EFFECTS OF 6-HYDROXYDOPA

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6-HYDROXYDOPA (2, 4, 5-trihydroxyphenylalanine)(6-OHDOPA), the carboxyl analog of 6-hydroxydopamine, is a drug whose usefulness lies in its ability to enter the brain after peripheral injection and cause a selective destruction of noradrenergic terminals (JACOBOWITZ and KOSTRZEWA, 1971; SACHS and JONSSON, 1972a). Studies on the pharmacologic effects of 6-OHDOPA show that this agent is capable of reducing the myocardial and the brain norepinephrine (NE) content of the mouse for several days (JACOBOWITZ and KOSTRZEWA, 1971; SACHS and JONSSON, 1972a; SACHS and JONSSON, 1972b; KOSTRZEWA and JACOBOWITZ, 1973; ONG *et al.*, 1969; BERKOWITZ *et al.*, 1970; KOSTRZEWA and JACOBOWITZ, 1972; CORRODI *et al.*, 1971).

PHARMACOLOGICAL STUDIES

A comparison of various routes of administration (KOSTRZEWA and JACOBOWITZ, 1973; BERKOWITZ *et al.*, 1970) clarifies the differences observed in NE brain levels after a single injection of 6-OHDOPA (SACHS and JONSSON, 1972a; BERKOWITZ *et al.*, 1970; CORRODI *et al.*, 1971). An intravenous (i.v.) injection was clearly far superior in depleting the brain NE content in a 24-hr period than the intraperitoneal (i.p.) or the subcutaneous route of administration (KOSTRZEWA and JACOBOWITZ, 1973). An i.v. dose of 100 or 150 mg/kg 6-OHDOPA caused a 30 or 50 per cent reduction, respectively, of NE in the brain after 24 hr (Fig. 1) (JACOBOWITZ and KOSTRZEWA, 1971; KOSTRZEWA and JACOBOWITZ, 1973). The brain dopamine content is not significantly changed for this time period after administration of 100 mg/kg and is reduced by 20 per cent 24 hr after 150 mg/kg. Therefore, the 100 mg/kg dose is the effective dose which is selective for noradrenergic neurons.

In peripheral organs, 6-OHDOPA, like 6-hydroxydopamine, produces variations in tissue susceptibility (KOSTRZEWA and JACOBOWITZ, 1972). After 100 mg/kg (24 hr i.v.), there was a 60 per cent and a 20 per cent reduction of the NE concentration in the ventricle and the submaxillary gland, respectively, whereas no significant changes were observed in the spleen or vas deferens for this time period. After three hours, reductions in the amine content are observed in the spleen, vas deferens and iris, which indicate a transient reversible depletion. It appears that a critical concentration of 6-OHDOPA is necessary for prolonged depletion of NE. Differences in blood flow could account for unequal effects on the NE content in various tissues.

The time course of depletion and recovery of brain amines after a single i.v. injection of 6-OHDOPA is indicated in Fig. 1 (JACOBOWITZ and KOSTRZEWA, 1971). A dose of 100 mg/kg reduced the NE content by 32 per cent after 1 day. After 66 days, the brain NE was still significantly decreased by 21 per cent. A larger dose (150 mg/kg) resulted in a 32 per cent decrease after 66 days (JACOBOWITZ and KOSTRZEWA, 1971). With the 100 mg/kg dose, the brain dopamine content was

