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.

November 5, 1975

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

R. E. BUCKINGHAM, *Pharmacology Department, Roche Products Ltd., Welwyn Garden City, Hertfordshire, AL7 3AY, U.K.*

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.

For implantation, rats were anaesthetized with ether and the skin shaved at the back of the neck and over the abdomen. A midline incision, 1 cm long was made through the skin approximately 2 cm behind the ears and a further incision, 4 cm long, made on the midline of the abdomen. The peritoneal cavity was opened and the viscera retracted to expose the major blood vessels. Cotton wool was used to remove connective tissue overlying the abdominal aorta but that overlying the vena cava was left intact. A trocar made of brass tubing (15 cm long, internal diameter 0·15 cm) and bevelled at one end, was passed through the psoas muscles lateral to the genito-femoral nerve and medial to the left ureter, and then subcutaneously towards the incision at the back of the neck, permitting exteriorization of both polythene cathers. The trocar was removed through the neck incision and the catheters sutured into the psoas muscles close to the aorta (Fig. 2). At the neck incision each catheter was passed through a collar made of 0·5 cm pp240 polythene tubing. Earlier, a small notch was made in each collar with a soldering iron. Collars, together with the catheters passing through them were firmly sutured into the neck muscles. Catheters were then filled with 0·9% w/v saline from a 2 ml syringe attached to a 23G needle and the ends of the pp25 tubing cut to a point to facilitate entry to the vessel lumen. Aortic blood flow was briefly stopped by application of a small artery clip rostral to the level of the iliolumbar vessels. A small hole was made in the aortic wall using a 25 G hypodermic needle approximately 1·5 cm rostral to the bifurcation. The aortic catheter was inserted through the puncture, and the artery clip removed immediately. No leakage occurred at the point of insertion, the elasticity of the aortic wall being sufficient to close the wound around the catheter. Considerable care was required in implanting the venous catheter. The best results were obtained when connective tissue overlying the vena cava was left intact. The end of the catheter was pushed firmly through the wall of the vessel without occluding the venous flow or puncturing the vessel wall with a hypodermic needle. This procedure must be performed positively since accidental removal of the catheter from the vein results in massive

FIG. 1. Polythene catheter construction.

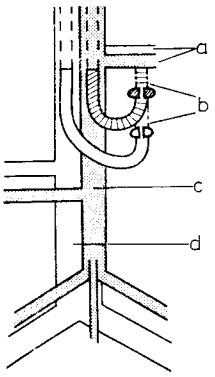

FIG. 2. Implantation of catheters in the major blood vessels. (a) Iliolumbar vessels. (b) Sutures into psoas muscles. (c) Abdominal aorta. (d) Posterior vena cava.

and uncontrollable haemorrhage. The length of pp25 tubing in each blood vessel was approximately 4 cm. The blood flow through each catheter was tested before sealing with a stilette. Stilettes consisted of domestic dress pins whose points had been removed by grinding and whose heads had been removed to prevent entanglement with the cage bar structure. The incisions were then closed and the animals housed individually and left to recover for 1–2 days before use. Asepsis and postoperative antibiotic therapy have not been found to be necessary.

During recordings of blood pressure, rats were placed in cylindrical tins (height 20 cm, outside diameter 20 cm). A polythene tubing connector (pp50) was joined to the implanted arterial catheter via a short length of hypodermic needle tubing (23G) and then to a pressure transducer via a hypodermic needle (23G). Between recordings, the connector was closed with a 1 ml syringe containing heparinized (heparin 50 I.U. ml^{-1}) 0·9% w/v saline. Similarly, remote intravenous injection or venous blood sampling could be performed via pp50 polythene tubing connected to the implanted venous catheter.

This method of arterial and venous catheterization is seen as a useful alternative to the method of Popovic & Popovic (1960). It has the advantage that it does not involve vessel occlusion and thereby blood stasis and the formation of thrombi is not encouraged. Rats prepared by this method have been used for recording blood pressure and receiving intravenous injections for periods of up to 2 weeks.

For this comparatively brief time span catheter blockade resulting from cellular deposition has not proved to be a problem. The most common reasons for failure of the arterial catheter appears to be the incidence of a fibrin plug distal to the join between the 2 sizes of polythene tubing, such that whilst it is possible to flush saline into the vessel, blood flow from the animal is restricted or totally inhibited. On examination of the arterial wall in contact with the catheter tip 1–2 weeks

after implantation we have observed some tissue erosion with white cell 'clumping' in the damaged area. These lesions have not been examined histologically. No significant problems have been encountered with the venous catheter. We have not adopted any protocol for regular flushing of implanted catheters for two reasons:

(i) We have been unable so far to find a suitable rapid method for frequent flushing. Conventional unsealing and re-sealing of each catheter for daily flushing damages the wall of the exteriorized portion of the catheter, thereby shortening its useful life.

