Increase in adrenal dopamine following 6-hydroxydopamine

6-Hydroxydopamine (6-OHDA) does not have the devastating effect on adrenomedullary cells that it has on peripheral adrenergic nerves. There are slight, if any, changes in the levels of rat adrenal catecholamines with a treatment schedule that reduces the content of noradrenaline in sympathetic nerves more than 80% (Thoenen & Tranzer, 1968). However, 6-OHDA may induce a reflex increase in nerve impulse flow to the adrenals (Mueller, Thoenen & Axelrod, 1969) and thus secondarily affect catecholamine synthesis and release. In this communication, stimulation of adrenal catecholamine synthesis, with an accompanying elevation of dopamine, is shown to occur following the administration of a single intraperitoneal dose of 6-OHDA.

Male Sprague-Dawley rats, 250 g, were given 6-OHDA HCl (200 mg kg^{-1} i.p.). Control animals injected with saline were run at representative time points and showed no difference compared to untreated animals. At various times after 6-OHDA, rats were killed with chloroform and the adrenals rapidly removed and quick-frozen on dry ice. One gland pair was used for each sample. The amines were extracted by homogenizing the glands in 0·4 N perchloric acid. Dopamine and adrenaline + noradrenaline were separated on a cation exchange column (Atack & Magnusson, 1970). Dopamine was analysed spectrophotofluorometrically (Atack, 1973) (6-OHDA does not interfere with this assay when combined with the column procedure, Atack, 1973), while adrenaline and noradrenaline were estimated by their native fluorescence against a noradrenaline standard.

Dopamine levels increased rapidly, reached a peak of about twice the control value at 24 h, and slowly decreased thereafter (Fig. 1). While adrenaline and noradrenaline levels tended to drop initially (Fig. 1), only the 24 h value (73% of control) was significant; that there was not a greater decrease may reflect partial replacement of the amines released by new synthesis, such as occurs during insulin stimulation (Bygdeman, Euler & Hökfelt, 1960). It should be noted that transynaptic induction of tyrosine hydroxylase has been demonstrated in the adrenals of rats treated with 6-OHDA (Mueller & others, 1969), and induction of dopamine β-hydroxylase may also occur (Thoenen, 1972).

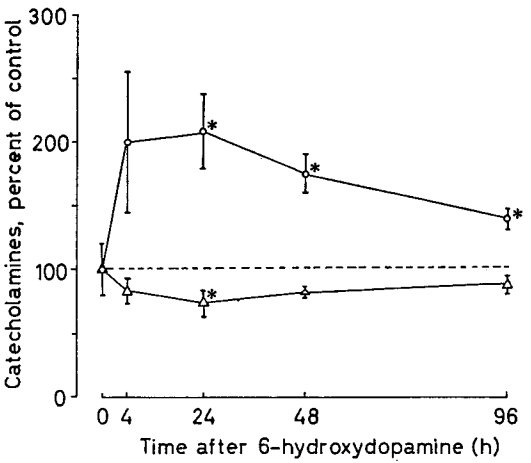

FIG. 1. Adrenal dopamine (○) and noradrenaline plus adrenaline (△) following a single injection of 6-hydroxydopamine (6-OHDA) 200 mg kg^{-1} i.p. Shown are the means ± s.e. of 3 determinations for each of 4 time points (control values are the means of 6 determinations). The control dopamine was 144 ± 14 ng per pair of adrenals. The control nordrenaline plus adrenaline was 25 ± 3 μg per pair. * Differs from control $P < 0.05$.

Increases in adrenal dopamine similar to that seen in the present data have been observed after several kinds of physiologic or pharmacologic stress (e.g. insulin hypoglycemia, Snider & Carlsson, 1972). Such increases can be prevented by adrenal denervation (Carlsson, Snider & others, 1973; Snider & Waldeck, 1974). Elevations in dopamine have also been seen in the carbachol-stimulated bovine adrenal (Snider, Brown & Winkler, unpublished data), in electrically stimulated adrenergic nerves of the rat (Snider, Almgren & Carlsson, 1973), and recently in the brain of rats after a 15 min period of vigorous foot shock (Brown, Snider & Carlsson, unpublished data). Most of the dopamine in these experiments is found in storage vesicles with characteristics similar, if not identical, to those containing noradrenaline or adrenaline (Snider & Carlsson, 1972; Snider & others, 1973). Furthermore, enzyme inhibition data indicate that this dopamine can be metabolized by dopamine β-hydroxylase, i.e. it is not located in a special dopamine "compartment" (Carlsson & others, 1973). These studies suggest that an increase in dopamine, mainly vesicle-bound, is produced by stimulation of adrenergic cells.

