On the long-lasting inhibitory effect of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP 4) on the active uptake of noradrenaline

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In a study of a series of tertiary *N*-haloalkyl-2-bromobenzylamine derivatives (Ross, Johansson & others, 1973) it was observed that the two β-chloroalkylamines *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP 4) and *N*-(2-chloropropyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP 6) potently inhibited the uptake of noradrenaline with long duration. Since phenoxybenzamine is an inhibitor of the noradrenaline uptake (Iversen, 1965; Iversen & Langer, 1969) and shares the property of DSP 4 and DSP 6 in being an alkylating agent we have now compared the inhibitory activities of phenoxybenzamine and DSP 4 on the noradrenaline uptake in the mouse brain.

The uptake of [^{3}H]noradrenaline in slices of the mouse brain was determined as described by Ross & Renyi (1967). The tissue slices (100 mg) were pre-incubated for 5 min and then for 5 min with [^{3}H]-noradrenaline (1×10^{-7} M). The radioactivity in the slices was taken as a measure of the uptake. The active uptake was defined as that part inhibited by 3×10^{-5} M cocaine.

In vitro, DSP 4 inhibited the uptake of noradrenaline in the cerebral cortex slices of the mouse by 50% at 3×10^{-7}M. It has about the same activity as that found for (+)-amphetamine (Ross & Renyi, 1967). The corresponding value for phenoxybenzamine was 6×10^{-6}M, which means that DSP 4 was about 20 times more active than phenoxybenzamine.

After a single injection of a high dose of DSP 4 (100 mg kg^{-1}, i.p.) the inhibitory effect on the uptake of noradrenaline in brain slices lasted for a very long time (Fig. 1). Phenoxybenzamine was much weaker than DSP 4 as an inhibitor of the noradrenaline uptake in the mouse brain *in vivo*. The inhibition of the uptake in cerebral cortex slices of the mouse was only significant 1 h after the injection of 50 mg kg^{-1}, i.p. (Fig. 1).

Since DSP 4 (100 mg kg^{-1}, i.p.) had no effect on the uptake of [^{3}H]dopamine in slices of striatum or on that of [^{3}H]5-hydroxytryptamine in slices of midbrain of the mouse, the inhibition of the noradrenaline uptake seems to be specific for this amine.

The dose response of the irreversible inhibitory effect of DSP 4 on the noradrenaline uptake in slices of mouse brain was examined (Fig. 2). The inhibition 24 h after the injection was higher than 50% for doses down to 25 mg kg^{-1}, intraperitoneally whereas 10 mg kg^{-1} produced no effect. Thus, the dose response curve was rather steep. It is obvious from this and other experiments that DSP 4 did not cause a complete block of the uptake but that the inhibition was limited to about 50–70% of the total cocaine sensitive uptake. This limitation may in part be due to the presence of dopaminergic neurons in the cerebral cortex slices (Thierry & Glowinski, 1973) which also take up noradrenaline, but this uptake is not inhibited by DSP 4.

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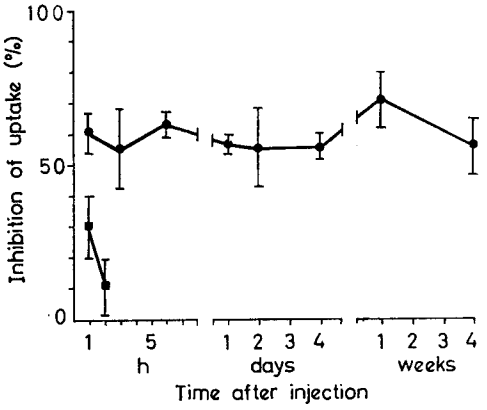

FIG. 1. Time curve of the inhibitory action of DSP 4 100 mg kg^{-1} (●) and phenoxybenzamine 50 mg kg^{-1} (■), on the uptake of [^{3}H]noradrenaline in mouse cerebral cortical slices. Each point is the mean of 4 animals. The vertical bars are s.e.m. The slices were incubated with 1×10^{-7} M of [^{3}H]noradrenaline for 5 min. Inhibition was determined in per cent of the cocaine sensitive uptake (0·084 ± 0·008 nmol g^{-1} in 5 min).

