Plasma noradrenaline levels as a measure of noradrenaline release

C.R. BENEDICT, MARIANNE FILLENZ, CLARE STANFORD & INMACULATA VALERO

Department of Clinical Pharmacology, University Laboratory of Physiology and Department of Psychology, Oxford

In order to monitor noradrenaline (NA) release over extended periods, cannulae were inserted into the right atrium of rats through the superior vena cava under anaesthesia. Tubing connected to the cannula runs subcutaneously and is brought to the surface in the midscapular region. Rats were allowed to recover from the anaesthesia and were kept on a standard laboratory diet. Blood samples of 0.25 ml were withdrawn and replaced with an equal volume of sterile saline. The blood was put into heparinized tubes containing 1.6 mg glutathione/ml blood and centrifuged; the serum was diluted 1:4 with distilled water and stored deep frozen. The NA content of the samples was assayed using a radioenzymatic method (Hörtnagl, Benedict, Grahame-Smith & McGrath, 1977). The NA content of plasma in untreated rats was 1.88 ± 0.08 ng/ml ($n=16$, C.V.$=0.58$). When desmethylimipramine (DMI; 10 mg/kg) and normetanephrine (NMN; 50 mg/kg) were given in a single intraperitoneal injection, to block neuronal and extraneuronal NA uptake mechanisms respectively, plasma NA rose to 5.26 ± 0.51 ng/ml ($n=9$). In order to discover whether there were changes related to the time of day, plasma NA was measured in a group of rats at hourly intervals between 1100 and 1700 hours. There were no statistically significant variations in plasma NA level throughout this period and the mean coefficient of variation for the 6 h period was 0.50, similar to that for the control value, which represents individual variation. Experiments were next carried out to measure the effect of the administration of NA. Plasma NA was measured at various time intervals after the intravenous administration of 100 μg/kg of NA; calculations carried out on the basis of these measurements showed that 1 min after NA administration only 4% of the administered dose was found in the circulation. There followed an approximately exponential decline in plasma NA levels with a $T_{\frac{1}{2}}$ of 1.5–2 minutes. In order to compare the fate of exogenous and endogenous NA rats were subjected to a 1 min swim stress. Blood samples taken before and at the end of the swim stress (water temperature 17°C) showed a rise in plasma NA from 2.33 ± 0.13 ng/ml to 9.44 ± 2.06 ng/ml. After the termination of the swim stress plasma NA levels fell steeply within the first 2 minutes. This initial rate of decline was affected very little by the administration of DMI (10 mg/kg) and NMN (50 mg/kg).

These preliminary experiments suggest that plasma NA in rats shows little variation between individuals or with time of day; although only a fraction of the released NA escapes uptake and reaches the blood stream, even short-lasting changes in release rate are reflected in plasma NA levels. NA in plasma has a very short half-life and even in the presence of DMI and NMN mechanisms for the rapid removal of NA remain.

Reference

HÖRTNAGL, H., BENEDICT, C.R., GRAHAME-SMITH, D.G. & McGRATH, B. (1977). Sensitive radioenzymatic assay for adrenaline and noradrenaline in plasma. *Br. J. clin. Pharmac.* (in press).

Effects of uptake blockade on cardiac responses of anaesthetized dogs to isoprenaline, noradrenaline, and sympathetic nerve stimulation

R.A. BROWN, ANNE M. SOLCA & J. WILSON

Department of Pharmacology, Fisons Ltd., Pharmaceutical Division, R. & D. Laboratories, Bakewell Road, Loughborough, Leicestershire

The greater muscle mass of the ventricular myocardium relative to that of the atria suggests that extraneuronal uptake (uptake 2) of catecholamines may perform a more substantial role in the termination of inotropic rather than chronotropic responses of the heart to catecholamines. In contrast, the higher density of sympathetic innervation in the sino-atrial and atrial tissues raises the possibility that neuronal uptake (uptake 1) may be more important in the termination of chronotropic than inotropic responses.

Accordingly, we have investigated the effects of differential and combined blockade of uptake 1 and uptake 2 on cardiac responses of anaesthetized dogs to (±)-isoprenaline, (−)-noradrenaline and electrical stimulation of the left ansa subclavia nerve.