(ii) We hold 50–60 catheterized animals at any given time and frequent flushing by conventional methods would be a time-consuming and, therefore, expensive exercise. In terms of cost-effectiveness we prefer to accept a small proportion of catheter failures and leave the animals undisturbed between time of implantation and time of usage (1–2 days) and between times of usage (2–3 days).

This technique has been used routinely in our laboratory for a considerble period with a low proportion of failures.

October 1, 1975

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The hydroxylation of *p*-tyramine in man

R. A. D. JONES, R. J. POLLITT*, *MRC Unit for Metabolic Studies in Psychiatry, University Department of Psychiatry, Middlewood Hospital, P.O. Box 134, Sheffield S6 1TP, U.K.*

Normal human urine contains *p*-hydroxymandelic acid, a metabolite of octopamine (Kakimoto & Armstrong, 1962). The production of octopamine from *p*-tyramine has been demonstrated in several animal tissues, including brain, and is particularly associated with the sympathetic nervous system (Carlsson & Waldeck, 1963; Brandau & Axelrod, 1972; Boulton & Wu, 1973). In rabbits after intraperitoneal injection of [^{14}C]-*p*-tyramine 7% of the radioactivity recovered in the urine was in the form of *p*-hydroxymandelic acid (Lemberger, Klutch & Kuntzman, 1966). In man, an increase in urinary *p*-hydroxymandelic acid after intravenous *p*-tyramine has been noted but it was not possible to determine whether this increase was due directly to conversion of infused tyramine to octopamine or to the release of endogenous octopamine stores (Bonham-Carter, Karoum & others, 1970). Surprisingly, no radioactive *p*-hydroxymandelic acid could be detected in the urine of four subjects receiving [1-^{14}C]-*p*-tyramine intravenously (Tacker, Creaven & McIsaac, 1972) suggesting that in man exogenous tyramine could not serve as a precursor for octopamine. We are investigating the metabolism of deuterium labelled *p*-tyramine in man using gas chromatography-mass spectrometry and report here the incorporation of label into both *p*-hydroxymandelic acid and 3,4-dihydroxyphenylacetic acid.

Two normal subjects (1 male, 1 female) were given 3,5-[$^{2}H_2$]-*p*-tyramine hydrochloride twice, once by mouth and once several weeks later intravenously. Urine was collected hourly for at least 3 h before the loads and at least 4 h afterwards; later collections were 2-hourly or overnight. Two subjects with manic-depressive psychosis were given intravenous tyramine only. The urinary acidic metabolites were extracted into ether and converted to the trimethylsilyl derivatives by standard methods (Dalgliesh, Horning & others, 1966). Gas chromatography-mass spectrometry with repetitive scanning was carried out using a modified Perkin-Elmer 270 instrument. Unlabelled compounds or α α,3,5-[$^{2}H_4$]-*p*-hydroxyphenylacetic acid, 3,5-[$^{2}H_2$]-*p*-hydroxymandelic acid and α α,2,5,6-[$^{2}H_5$]-3,4-dihydroxyphenylacetic acid were used as internal standards for quantitation. The identity of the metabolites rests on coincidence with either natural or deuterium labelled internal standards on at least three stationary phases (OV3, OV17 and OV225). The deuterated compounds emerge a few seconds before the natural compounds. The *p*-hydroxyphenylacetic acid was monitored using the *m/e* 296 fragment and the *p*-hydroxymandelic and 3,4-dihydroxyphenyl acetic acids using *m/e* 267 and 384 respectively.

Results for the two normal subjects are summarized in Table 1 and Fig. 1. The two patients gave similar results on the intravenous load, with incorporation of label into *p*-hydroxymandelic acid and 3,4-dihydroxyphenylacetic acid. With the 3,4-dihydroxyphenylacetic acid, low levels of labelling could not be measured accurately on our machine as the large peak at *m/e* 385 in the natural compound coincides with the M^+ peak of the deuterium labelled compound. The presence of only one deuterium atom in the labelled 3,4-dihydroxyphenylacetic acid agrees with the findings of Nagatsu, Levitt & Udenfriend (1964) that the NIH-shift does not occur between the 3 and 2 positions during the hydroxylation of tyrosine.

* Correspondence.