Lack of effect of protriptyline on the 6-hydroxydopamine -induced noradrenaline depletion in the rat spinal cord

6-Hydroxydopamine (6-OH–DA) is thought to be a specific toxic compound for catecholamine neurons (for review see Malmfors & Thoenen, 1971). The specificity is considered to be the result of an uptake of 6-OH–DA into peripheral adrenergic nerves (Jonsson & Sachs, 1970) and catecholamine neurons in the central nervous system (Schubert, Kreutzberg & others, 1973). After uptake, 6-OH–DA causes neuronal degeneration by uncoupling of oxidative phosphorylation (Wagner, 1971), formation of hydrogen peroxide (Heikkila & Cohen, 1972) and/or formation of quinones (Saner & Thoenen, 1971).

Recent studies indicate that the action of 6-OH–DA injected into the central nervous tissue is not specific. The lesion observed after a 6-OH–DA injection could not be distinguished from that after electrocoagulation (Poirier, Langelier & others, 1972).

The catecholamine concentration mechanism in the cell membrane of peripheral adrenergic nerves (Hillarp & Malmfors, 1964) and of monoamine neurons in the central nervous system (Hamberger & Masuoka, 1965) is efficiently inhibited by protriptyline (PTP) (Carlsson & Waldeck, 1965; Carlsson, Fuxe & others, 1966). If the effect of 6-OH–DA is specific for catecholamine neurons after injection into the tissue of the central nervous system, the depletion of noradrenaline should be antagonized by PTP. This has been shown after intraventricular administration of 6-OH–DA (Evetts & Iversen, 1970). No results have been reported, however, on the effect of PTP on the monoamine neuron degeneration induced by 6-OH–DA injected into the brain or the spinal cord when much higher tissue concentrations of 6-OH–DA can be expected than after intraventricular or intravenous injections. Therefore the effect of PTP on 6-OH–DA-induced degeneration in the spinal cord was explored.

Protriptyline hydrochloride dissolved in 0·9% NaCl was administered (25 mg kg^{-1} i.p.) (doses expressed as free base) to male Sprague-Dawley rats weighing 180–200 g. After 30–45 min the spinal cord was exposed at the level of C7 under pentobarbitone sodium (Nembutal)anaesthesia (40 mg kg^{-1}, i.p.) and 2 μl (6μg μl^{-1}) of 6-OH–DA-hydrobromide dissolved in 0·9% NaCl with 1% ascorbic acid was injected into the spinal cord on each side of the central canal and 2 μl of the same solution was administered under the pia mater bilaterally. Control animals were similarly prepared but with injection of solvent instead of 6-OH–DA, PTP or both. Rats pretreated with PTP slept several hours longer than those without PTP treatment.

After 30 days the rats were killed by thoracotomy and exsanguination under light chloroform anaesthesia. The spinal cord was rapidly removed and divided into two parts, cranial and caudal to the injection site. A piece of the cord, about 5 mm in length around the injection site was excised and discarded. Noradrenaline was determined in both parts of the cord after cation exchange chromatography as described previously (Bertler, Carlsson & Rosengren, 1958; Häggendal, 1963; Andén & Magnusson, 1967). Statistical analysis was made by Student's *t*-test.

After recovery from the operation both the 6-OH–DA treated and the control animal appeared grossly normal with normal posture, movements and sensibility and without disturbance of the urination and defaecation. The noradrenaline in the spinal cord caudal to the injection site was significantly ($P<0·001$) decreased in the 6-OH–DA treated groups after 30 days. There was no difference ($P>0·5$) between the 6-OH–DA group pretreated with PTP and that without PTP pretreatment (Table 1).

The results show that 6-OH–DA injected into the tissue of the central nervous system at the concentration used here produces the same biochemical effects before and after PTP treatment. Assuming that PTP inhibits the uptake of 6-OH–DA into

Table 1. *Noradrenaline concentratians ($\mu g\ g^{-1}$) in the spinal cord of rats 30 days after injection of 6-OH-DA (2 × 2 μl, 6 $\mu g\ \mu l^{-1}$) or 0·9% NaCl into the spinal cord at the level of C7 after pretreatment with protriptyline (PTP) (25 mg kg^{-1}) or NaCl i.p. Cr = cranial, Cau = caudal to the injection site of 6-OH-DA.*

	PTP + 6-OH-DA		NaCl + 6-OH-DA		PTP + NaCl		NaCl + NaCl	
	Cr	Cau	Cr	Cau	Cr	Cau	Cr	Cau
x	0·32	0·03	0·26	0·03	0·28	0·31	0·36	0·34
s.e.m.	0·01	0·01	0·03	0·01	0·01	0·05	0·02	0·02
n	5	5	22	22	2	2	16	16

noradrenaline neurons, the present findings indicate that the action of 6-OH-DA is not quite specific. 5-Hydroxytryptamine in the spinal cord in rats treated with 6-OH-DA in exactly the same way as in this experiment (unpublished observations) also shows a decrease, but only down to 45% of normal values. The fact that the 6-OH-DA groups did not differ from the controls in the gross behaviour seems to argue against a totally unspecific effect, as the noradrenaline neurons are spread over a large area in the transverse section of the rat spinal cord (see Dahlström & Fuxe, 1965). A possible explanation is that thin fibres are preferentially affected. The fact that 5-hydroxytryptamine neurons are less affected than noradrenaline neurons could be due to their different distribution in the cord (see Dahlström & Fuxe, 1965).

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