Beagle dogs of either sex were studied (10.5–16.0 kg) under sodium pentobarbitone anaesthesia (30 mg/kg i.v.). The animals were artificially respired and arterial PO_2, PCO_2 amd pH were checked repeatedly during the experiments and maintained within normal physiological limits. Both cervical vago-sympathetic nerve trunks were sectioned and the chest was opened in the mid-line. Left ventricular pressure, dp/dt max., aortic blood pressure and heart rate were obtained by use of Millar catheter tip pressure transducers, an appropriate differentiator and ratemeter and registered on a Devices M19 8-channel recorder.

Cocaine hydrochloride (5 mg/kg i.v., and 1 mg/kg every 45 min) was used to block uptake 1 (Trendelenburg, 1959), and (±)-metanephrine (40 μg kg^{-1} min^{-1} i.v.) to block uptake 2 (Burgen & Iversen, 1965). Dose-response curves to i.v. bolus injections of isoprenaline (50 ng/kg–1 μg/kg) and noradrenaline (100 ng/kg–2 μg/kg) and frequency-response curves (supramaximal voltage, 5 ms pulse width, 1.0–20 Hz) of electrical stimulation of the left ansa subclavia nerve established under control conditions were compared with those obtained during individual and combined administration of the uptake antagonists. Statistical testing was by analysis of variance.

Cocaine potentiated responses to noradrenaline or nerve stimulation, but did not affect those to isoprenaline. Metanephrine potentiated positive inotropic responses to all doses of isoprenaline ($P < 0.05$) and positive chronotropic responses were enhanced at doses ⩾100 ng/kg. Positive inotropic responses to noradrenaline were enhanced significantly at doses ⩾250 ng/kg, and chronotropic responses at doses ⩾1 μg/kg. Metanephrine did not affect positive inotropic responses to sympathetic nerve stimulation of any frequency and cardiac responses to noradrenaline or nerve stimulation obtained after administration of cocaine were not further modified by the concurrent administration of metanephrine. Similarly, responses to isoprenaline in the presence of both cocaine and metanephrine were similar to those obtained in the presence of metanephrine alone, but subsequent administration of cocaine to those preparations already receiving metanephrine still potentiated cardiac responses to noradrenaline or nerve stimulation.

Our results confirm the known effects of cocaine on cardiovascular responses to noradrenaline and sympathetic nerve stimulation and establish that metanephrine enhances cardiac responses to isoprenaline *in vivo*. However, plots of dp/dt max. against heart rate demonstrate that cocaine preferentially enhances chronotropic responses to noradrenaline, but that metanephrine does not discriminate between inotropic and chronotropic effects of isoprenaline.

References

BURGEN, A.S.V. & IVERSEN, L.L. (1965). The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br. J. Pharmac. Chemother.*, **25**, 34–49.

TRENDELENBURG, U. (1959). The supersensitivity caused by cocaine. *J. Pharmac. exp. Ther.*, **125**, 55–62.

Studies on the rebound hypertension after clonidine withdrawal in conscious hypertensive cats, rats, and dogs

I. CAVERO, S. FÉNARD, L. FINCH,† F. LEFEVRE & A. ROACH

Cardiovascular Group Synthelabo, Paris & †School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP

Clonidine is a potent antihypertensive agent which is known to act on central cardiovascular sites and its effect on these centres is thought to be due to stimulation of central α-adrenoceptors (Schmitt, Schmitt & Fénard, 1973; Finch, 1974). The reports of a severe rebound rise in blood pressure if the drug is withdrawn abruptly remains a problem in the treatment of patients and also its mechanism is poorly understood (Hunyor, Hansson, Harrison & Hoobler, 1973).

In both spontaneous hypertensive and normotensive rats ($n = 8$), clonidine (2 × 0.2 mg/kg s.c. for 14 days) produced a marked fall in blood pressure measured by the indirect tail cuff method: on withdrawal of clonidine (15 days), the blood pressures returned to normal.

In conscious renal hypertensive cats ($n = 6$) with blood pressure recorded from cannulae chronically implanted into the thoracic aortae (Finch, 1974), treatment with clonidine (3 × 0.025 mg p.o. for 10 days) also produced a sustained fall in mean blood pressure (40 mmHg). However, after completion of the clonidine treatment (day 11), 4 cats exhibited a