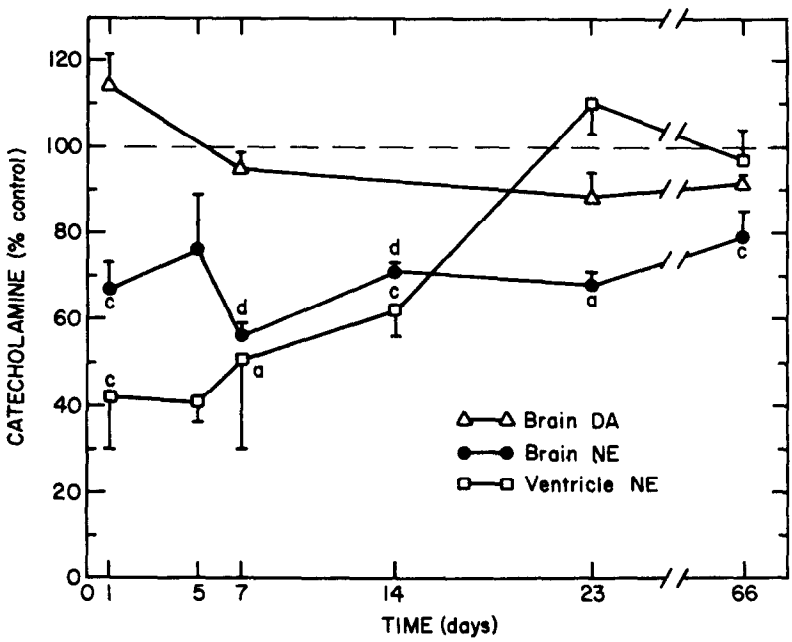

FIG. 1.—Time-course curves after a single intravenous injection of 100 mg/kg 6-hydroxydopa (6-OHDOPA). Each point represents the mean norepinephrine (NE) or dopamine content (μg/g $\pm$ S.E.M.) of an average of five treated and six control mice. Results are expressed as a percentage of the mean of control values for each time period. The mean control values of NE and dopamine in the brain are 0·38 and 0·61 μg/g, respectively, and 0·76 μg/g in the ventricle. Letters indicate significance (p values: a $< 0{\cdot}05$, b $< 0{\cdot}01$, c $< 0{\cdot}005$, d $< 0{\cdot}001$).

not significantly altered. In the ventricle, the NE content was reduced by 38 per cent by 14 days after 6-OHDOPA; at this dose level, it was normal by 23 days (Fig.1).

The effects of various drugs on the NE-depleting action of 6-OHDOPA were studied (KOSTRZEWA and JACOBOWITZ, 1973; ONG *et al.*, 1969; KOSTRZEWA and JACOBOWITZ, 1972). The action of 6-OHDOPA (100 mg/kg) in depleting the NE content of the brain and of the heart could be prevented by D- and L-amphetamine, desipramine and chlorpromazine. These agents have been shown to be inhibitors of the membrane pump for uptake of catecholamines (AXELROD *et al.*, 1961). Since amino acids such as tyrosine and dopa are not inhibited by blockers of the amine uptake mechanism, it would seem that 6-OHDOPA, an amino acid, is converted extraneuronally to 6-hydroxydopamine, which is then inhibited from uptake into adrenergic nerves by the above blocking agents.

Tranylcypromine, a monoamine oxidase inhibitor, failed to alter the effect of 6-OHDOPA on the NE content in the ventricle and in the brain, as compared with saline-injected controls. However, this could be misleading in terms of potential destructive effects of 6-OHDOPA on adrenergic neurons. An increase in the amine content of the remaining neurons after administration of a monoamine oxidase inhibitor might suggest a failure of this drug to influence the organ content of NE (KOSTRZEWA and JACOBOWITZ, 1972; KOSTRZEWA and JACOBOWITZ, 1973). However, SACHS and JONSSON (1972a, 1972b) showed that pretreatment of mice with nialamide

(100 mg/kg, i.p.) 2 hr prior to an i.p. injection of 6-OHDOPA resulted in a significant reduction of [^{3}H]NE uptake in the atrium, cortex and iris after 1 and 7 days. Although an i.p. injection of 6-OHDOPA was not effective in altering the [^{3}H] amine uptake, the combination of nialamide (100 mg/kg, i.p.) with 6-OHDOPA (100 mg/kg, i.p., three injections at 24-hr intervals) 2 hr before the latter drug appears to result in a long-lasting reduction of endogenous brain NE and of [^{3}H] amine uptake in the cortex and atrium. It would therefore appear that monoamine oxidase is involved in the inactivation of 6-OHDOPA. Administration of tranylcypromine (5 mg/kg, i.p.) 1 hr prior to 6-OHDOPA (100 mg/kg, i.v.) was useful for histochemical studies in that an enhancement of catecholamine fluorescent intensity of preterminal axons was observed (JACOBOWITZ and KOSTRZEWA, 1971).

