Assay of picomole quantities of polyamines by high pressure ion-exchange chromatography

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With the advent of the very sensitive assay based on dansylation and thin layer chromatography conventional amino-acid analysis by ion-exchange chromatography and ninhydrin detection has fallen out of favour. The purpose of the present demonstration is to show that more recent developments such as the use of microparticulate resins and fluorescence derivatisation have made ion-exchange chromatography much more competitive in terms of sensitivity whilst the attraction of automation of the assay and relatively simple sample preparation remains.

Although we are mainly concerned with the assay of the polyamines spermidine and spermine the methodology used is obviously equally applicable to the assay of most amino-acids including GABA. Separations are carried out at 75°C on a 15 cm × 3.2 mm column of a strongly acidic cation exchange resin with a styrene-divinyl benzene polymer lattice incorporating 7.5% cross-linkage and with a bead diameter of 7–10 μm. Buffer selection is controlled by a 6 position solenoid operated rotary valve (Rheodyne 50-03) which feeds a high pressure pump (Altex 110). A solenoid operated six port rotary valve with an external loop (Rheodyne 70-10) permits injection of sample on the high pressure side of the pump. Loop filling is automated using a turntable sampling unit (Chemlab CS 40) and a peristaltic pump. After separation, the eluant from the column is reacted with o-phthalaldehyde and 2-mercaptoethanol in borate buffer pH 9 (Roth & Hampai, 1973; Benson & Hare, 1975) which is delivered at the same flow rate as the column effluent by a pulse damped Milton-Roy pump (Magnus Scientific). After passage through a short delay coil the resulting fluorescence is monitored by a flow cell fluorometer (Gilson). The fluorometer output is electronically integrated (Infotronics CRS 309) and displayed on a two channel potentiometric recorder. Automated operation of the analytical sequence is controlled by a programming unit with a film loop timer (Chemlab).

Full details of the analytical procedure were presented at the demonstration.

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A method for injecting drugs into the nucleus tractus solitarii of conscious rats

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Noradrenaline, α-methylnoradrenaline and adrenaline have been shown to depress heart rate and blood pressure following bilateral injection into the region of the nucleus tractus solitarii (N.T.S.) of anaesthetised rats. These depressor responses are inhibited by prior administration of α-adrenoceptor antagonists into the N.T.S. (De Jong, 1974 & 1976; De Jong, Nijkamp & Bohus, 1975; De Jong & Nijkamp, 1976; Nijkamp & De Jong, 1975; Struyker Boudier, Smeets, Brouwer & Van Rossum, 1975).

A method is described which enables bilateral injection of drugs to be made into the region of the N.T.S. of conscious rats and therefore eliminates the effect of anaesthesia.

Male Sprague-Dawley rats (weight range 200–250 g) were anaesthetised with sodium pentabarbitone (60 mg/kg, i.p.). The animals were secured in a Neuman sterotaxic frame (incisor bar + 5 mm). The dorsal surface of the skull was exposed. Two holes were made in the skull anterior to lambda using a size 4 dental burr; 10 B.A. × 1/8 inch steel plated cheese headed screws were screwed into these holes to act as anchors for the guide tubes. The guide tubes (containing injection cannulae) were clamped in a small vice and lowered into the region of the N.T.S. (A.P. − 6.0 mm; Lat ± 0.7 mm; Vertical + 0.7 mm from ear bar zero) through previously drilled holes. The guide tubes were cemented to the anchor screws and skull using dental cement. The wound was then closed up, and stylets were placed in the guide tubes.

The animals were allowed to recover for two days before the descending aorta was cannulated for direct blood pressure (b.p.) and heart rate (h.r.) monitoring using the method described by Weeks & Jones (1960). After a two day recovery period the rats were used for a period of up to two weeks.

The guide tubes, stylets and injection cannulae were made to the specifications shown in Figure 1. The holes drilled in the perspex for the guide tubes were made with a size 72 (0.025 inch) drill bit which made holes slightly smaller than the 22 gauge guide tubes. 22 Gauge × 1 inch hypodermic needles were then forced through these holes which made glueing unnecessary. The Luer fittings were broken off and the needles were ground flush with the perspex and to the correct length with a 45° bevel.

This relatively simple method enables examination of the effect of drugs injected bilaterally into the N.T.S. on b.p. and heart rate of conscious rats. Bilateral electrolytic lesions of the N.T.S. using the coordinates given in this paper have also been attempted and they produced acute fulminating hypertension suggesting that the injection site is clearly in the region of the N.T.S. Electrodes and electrical parameters used to produce the lesions were as described by Doba & Reis (1973).

The effect of bilateral injection of adrenoceptor agonists and antagonists into the region of the N.T.S. on b.p. and heart rate of conscious rats using the method described above will be demonstrated.

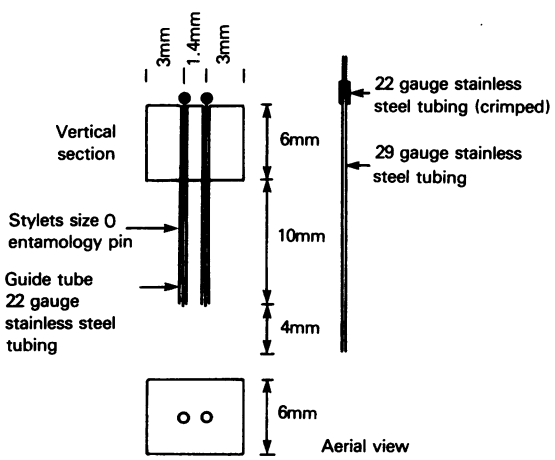

Figure 1 Dimensions of the guide tubes and injection cannula used for bilateral injection of drugs into the nucleus tractus solitarii.

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A novel modification of the isolated perfused heart preparation

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Several attempts have been made to modify the Langendorff perfused heart preparation so that it performs external work (see Morgan, Neely, Wood, Liebecq, Liebermeister & Park, 1965; Flynn, Gristwood & Owen, 1977). These attempts have concentrated on the principle of delivering perfusate to the left ventricle via the left atrium. We have designed a preparation which permits the right ventricle to do pumping work on fluid delivered via the right atrium. The coronary circuit is perfused separately via the aorta.

The thorax of a freshly killed rat was opened and the aorta cannulated. Ice-cold physiological saline (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM;