Effects of protriptyline on the depletion of catecholamines induced by 6-hydroxydopamine in the brain of the rat

The compound 6-hydroxydopamine produces a long-lasting depletion of catecholamines when injected intraventricularly into the rat brain. Only catecholamine-containing neurons are affected; brain dopamine being less severely depleted than noradrenaline. This effect appears to be associated with a degeneration of nerve terminals (Bloom, Algeri & others, 1969; Uretsky & Iversen, 1970; Bartholini, Richards & Pletscher, 1970).

The effects of 6-hydroxydopamine and other catecholamine depleting agents on the mouse heart are antagonized by desipramine and protriptyline (Stone, Porter & others, 1964). Desipramine and related drugs are thought to antagonize the peripheral effects of 6-hydroxydopamine by blocking its uptake into sympathetic nerves, which appears to be a prerequisite for the depleting action (Malmfors & Sachs, 1968; Thoenen & Tranzer, 1968). Desipramine and protriptyline are known to be potent competitive inhibitors of the neuronal catecholamine uptake process in sympathetic nerves (Carlsson & Waldeck, 1965; Iversen, 1967; Berti & Shore, 1967).

Desipramine also blocks the uptake of noradrenaline after intraventricular injection of the labelled catecholamine into the rat brain (Glowinski & Axelrod, 1964). Studies of catecholamine uptake in tissue slices from various regions of the rat brain show that noradrenaline uptake is inhibited by desipramine in regions in which noradrenaline neurons predominate, but is less effective in the neostriatum in which dopamine neurons are the most abundant (Häggendal & Hamberger, 1967; Snyder, Green & Hendley, 1968). Fuxe & Ungerstedt (1968), using fluorescence microscopy, found that desipramine and protriptyline inhibited the accumulation of catecholamines by noradrenaline containing neurons but not by dopamine-containing neurons.

In view of these findings it seemed possible that desipramine or protriptyline might selectively antagonize the effects of 6-hydroxydopamine on noradrenaline-containing neurons in the brain, without affecting the actions of the drug on dopamine-containing neurons.

Protriptyline hydrochloride dissolved in saline was administered intraperitoneally (doses expressed as the hydrochloride) to male albino Wistar rats weighing about 160 g. After treatment with protriptyline, 6-hydroxydopamine was given intraventricularly (doses expressed as free base) (Uretsky & Iversen, 1970). All rats were prepared for intraventricular injection (Noble, Wurtman & Axelrod, 1967) on the day before treatment, to facilitate the timing of the protriptyline-6-hydroxydopamine schedule. Animals were killed 4 days after treatment, and their brain removed and weighed before the extraction and fluorimetric assay of dopamine and noradrenaline (Uretsky & Iversen, 1970). Each experiment involved four groups of animals. 1. Saline + artificial csf. 2. Protriptyline + artificial csf. 3. Saline + 6-hydroxydopamine. 4. Protriptyline + 6-hydroxydopamine.

In the first experiment the time course of the effect of protriptyline (10 mg/kg) on 6-hydroxydopamine (250 μg/rat) was investigated, with intervals from 15 to 180 min between administration of the two drugs. Protriptyline indeed antagonized the 6-hydroxydopamine induced depletion of brain noradrenaline at all time intervals (Fig. 1). Brain dopamine, on the other hand, was significantly protected from the actions of 6-hydroxydopamine only at the 60 and 90 min intervals; this may reflect changes in the concentration of protriptyline in the brain with time. Using the optimum interval of 120 min, the effects of 5, 10 and 15 mg of protriptyline/kg on the catecholamine depletion induced by 250 μg of 6-hydroxydopamine were compared (Fig. 2). The optimum dose for protection of noradrenaline was 15 mg of

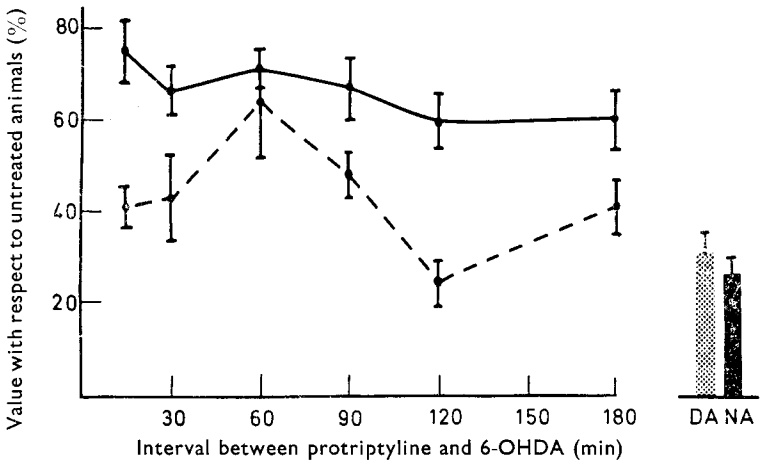

FIG. 1. The effect of 250 μg of 6-hydroxydopamine (6-OHDA) on brain noradrenaline (NA) and dopamine (DA) (solid lines and dotted lines respectively) when administered at various times after treatment with protriptyline hydrochloride (10 mg/kg). The histograms represent the depletions induced by 250 μg of 6-OHDA in saline pretreated animals. All values are expressed as percentages of those from untreated controls (i.e. saline i.p. + artificial csf intraventricularly), and are means and s.e. for not less than five animals.

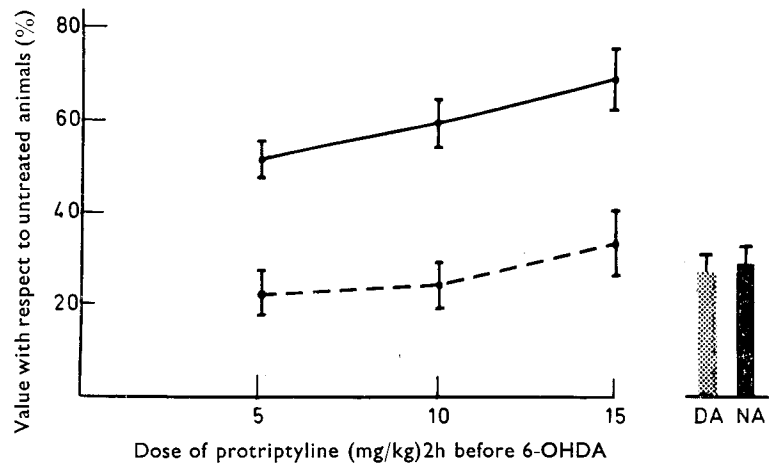

FIG. 2. The effect of varying the pretreatment dose of protriptyline hydrochloride on the catecholamine depletons induced by 250 μg of 6-OHDA, with an interval between drugs of 120 min. Other details as for Fig. 1.

protriptyline/kg. None of the doses of protriptyline, however, had any significant effect on the 6-hydroxydopamine-induced depletion of dopamine. This selective effect of protriptyline pretreatment was most obvious in the third experiment, in which the dose of 6-hydroxydopamine was varied (Fig. 3). For example, a dose of 100 μg given 120 min after treatment with protriptyline (15 mg/kg) produced no significant change in the level of noradrenaline, although dopamine was reduced to 50% of the normal level. The same dose given to saline-pretreated animals produced a depletion of noradrenaline to 47% and of dopamine to 55% of normal levels. In all three experiments the levels of brain catecholamines 4 days after treatment

with protriptyline alone (5–15 mg/kg) were not significantly altered. Overall means for dopamine and noradrenaline levels in control animals were 0·53 ± 0·045 μg/g and 0·34 ± 0·028 μg/g respectively (corrected for recovery).

