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The influence of 6-hydroxydopamine on mouse brain acetylcholinesterase and glutamic acid decarboxylase activity

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Classical approaches to the study of function in the nervous system include biochemical inactivation and anatomical lesioning. The finding that 6-OHDA destroys catecholamine containing neurones has therefore been the subject of many studies. In adult animals the catecholamine containing nerve endings destroyed by 6-OHDA show no tendency for regeneration [1]. The intraventricular (i.v.) administration of 6-OHDA is initially associated with various behavioural changes, yet within days it is difficult to distinguish the experimental animals from the controls [2]. It seems possible that the residual catecholamine containing neurones, or other neural systems, are taking over the function of the destroyed neurones. The possibility of an influence of 6-OHDA on brain AChE (Acetyl-CoA: choline *O*-acetyltransferase E.C.3.1.1.7.) and GAD (L-glutamate 1-carboxylyase E.C.4.1.1.15) activity was examined since these enzymes are concentrated in nerve endings and play key roles in other putative transmitter systems.

Materials and methods

The i.v. injection technique of Brittain and Handley [3] was used to inject 200 μg of 6-OHDA (Labkemi, Goteberg, Sweden), made up in 0·1% (w/v) ascorbic acid in 5 μl. Female mice of the LAC/G strain, of approx. 25 g wt were used. The control animals received an equivalent volume of 0·1% ascorbic acid. All injections were administered 3 days prior to biochemical assay. One half of a mouse brain was used to determine GAD activity according to Lowe *et al.* [4], and the other half of the brain was used to assay AChE according to Ellman *et al.* [5]. Protein was assayed by the method of Lowry *et al.* [6].

Three mouse brains, frozen in liquid nitrogen, were pooled and used for the determination of the biogenic amines, NA, DA and 5HT, which were measured according to Brownlee and Spriggs [7].

Results and discussion

Following the injection of 6-OHDA the behaviour of the mice alternated between short periods of hyperexcitability and longer periods of sedation. About one mouse in eight entered into convulsions and died. A report has appeared describing the effects of 6-OHDA in the mouse [8]. These authors reported that doses in excess of 50 μg produced a high mortality rate. In our experiments the dose of 200 μg killed 12–15 per cent of the mice and a further 10 per cent showed varying degrees of ataxia. After three days the behaviour of the remainder of the mice was almost indistinguishable from saline-injected controls and these mice were used for the biochemical assays.

The i.v. injection of 6-OHDA 3 days prior to assay resulted in a statistically significant decrease in whole brain DA ($P < 0.001$) and NA ($P < 0.001$), although no significant change in the level of 5-HT was produced (Table 1). This decrease in brain catecholamines was associated with

Abbreviations used: 6-OHDA (6-hydroxydopamine), GAD (L-glutamic acid 1-carboxylyase E.C.4.1.1.15), ChaC (Acetyl-CoA: choline *O*-acetyltransferase E.C.2.3.1.6), DA (dopamine), NA (noradrenaline), 5-HT (5-hydroxytryptamine).

Table 1. The influence of 6-OHDA on the levels of mouse brain biogenic amines

	μg Amine/g brain tissue ± S.D.		
	5-HT	DA	NA
Control (6)	0·485 ± 0·070	0·936 ± 0·109	0·569 ± 0·042
6-OHDA (6)	0·573 ± 0·124	0·197 ± 0·134*	0·074 ± 0·017*

In mice 6-OHDA was injected by the i.v. route 3 days prior to the assay of brain tissue, frozen in liquid nitrogen, for 5-HT, DA and NA. The number of observations is given in brackets. The differences between mean values were analysed using Student's *t*-test, *P < 0·001.

Table 2. The influence of 6-OHDA on mouse brain GAD and AChE activity

	GAD (μmoles GABA produced/g protein per hr ± S.D.)	AChE (μmoles ATCh hydrolysed/g proteins per min ± S.D.)
Control (6)	444·29 ± 33·34	29·85 ± 1·34
6-OHDA (6)	384·60 ± 36·33*	25·90 ± 1·53†

Three days following the i.v. injection of 6-OHDA, half mouse brains were assayed for GAD and AChE activity. The number of observations is given in brackets. The differences between mean values were analysed using Student's *t*-test, *P < 0·02, †P < 0·001.

a decrease in both AChE activity (P < 0·001) and GAD (P < 0·02) (Table 2).

A relationship between brain NA and ACh has been reported by a number of workers. The i.v. injection of NA has been found to lead to an increase in brain ChAc activity [9], a similar finding has been found following the administration of amphetamine [10, 11]. Sethy and Van Woert [12] found that the chronic administration of L-DOPA resulted in raised ACh levels in the brain. Herman *et al.* [13] gave i.v. injections of ACh and after 5 min found decreased levels of NA in four areas of the brain, and an increased level in a fifth. Phillipu [14] found that ACh increased the rate of NA release from the hypothalamus. The present finding that 6-OHDA-induced decrease in brain catecholamines are associated with lower AChE activity is therefore consistent with a number of findings implying that the cholinergic system and the catecholamines are in some way interrelated.

There is also evidence that a relationship exists between GABA and the catecholamines. Amino-oxyacetic acid, a drug which increases GABA levels [15], also produces changes in the levels of brain NA and DA [ref. 16; and Benton, unpublished results]. *In vitro* studies showed that GABA released NA from cortical and hypothalamic slices [17]. Nicklas and Berl [18] have reported that following 6-OHDA administration, a small compartment of glutamate and its associated metabolites was no longer present in brain tissue and they suggested that this glutamate compartment is associated with catecholamine containing neurones. The present finding that following 6-OHDA administration GAD activity is decreased, is therefore consistent with these findings.

It is possible that these changes in the GABA and cholinergic systems are not directly due to the action of 6-OHDA, but rather are reactions to the changes in the catecholamines produced by the drug. Such changes will be important in our understanding of the mechanism of 6-OHDA's action.

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Effects of amphetamine and its hydroxylated derivatives on newly synthesized hypothalamic norepinephrine; study *in vitro*

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Sympathetic stimulations release norepinephrine from a newly synthesized pool [1, 2] as do amphetamine and its hydroxylated derivatives in peripheral tissues [3–5]. In this report we present evidence that newly synthesized norepinephrine is preferentially released in brain tissue by both amphetamine and its metabolites. The latter have a longer biological half-life in the brain and this may explain some aspects of the pharmacological action of amphetamine [6, 7].

Male rats (C. Rivers) received intraventricular (i.v.) injections of 10 μCi/kg of ^{3}H-dopamine (specific radioactivity: 17·5 Ci/m-mole). The animals were killed after 15 min by decapitation and the brain was rapidly removed. The hypothalamus was isolated and sliced into sections. These sections were incubated for 45 min in Mac Ilwain medium with one of the following drugs: *d*-amphetamine (De Laire), *dl*-hydroxyamphetamine (SKF), or *dl*-α-methyloctopamine (Aldrich) at a concentration of 2×10^{-6} moles/ml. Control slices were incubated under the same conditions, but without sympathomimetic amines. At the end of incubation (45 min) the hypothalamic sections were homogenized in 5 ml of $HClO_4$ 0·4 N at 0°. The supernatant was acidified in the same way.

The ^{3}H-norepinephrine was isolated from ^{3}H-dopamine by filtering through a Dowex 50 WX 4 (H+) column [8]. The norepinephrine fraction was purified by alumin adsorption [9]. The endogenous norepinephrine was determined by fluorometric method. There is a 90 per cent recovery using this method [5].

The endogenous norepinephrine levels (μg/g) in hypothalamic slices and the supernatant are shown in Fig. 1. In addition, the newly formed ^{3}H-norepinephrine is expressed as a per cent of total radioactivity.

Amphetamine does not change the level of endogenous norepinephrine in the tissues, even though it does increase the concentration of the transmitter in the supernatant. On the other hand, hydroxyamphetamine and α-methyloctopamine, a false neurotransmitter [10], deplete the tissue of norepinephrine while increasing the level of transmitter in the supernatant. This well established tissue depletion [11, 12] is the result of granular penetration by the hydroxylated derivatives [13]. These findings for endogenous norepinephrine are qualitatively similar to those for newly formed ^{3}H-norepinephrine. There is a relationship between the increase of norepinephrine in the supernatant under the influence of the three amines, and its decrease in the tissue when the slices are incubated with either hydroxyamphetamine or α-methyloctopamine. In the case of amphetamine, the loss of tissue norepinephrine is not significant. In all cases (Table 1) the ratio of the specific radioactivity of the hypothalamic slices and that of the supernatant shows that there is a preferential release of newly synthesized norepinephrine. Despite these similarities, there are large differences in the mechanism of the three amines' activity. With both amphetamine and α-methyloctopamine there is a decrease in the specific radioactivity of tissue norepinephrine and an increase in the specific radioactivity of supernatant norepinephrine. This increase is not very important. It is partially masked by the more important release of endogenous transmitter, resulting from the granular penetration of the false transmitter. In the case of hydroxyamphetamine, there is an

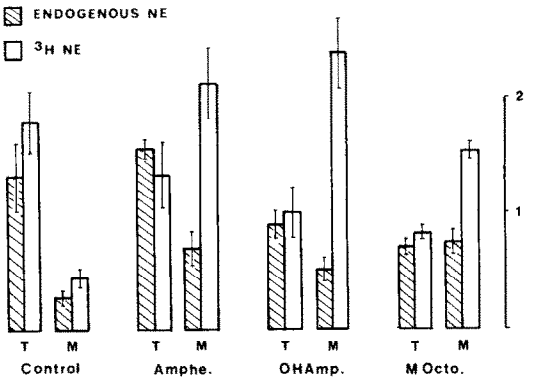

Fig. 1. Endogenous and tritiated norepinephrine (NE) released by amphetamine (Amphe), hydroxyamphetamine (OH-Amp) and α-methyloctopamine (M.Octo.) from hypothalamic slices. In tissues (T) and medium (M), endogenous NE was expressed in μg/g; ^{3}H-NE was expressed as per cent of the whole radioactivity (n = 6).