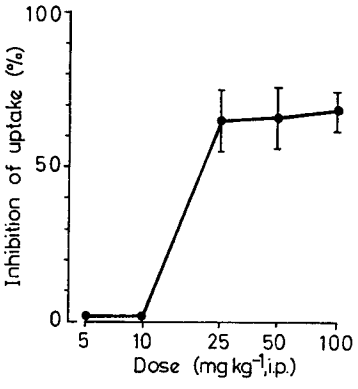

FIG. 2. Dose response curve of the inhibitory action of DSP 4 on the uptake of [^{3}H]noradrenaline in mouse cerebral cortical slices 24 h after its injection. Each point is the mean of 4 animals. The vertical bars are s.e.m.

Table. 1. *Antagonizing effect of (±)-amphetamine sulphate on the irreversible inhibition of noradrenaline uptake in mouse cerebral cortex slices produced by DSP 4.*

Treatment	Dose mg kg^{-1} i.p.	Noradrenaline uptake mol g^{-1} in 5 min ± s.e.m.	Inhibition of active uptake†%
1. Saline	—	0·148 ± 0·008	—
2. DSP 4	100	0·102 ± 0·005	52*
3. (±)-Amphetamine sulphate	10	0·131 ± 0·004	19
4. (±)-Amphetamine sulphate + DSP 4	10 100	0·121 ± 0·002	33*

*0·05 > P > 0·01 (2 compared to 4; Student's t-test).
†Active uptake in the controls (=cocaine sensitive uptake): 0·088 nmol g^{-1} in 5 min. The mice were injected with (±)-amphetamine sulphate (10 mg kg^{-1}, i.p.) 15 min before DSP 4 and killed 24 h later. The slices were incubated with 1 × 10^{-7}M [^{3}H]noradrenaline for 5 min. Each value is the mean ± s.e.m. of 4 determinations (animals).

The inhibitory effect of DSP 4 under the brief incubation conditions used in these experiments indicates that it acted on the uptake at the level of the neuron membranes. Compounds interfering with the neuronal storage mechanisms for noradrenaline, e.g. reserpine, have only a weak effect under these conditions (Ross & Renyi, 1966). More support for this hypothesis was provided by the observation that (±)-amphetamine sulphate, an uptake inhibitor at the membrane level (Ross & Renyi, 1967), injected 15 min before DSP 4 significantly antagonized the irreversible effect of DSP 4 (Table 1).

Like phenoxybenzamine, DSP 4 also blocks α-adrenoceptors (Ross & others, 1973). To judge from preliminary experiments in rats the block of the vasopressor effect produced by noradrenaline, the duration of this effect is much shorter than that of the inhibition of noradrenaline uptake in brain neurons.

The results presented in this report show that DSP 4 is a remarkable long-acting and specific inhibitor of the noradrenaline uptake in the mouse brain. If this effect is due to alkylation of the noradrenaline uptake receptors or to degeneration of noradrenergic neurons is now being investigated. In any case DSP 4 may become a valuable pharmacological tool since it readily penetrates the blood brain barrier and seems to be quite specific to the noradrenergic neurons.

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Indwelling catheters for direct recording of arterial blood pressure and intravenous injection of drugs in the conscious rat

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Methods have previously been described for the implantation of polythene catheters in the carotid artery (Popovic & Popovic, 1960) and abdominal aorta (Weeks & Jones, 1960) of rats for the recording of arterial blood pressure in conscious animals. Popovic & Popovic (1960) also described the implantation of a catheter in the right superior vena cava via the right external jugular vein. This communication describes modifications of the method for recording blood pressure from the abdominal aorta and also the implantation of a polythene catheter in the abdominal vena cava permitting remote intravenous administration of drugs or repeated sampling of venous blood of conscious rats.

Both catheters were constructed from polythene tubing (Portex) obtained from Jencons Scientific Ltd., Hemel Hempstead, Hertfordshire. A 15 cm length of pp50 polythene tubing was fused to a shorter length of pp25 tubing (5 cm) using a hot soldering iron; lengths of fuse wire passed through both sizes of polythene tubing were used to maintain the patency of the catheter during the fusing process. The open end of the pp25 tubing was then heat-sealed and the join in the catheter tested for leaks by injecting water from a syringe attached to the pp50 tubing. The heat-seal was then cut away and the patency of the catheter confirmed by rapid injection of water through the tubing. Each catheter was then bent by dipping into boiling water (Fig. 1). The pp25 tubing was bent tightly close to the join and the pp50 tubing similarly bent 6–10 cm from the join, depending upon the size of rat to be catheterized. Bending of the exteriorized portion of the catheter enables posterior projection and thereby affords some protection against damage.