The peripherally acting dopa decarboxylase inhibitor, MK-486, protected the mouse ventricle against the NE-depleting effect of 6-OHDOPA. The NE level of the brain was significantly reduced by an additional 14 per cent after the combination of MK-486 and 6-OHDOPA. It would thus appear that inhibition of decarboxylation of 6-OHDOPA to form 6-hydroxydopamine would make more 6-OHDOPA available in the brain and would thereby result in a greater reduction of NE. MK-485, another peripheral decarboxylase inhibitor, did not potentiate the NE depletion in the rat brain after an i.p. injection of 6-OHDOPA (250–400 mg/kg) (CORRODI *et al.*, 1971) or decrease the uptake of [^{3}H]NE after nialamide and 6-OHDOPA in slices of mouse cortex (SACHS and JONSSON, 1972a). The inability of MK-485 to alter the 6-OHDOPA effects in the latter studies may be due to the appearance of enlarged, distorted nonterminal adrenergic axons (JACOBOWITZ and KOSTRZEWA, 1971; SACHS and JONSSON, 1972a), which could conceivably serve to store or take up increased amounts of NE at a time when there are fewer terminals. Thus, a compartmental relocation of adrenergic nerve uptake and storage sites may serve to mislead in the interpretation of the effects of both dopa decarboxylase and monoamine oxidase inhibitors.

6-OHDOPA had no effect on the monoamine oxidase and choline acetyltransferase activities in the mouse telencephalon (cortex and striatum regions), brain stem and cerebellum after two and 14 days. Catechol-*O*-methyltransferase activity is reduced slightly in the brain stem after 14 days. Acetylcholinesterase activity is reduced by 20 and 30 per cent in the telencephalon 2 and 14 days, respectively, after 6-OHDOPA. Since choline acetyltransferase activity is not altered, it would appear that cholinergic nerves are not affected by 6-OHDOPA. It is suggested that the acetylcholinesterase, which is reduced by 6-OHDOPA, is present within the adrenergic nerves of the cortex and/or the striatum.

The serotonin content of the brain has been reported to be unchanged (BERKOWITZ *et al.*, 1970), slightly increased 4 hr after a massive dose (CLARKE *et al.*, 1972) or slightly reduced in the cortex after nialamide and 6-OHDOPA treatment (SACHS and JONSSON, 1972a). After the latter treatment, however, the uptake of [^{14}C] serotonin was 35 per cent above the nialamide control value. It would therefore appear that serotonergic nerves are not impaired after 6-OHDOPA treatment.

It is of interest that 6-OHDOPA failed to decrease the tyrosine hydroxylase activity in rat brain parts one day or one week after injection although reductions in NE content ranging from 54 to 24 per cent of the control value were observed (THOENEN, 1972). This suggested that 6-OHDOPA administration does not result

in destruction of adrenergic neurons. However, as appeared above in those studies which showed a lack of the NE-depleting effects of 6-OHDOPA caused by dopa decarboxylase and monoamine oxidase inhibitors, a misleading interpretation could arise if a buildup of tyrosine hydroxylase occurs in nonterminal adrenergic axons. Such an accumulation of tyrosine hydroxylase results after a ligation of adrenergic nerve trunks (WOOTEN and COYLE, 1973).

In an effort to demonstrate an actual destruction or impairment of neuronal function rather than a simple depletion of NE stores, hearts of control rats and of those treated with 6-OHDOPA (100 mg/kg, i.v., followed 1 hr later by 100 mg/kg, i.p.) and killed 24 hr after the first injection were perfused with 1 μg/ml NE (KOSTRZEWA and JACOBOWITZ, 1972). The hearts of 6-OHDOPA-treated rats with normally undetectable amounts of NE took up 46 per cent less NE than the control rat hearts. In addition, perfusion of the 6-OHDOPA-treated rats with NE did not increase the number of histochemically observable adrenergic nerve terminals. A buildup of catecholamine fluorescence was observed in swollen preterminal axons of the iris of these rats. This buildup is very similar to that observed with 6-hydroxydopamine (MALMFORS and SACHS, 1968; GOLDMAN and JACOBOWITZ, 1971) and suggests that actual destruction of adrenergic nerves can occur after 6-OHDOPA administration.

A more recent interesting development is the ability of 6-OHDOPA, injected into either pregnant rats or their newborn offspring, to produce long-lasting depletion of whole brain NE. No change was found in the brain dopamine content (ZIEHER and JAIM-ETCHEVERRY, 1973). NE in the tele-diencephalon and in the hypothalamus was significantly reduced three to seven months after birth. The NE content of the brain stem, i.e. the region containing the noradrenergic cell bodies, was significantly increased during this time period. There was no reduction in the NE content of the heart and of the salivary glands of rats whose mothers received 6-OHDOPA during the period of gestation (18–20 days). This observation gives promise of a method whereby separation of central from peripheral effects on noradrenergic neurons can be accomplished with 6-OHDOPA.

BEHAVIORAL STUDIES

To facilitate the study of the various parameters of behavior, rats were injected with 6-OHDOPA in the lateral cerebral ventricles in order to avoid the peripheral effects on adrenergic neurons (RICHARDSON and JACOBOWITZ, 1973). Intraventricular injection of 6-OHDOPA produced a graded dose-dependent reduction of NE in various brain parts. Dopamine levels were not altered by the doses of 6-OHDOPA used (45–180 μg). A working dose of 90 μg was chosen, and a time course of depletion and recovery was studied in various regions of the brain and of the spinal cord (Fig. 2). Telencephalic NE remained at 60 per cent of the control levels for 14 days and had returned to normal levels by 70 days after a single injection. The NE content continued to fall in the diencephalon for 14 days and was still significantly reduced by 33 per cent after 70 days. NE levels in the cerebellum continued to fall for 14 days and remained reduced by 57 per cent after 70 days. Hindbrain NE was at its lowest level two days after injection and had returned very gradually to control values after 70 days. The spinal cord NE levels had progressively declined to 33 per cent of the control values by the end of 14 days. Brain dopamine levels were not affected for up to 70 days after the intraventricular injection.

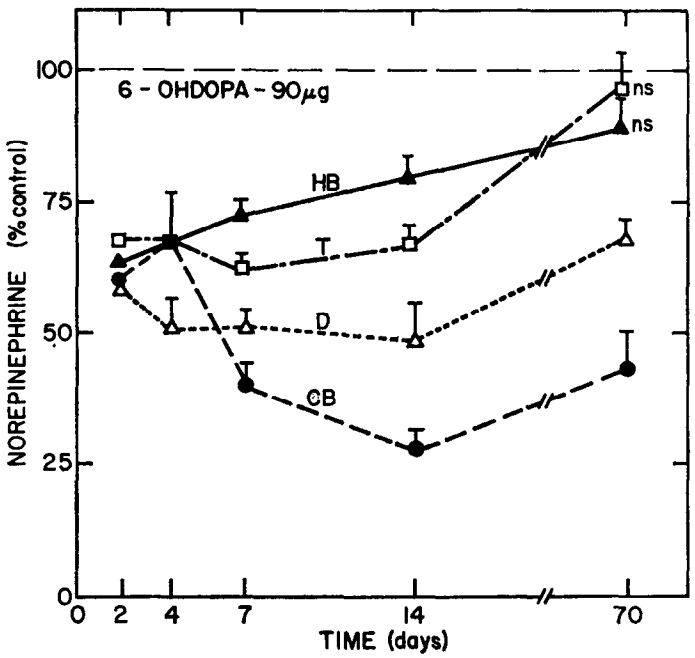

FIG. 2.—Time-course curves after a single intravenous injection of 90 μg 6-hydroxydopa (6-OHDOPA) of the norepinephrine (NE) content in the hindbrain (HB), telencephalon (T), diencephalon (D) and cerebellum (CB) of the rat brain. Each point represents the mean ± S.E.M. of 6–16 drug-treated rats as compared with 6–11 diluent-injected control rats; "n.s." refers to those points that are not statistically different from controls.

Injection of 90 μg 6-OHDOPA reduces food and water consumption for three to five days, but returns to normal at a time when NE levels in the diencephalon are still greatly reduced.

The level of emotionality was quantified by a modification of the rating scale devised by BRADY and NAUTA (1955) to measure the septal rage syndrome. Emotionality scores were increased in rats after an intraventricular injection of 6-OHDOPA and returned to normal after two months, when NE levels in the telencephalon and hindbrain had also returned to normal (Fig. 2). Further studies are needed to determine whether the telencephalon, which includes the limbic system, and/or hindbrain, which includes the reticular formation, may be involved with the inhibition of emotional reactivity.

A significant increase in shock-induced aggression occurred in rats four days after an intraventricular injection of 90 μg 6-OHDOPA (THOA *et al.*, 1973). It may be suggested that shock-induced aggression is modulated through a central noradrenergic system.

HISTOCHEMICAL STUDIES

The catecholamine histofluorescence method of Falck and Hillarp (FALCK, 1962; FALCK *et al.*, 1962; FALCK and OWMAN, 1965) was used to examine the effects of 6-OHDOPA on the brain. Mice were treated with 6-OHDOPA (100 or 150 mg/kg,

i.v.) and observed after 1–23 days (JACOBOWITZ and KOSTRZEWA, 1971). After 24 hr, there was a variable decrease in the number of varicosities in regions known to contain noradrenergic fibers (DAHLSTRÖM and FUXE, 1964; DAHLSTRÖM and FUXE, 1965; FUXE, 1965). The most striking observation was the appearance of an abundance of intensely fluorescent nonterminal smooth axons in the reticular formation of the pons-medulla and mesencephalic regions.

Pretreatment with monoamine oxidase inhibitors resulted in an increase in the intensity of fluorescence of the nonterminal axons (JACOBOWITZ and KOSTRZEWA, 1971; SACHS and JONSSON, 1972a; SACHS and JONSSON, 1972b), which was reminiscent of that observed after central regional ablation or stereotaxic lesions (ANDEN *et al.*, 1965; ANDEN *et al.*, 1966). There were no obvious changes in the fluorescence content of dopamine-containing regions. This, coupled with the fact that only the NE content of the brain was reduced after injection of 6-OHDOPA, led to the suggestion that the nonterminal axons were primarily noradrenergic (JACOBOWITZ and KOSTRZEWA, 1971). The accumulation of catecholamine in nonterminal axons in the brain, which normally are not visible or are in some regions just at the limit of fluorescence observation, provided an opportunity to map out the nonterminal noradrenergic axons of the brain. Such pathways were obtained by mapping onto projections of cresylviolet-stained slides. The noradrenergic cell bodies (A1, A2, A7, locus coeruleus) and the dopaminergic cell bodies in the substantia nigra did not appear to be different from those of the controls. A major trunkline of large axons was observed bilaterally through the reticular formation that descends towards the median forebrain bundle (MFB), which carries axons to the hypothalamus, septal region and cerebral and olfactory cortices. This major reticular formation tract in the mouse is probably equivalent to the dorsal noradrenergic bundle described by UNGERSTEDT (1971) in the rat. Nonterminal processes were no longer seen two weeks after injection of 6-OHDOPA. Noradrenergic terminals appeared normal in most regions except the cortex, hippocampus and cerebellum. The reappearance of varicose terminals suggests that regeneration of noradrenergic nerves occurs.

Histochemical observations of rat brains were made after intraventricular injections of 90 μg 6-OHDOPA (RICHARDSON and JACOBOWITZ, 1973). After 24 hr, a reduction in the number and intensity of fluorescence of varicose nerve fibers was observed in regions containing noradrenergic terminals. There was a greater decrease in varicose terminals in the regions more proximal to the ventricular system (e.g. periventricular, paraventricular and dorsomedialis nuclei of the hypothalamus). Regions containing dopamine nerve terminals and all monoaminergic cell bodies (NE, serotonin, dopamine) in the hindbrain appeared normal. As seen in the mouse brain one day after an i.v. injection of 6-OHDOPA, the most prominent observation was the appearance of many nonterminal axons with an intense fluorescence (RICHARDSON and JACOBOWITZ, 1973; THOA *et al.*, 1973). The axonal trunks contained swollen and enlarged segments. The biochemical and histochemical evidence indicates that these nonterminal axons are noradrenergic processes. After 4 and 7 days, small axonal sprouts of varicose nerve fibers were observed to be budding off the main trunks. After 14 days, regions of terminal regeneration were observed in the forebrain. It therefore appears that the regrowth of noradrenergic nerves results from axonal and/or perhaps terminal sprouting and probably accounts for the return to normal levels of telencephalic and hindbrain NE 70 days after injection

of 6-OHDOPA. It also seems that reinnervation to the cerebellum is very inefficient after an intraventricular injection of 6-OHDOPA in the rat. Possibly, the cerebellar penduncles are somehow not conducive to regeneration of adrenergic neuronal processes. The suggestion that individual NE cell bodies of the locus coeruleus can innervate both the cerebral cortex and the cerebellum (ANDEN *et al.*, 1967; OLSON and FUXE, 1971; UNGERSTEDT, 1971) may explain the increased rate of regeneration of noradrenergic nerves to the telencephalon. An increased transport of NE in the remaining axonal trunks could serve to facilitate the regeneration of nerves to the telencephalon.

