance responding (SMITH *et al.*, 1973a). These deficits were not observed in animals in which brain NE was preferentially reduced. However, depletion of brain NE in immature rats facilitated performance early in acquisition of the shuttle-box avoidance task and produced hyperactivity during habituation to circular activity cages. Depletion of NE in neonates did not alter brain serotonin, suggesting that these behavioural changes may indeed be due to altered noradrenergic function.

AMPHETAMINE-INDUCED BEHAVIOUR AFTER 6-OHDA TREATMENT

In accord with the view that catecholamines are important for the behavioural actions of amphetamine (HANSON, 1967), HOLLISTER *et al.* (1973) recently found that amphetamine induced motor activity was reduced following depletion of both catecholamines in brain with 6-OHDA (Table 1). Stereotypic behaviour produced by amphetamine was likewise reduced. While depletion of NE did not reduce amphetamine-stimulated motor activity and stereotypies, depletion of DA did antagonise these activities. In contrast to antagonism of the pharmacological actions of amphetamine, preferential depletion of DA with 6-OHDA markedly potentiated DOPA stimulated motor activity (Table 1).

SUMMARY

Adult and neonatal rats treated with 6-OHDA to reduce NE, DA or both catecholamines have been used to examine the role of brain catecholamines in several behaviours (Table 1). The data implicated DA-containing fibres in the maintenance of several diverse functions including consummatory behaviour, active avoidance responding and self-stimulation of brain. Motor activity and stereotypies induced by amphetamine also appear to be dependent upon brain DA. Table 1 shows that brain NE at this time has been associated only with temperature control. Present findings are consistent with proposals (EVERETT and WIEGAND, 1962; SEIDEN and CARLSSON, 1963) suggesting alternative roles for DA not clearly related to its usual association with extrapyramidal function.

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