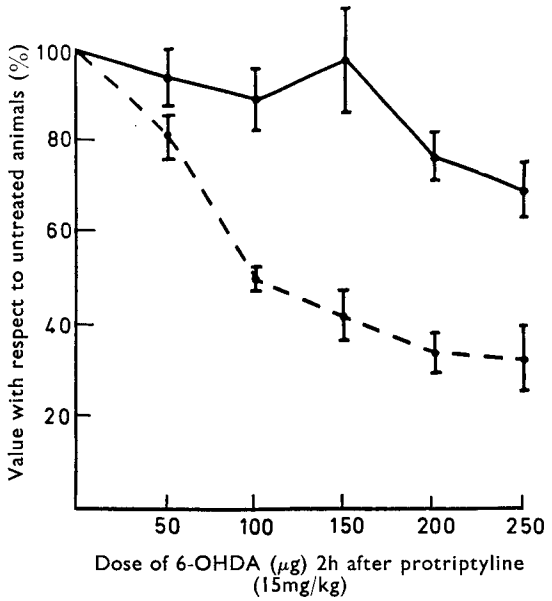

FIG. 3. Depletions of NA and DA induced by various doses of 6-OHDA given 120 min after treatment with protriptyline hydrochloride (15 mg/kg). Details as for Fig. 1.

By pretreatment with protriptyline, it is thus possible to produce animals in which dopamine-containing neurons are chemically lesioned by 6-hydroxydopamine while noradrenaline-containing neurons remain intact. The findings of Coyle & Snyder (1969) indicate that benztropine (cogentin) is a more potent inhibitor of dopamine uptake than of noradrenaline uptake in the rat brain. By pretreatment with benztropine we believe it may be possible to antagonize the effect of 6-hydroxydopamine on dopamine neurons without altering its action on noradrenaline neurons. Studies of the behaviour of animals treated in these various ways are in progress (Evetts, Uretsky & others, 1970).

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Effects of 6-hydroxydopamine on the perfused rat mesentery preparation

We have recently shown in the pithed rat that after pretreatment with 6-hydroxydopamine, an agent producing chemical sympathectomy (Thoenen & Tranzer, 1968), supersensitivity develops to injected noradrenaline (Finch & Leach, 1970). Similar results have been obtained in the spinal cat by Haeusler, Haefely & Thoenen (1969), who observed a 10–30 fold increase in the responses to noradrenaline. In contrast to the cardiovascular responses, the isolated heart exhibited only a 3·5 times greater sensitivity to noradrenaline. It was, therefore, decided to investigate whether 6-hydroxydopamine could produce supersensitivity in the vascular beds.

Male C.S.E. rats, 300–350 g, were given intravenously 6-hydroxydopamine (A. B. Kistner, Gotenborg) (2 × 50 mg/kg on day 1 and 2 × 100 mg/kg on day 7). Perfusion experiments were made on day 10. Mesenteric vessels were isolated and perfused with Krebs solution (McGregor, 1965). Fibres of the periarterial nerve plexus were stimulated at supramaximal voltage (30–40 V), pulse duration 1 ms and a frequency of 6–25 Hz repeated every 2 min. To investigate changes in sensitivity to exogenously administered drugs, noradrenaline and adrenaline (as base) were injected into the perfusate every 3 min in doses of 0·01–1·0 μg.

After pretreatment with 6-hydroxydopamine, the vasoconstrictor responses of the mesenteric preparation to sympathetic nerve stimulation were abolished at low frequencies of stimulation (6, 12 Hz) and markedly reduced at 25 Hz. Noradrenaline sensitivity after treatment with 6-hydroxydopamine was increased by 10–12 times (Fig. 1A). Similar results were obtained for adrenaline. When desipramine (10^{-8} g/ml) was added to the perfusion fluid, no further increase in sensitivity to noradrenaline occurred in the preparations pretreated with 6-hydroxydopamine (Fig. 1B). Control preparations, however, showed potentiated responses similar in extent to those seen in 6-hydroxydopamine-treated preparations.

These results suggest that 6-hydroxydopamine produces chemical sympathectomy of the mesenteric blood vessels. Supersensitivity to injected noradrenaline also occurred and there was no further potentiation after desipramine pretreatment. It would seem, therefore, that chemical sympathectomy abolishes the normal physiological uptake process by day 10 and produces a pre-junctional supersensitivity to noradrenaline. These results are only in partial agreement with those reported by McGregor & Phelan (1969) who found that 6-hydroxydopamine abolished the vasoconstrictor responses to nerve stimulation but did not alter the noradrenaline sensitivity of the perfused mesenteric arteries. Their treatment with 6-hydroxydopamine (30 mg/kg, i.p.) produces only an incomplete depletion of endogenous catecholamines