Because of the accumulation of fluorescent catecholamine in the nonterminal axons, noradrenergic neuronal pathways of the brain were capable of being mapped (JACOBOWITZ and RICHARDSON, in preparation). Rat brains were studied two to seven days after a single intraventricular injection of 6-OHDOPA (90 μg) with or without pretreatment with tranylcypromine two hours prior to sacrifice. The dorsal-ascending noradrenergic bundle (UNGERSTEDT, 1971) was revealed in the mesencephalon and followed to its point of union with the ventral and dopaminergic tracts in the MFB (Fig. 3). This bundle has previously been cited as giving off branches to the cerebral cortex, hippocampus, colliculus, thalamus and geniculate bodies (UNGERSTEDT, 1971; OLSON and FUXE, 1971).

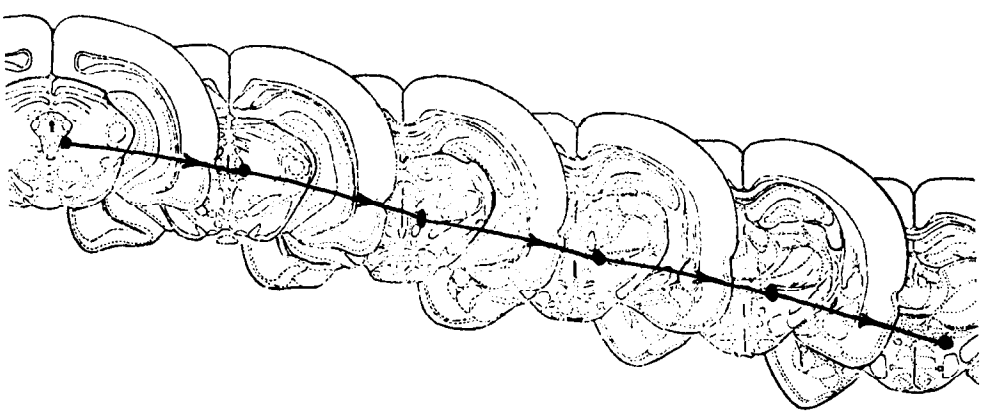

FIG. 3.—Projection of the dorsal adrenergic projection. Black circles indicate exact localization of the dorsal bundle. See KÖNIG and KLIPPEL (1963) brain atlas for coordinate information.

The fasciculus longitudinalis dorsalis (Schütz), pars tegmentalis, was found to contain a noradrenergic tract that appeared at the level of the mesencephalic-metencephalic junction (Fig. 4). This tract was followed rostrally through the substantia grisea as the fasciculus longitudinalis dorsalis (Schütz) in the mesencephalon. The latter bundle descends as the fibrae periventriculares hypothalami at the level of the posterior mamillary body and continues rostrally to the nucleus dorsomedialis (hypothalami), where it can no longer be followed. It would seem that this tract contributes noradrenergic fibers to the nucleus dorsomedialis and possibly to the periventricular nucleus.

Preterminal axonal accumulation of amine can be observed along the length of the MFB. At various levels, a small number of distorted axons can be followed

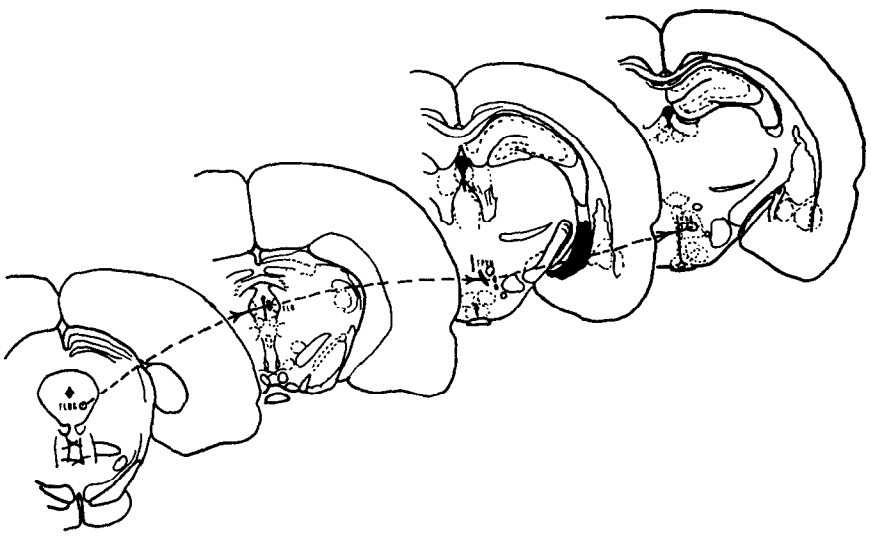

FIG. 4.—Projection of the fasciculus longitudinalis dorsalis (Schütz), pars tegmentalis (FLDG). Dashed-arrow line projects rostrally to the levels of the fasciculus longitudinalis dorsalis (Schütz), fibrae periventriculares hypothalami (FPVH) and nucleus dorsomedialis (hd). See KÖNIG and KLIPPEL (1963) for coordinate information.