Why does the level of dopamine increase? A possible explanation becomes apparent when the kinetics of catecholamine biosynthesis are considered. When adrenergic systems are neurogenically stimulated, the rate of synthesis is increased, often markedly, in relation to the unstimulated control (e.g. Alousi & Weiner, 1966). In the peripheral nervous system of the rat, variations in synthesis rate of as much as 20-fold can occur (Snider, Brown & Carlsson, unpublished data). The activity of the enzymes, tyrosine hydroxylase dopa decarboxylase and dopamine β-hydroxylase, likewise increases several fold. However, the mechanisms by which the increases in enzyme activity occur are different. In the case of the rate-limiting enzyme, tyrosine hydroxylase, the reaction is zero-order with respect to the substrate, i.e. the enzyme is normally saturated with tyrosine and the reaction is independent of the tyrosine concentration (Udenfriend, 1966). The velocity of the enzyme reaction, as well as the overall synthesis rate, is probably increased by some form of modulation of the enzyme related to an increase in the nerve impulse flow (Cloutier & Weiner, 1973). On the other hand, the dopa decarboxylase and dopamine β-hydroxylase reactions appear to be first-order and the velocity of the reactions is proportional to the substrate concentration: a higher concentration of dopa or dopamine results in a higher rate of formation of noradrenaline (Levitt, Spector & others, 1965). A corollary of this is that a higher rate of noradrenaline synthesis is accompanied by higher concentrations of dopa and dopamine. This provides a reasonable explanation for the increased levels of dopamine in the present experiment and in other stimulated adrenergic tissues. It would also explain increases in adrenal dopa that we have seen after stimulation (Snider, Brown & Carlsson, unpublished data). However, the possibility that 6-OHDA enters adrenomedullary cells and interferes with dopamine catabolism cannot be entirely excluded

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Enhancement of noradrenaline turnover in rat brain by L-dopa

L-3,4-Dihydroxyphenylalanine (L-dopa) has been postulated to enhance the turnover of noradrenaline in the brain. The evidence for this effect is based on two kinds of changes induced by L-dopa, i.e. (i) the decreased accumulation of cerebral [^{14}C]noradrenaline (^{14}C-NA) after intravenous administration of labelled tyrosine (Persson & Waldeck, 1971) and (ii) the accelerated disappearance from the brain of ^{14}C-NA injected into the cerebral ventricles (Chalmers, Baldessarini & Wurtman, 1971; Romero, Chalmers & others, 1972). However, it cannot be excluded that tyrosine and L-dopa compete for penetration through the blood-brain barrier and/or into the neurons and that labelled tyrosine might not mix homogeneously with the endogenous tyrosine. Furthermore, ^{14}C-NA injected intraventricularly possibly accumulates not only in noradrenergic neurons but also in other structures (e.g. dopaminergic and 5-hydroxytryptaminergic neurons, capillary walls, glial cells). Therefore the disappearance of the labelled amine may not exactly reflect its release from noradrenergic neurons. For these reasons, changes of endogenous cerebral noradrenaline induced by L-dopa have been investigated.

Male Wistar rats (Füllinsdorf) 150 g, were administered various doses of L-dopa, alone or 30 min after injection of either 5 mg kg^{-1} FLA63 (bis(4-methyl-1-homopiperazinyl-thiocarbamyl)disulphide) or 50 mg kg^{-1} benserazid (N^1-DL-seryl-N^2-(2,3,4-trihydroxybenzyl)-hydrazine, RO 4-4602) or their combination. Some animals received FLA63 alone 1½ or 4½ h before death. Untreated animals served as controls. All injections were given intraperitoneally. The FLA63-treated rats were maintained at 30° to prevent hypothermia. After decapitation, the whole brain was homogenized in perchloric acid. Endogenous noradrenaline was isolated by adsorption on alumina (Anton & Sayre, 1962), separated from L-dopa and dopamine by column chromatography on Dowex 50W × 8 (Bertler, Carlsson & Rosengren, 1958) and measured spectrophotofluorimetrically (Laverty & Taylor, 1968). In addition, determinations of cerebral 3-methoxy-4-hydroxyphenylethyleneglycol-sulphate (MOPEG) in the whole brain 2 h after administration of L-dopa were made (Meek & Neff, 1972).

In agreement with previous results (Constantinidis, Bartholini & others, 1968), L-dopa alone or in combination with benserazid, an inhibitor of extracerebral decarboxylase (Bartholini & Pletscher, 1968), did not change the levels of cerebral noradrenaline compared to controls. However, 1 and 4 h after FLA63, an inhibitor of dopamine-β-hydroxylase (Corrodi, Fuxe & others, 1970), the amine content decreased significantly ($P<0.001$). The FLA63-induced diminution of cerebral noradrenaline was markedly enhanced by L-dopa alone or in combination with benserazid (Table 1). This enhancement depended on the dose of L-dopa, and in the range of 20–100 mg kg^{-1} L-dopa was more marked in the presence than in the absence of benserazid (Fig. 1).