to regions of terminations. Innervation of the amygdala emanates from the MFB, courses rostrally via the ansa lenticularis and the stria terminalis (pars infracommissuralis, pars precommissuralis) through the nucleus interstitialis striae terminalis, all of which turn caudally at the level of the preoptic nucleus to follow the dorsal stria terminalis to the level of the posterior hypothalamus, where it descends into the amygdaloid complex (Fig. 5). The rosto-caudal direction of the dorsal stria terminalis was previously described (UNGERSTEDT, 1971). It is not clear whether an additional route to the amygdala exists from the MFB via the commissura supraoptic dorsalis, pars ventralis (Meynert) and internal capsule (pars retrolenticularis).

The septal area is innervated by noradrenergic nerves via the MFB through the tractus diagonalis (Broca) and tractus septohypothalamicus (Fig. 5). The nucleus accumbens appears to contain noradrenergic processes from the MFB by way of the tractus striohypothalamicus (Fig. 5). It would be of interest to know whether the latter processes provide terminal ramifications of noradrenergic terminals or are merely axons on passage through the nucleus accumbens, a region currently regarded as primarily dopaminergic (DAHLSTRÖM and FUXE, 1965; UNGERSTEDT, 1971).

The hippocampus receives innervation via the MFB by way of the tractus diagonalis through the septum along the fornix superior in a caudal direction beneath the corpus callosum (Fig. 6). Another possible path is along the tractus corticohabenularis medialis from the stria medullaris into the fornix column and caudally with the fornix superior to the hippocampus (Fig. 6). A tract appears to traverse the stria medullaris in a caudal direction. The destination of these processes is not clear, although it is suggested here that these axons innervate the thalamus.

The cortex receives innervation by several routes: (1) Via the MFB and tractus

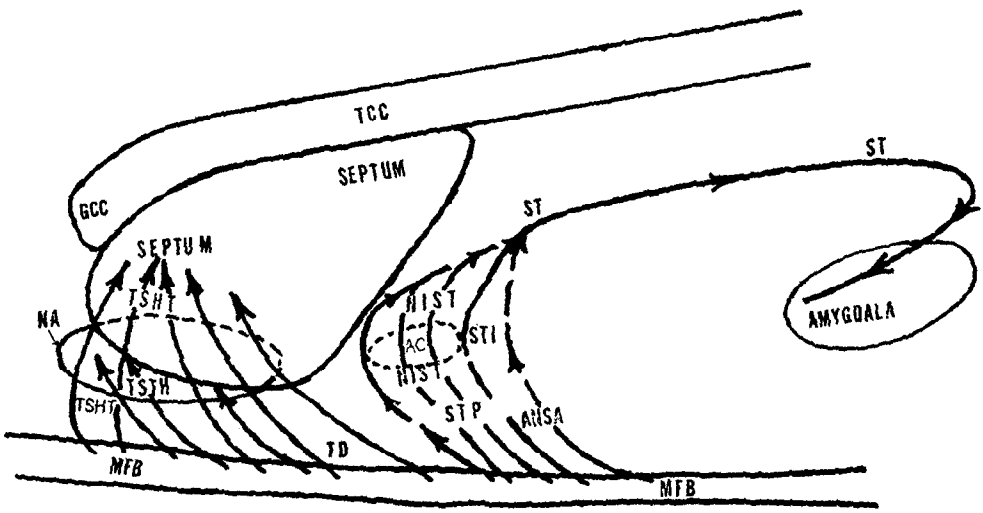

FIG. 5.—Schematic parasagittal projection of noradrenergic fiber tracts to the amygdala, septum and nucleus accumbens (NA). Abbreviations: anterior commissure (AC); ansa lenticularis (ANSA); genu corporis callosi (GCC); median forebrain bundle (MFB); nucleus interstitialis striae terminalis (NIST); stria terminalis (ST); stria terminalis, pars infracommissuralis (STI); stria terminalis, pars precommissuralis (STP); truncus corporis callosi (TCC); tractus diagonalis Broca (TD); tractus septohypothalamicus (TSHT); tractus striohypothalamicus (TSTH).

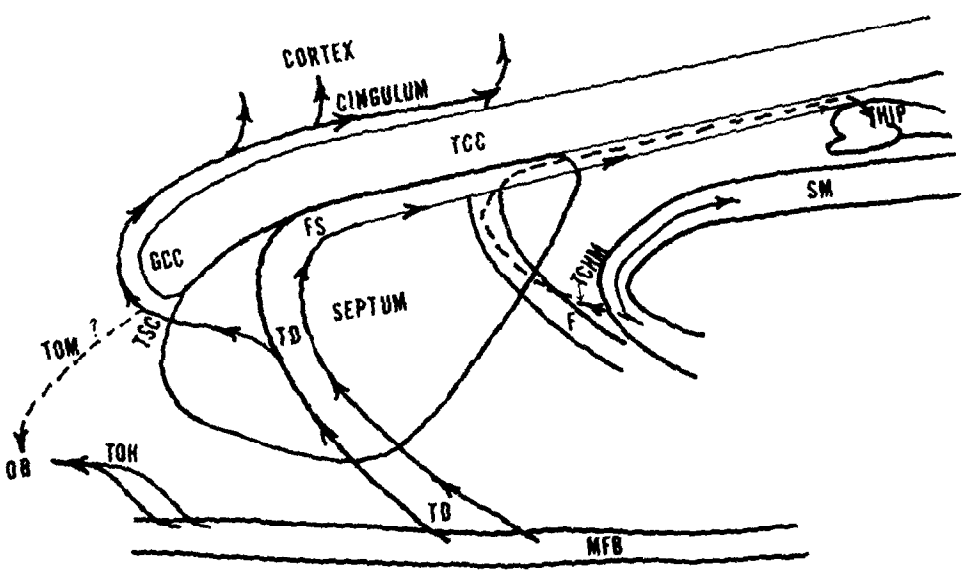

FIG. 6.—Parasagittal projection of noradrenergic projection fiber tracts to the cortex, hippocampus and olfactory bulbs. Abbreviations: columna fornicis (F); fornix superior (FS); genu corporis callosi (GCC); hippocampus (HIP); median forebrain bundle (MFB); olfactory bulbs (OB); stria medullaris thalami (SM); truncus corporis callosi (TCC); tractus corticohabenularis medialis (TCHM); tractus diagonalis (Broca) (TD); tractus olfactohypothalamicus (TOH); tractus olfactorius medialis (TOM); tractus septocorticalis (TSC).

diagonalis through the septum and around the genu of the corpus callosum by way of the tractus septocorticalis and caudally through the cingulum (Fig. 6). (2) Via the MFB through the commissura supraoptica dorsalis, pars ventralis (Meynert), into the internal capsule, which then courses through the caudate-putamen. (3) Via the dorsal bundle through Forel's fields H1 and H2 and zona incerta at the level of the nucleus subthalamicus. These processes enter the internal capsule which then penetrates the caudate-putamen and corpus callosum into the cortex.

The olfactory bulbs receive noradrenergic nerves from the MFB via the tractus olfacto-hypothalamicus and possibly from the tractus olfactorius medialis (Fig. 6).

SUMMARY

After a single i.v. injection, 6-OHDOPA is capable of causing destruction and/or functional impairment of noradrenergic nerve terminals in both the central and the peripheral nervous system. By analogy with the effects of 6-hydroxydopamine and histochemical observations of preterminal accumulation of neurotransmitters, the conclusion that 6-OHDOPA causes degenerative changes in the terminal plexus is derived. There is no question that the final determination of neuronal degeneration should come from electron microscopic studies. The accumulation of histochemically observable catecholamine fluorescence in nonterminal axons, the time course of depletion and recovery of NE within various peripheral organs and impairment of uptake of exogenously administered NE support the conclusion that 6-OHDOPA has the potential to cause selective destruction of noradrenergic terminals.

6-OHDOPA is a valuable tool for the investigation of central pathways and functional organization of the noradrenergic neurons in the brain. Injection of 6-OHDOPA into the cerebral ventricles can produce a highly specific reduction in the NE content of the central nervous system while leaving dopaminergic neurons unaffected. This factor will be useful for the exploration of the behavioral contributions of central noradrenergic neurons.

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