Results were similar to those obtained by measuring NA overflow. However, as 0.2 Hz was used, the rate-dependent increase in [^{3}H]-catechols in the presence of cocaine (10 μM), oestradiol (3.7 μM) and phentolamine (10 μM) was more clearly seen.

The selective pre-junctional α-adrenoceptor agonist clonidine (11.2 nM) inhibited the twitch by 68.3% at 0.2 Hz, by 24.0% at 1.0 Hz and 0.3% at 10 Hz. Clonidine (11.2 nM) reduced the overflow of [^{3}H]-catechols at 0.2 Hz but not at 1.0 Hz or 10 Hz.

The results, obtained from the measurement of NA overflow and the effect of clonidine, demonstrate the presence of a pre-synaptic α-adrenoceptor in the mouse vas deferens.

Supported by the University of London Central Research Fund. We thank Dr P. Sever and Miss B.A. Osikowska for advice about the NA assay. NBS is an MRC Scholar.

References

HENRY, D.A., STARMAN, B.J., JOHNSON, D.G. & WILLIAMS, R.H. (1975). A sensitive radioenzymatic assay for norepinephrine in tissues and plasma. *Life Sci.*, **16**, 375–384.

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmac.*, **23**, 1793–1800.

MARSHALL, I., NASMYTH, P.A., NICHOLL, C.G. & SHEPPERSON, N.B. (1977). Pre-synaptic α-adrenoceptor regulation of the twitch response of the mouse vas deferens. *Br. J. Pharmac.*, **59**, (in press).

MARSHALL, I., NASMYTH, P.A. & SHEPPERSON, N.B. (1977). Pre-synaptic α-adrenoceptors and the inhibition by cocaine and oestradiol of the twitch response of the mouse vas deferens. *Br. J. Pharmac.*, **59**, (in press).

STARKE, K., ENDO, T. & TAUBE, H.D. (1975). Pre- and post-synaptic components in effect of drugs with α-adrenoceptor affinity. *Nature, Lond.*, **254**, 440–441.

Release of [^{3}H]-noradrenaline by field stimulation and by drugs from the anococcygeus muscle

J.C. McGRATH & H.J. OLVERMAN

Institute of Physiology and Department of Pharmacology, University of Glasgow, Glasgow, G12 8QQ

Incubation of rat anococcygeus with [^{3}H]-noradrenaline (^{3}H-NA) results in accumulation of the [^{3}H]-NA in the adrenergic nerve terminals (Nash, Gillespie & Robertson, 1974). This preparation is thus of potential use for the study of mechanisms involved in the release of NA.

Single rat anococcygeus muscles were incubated in a 3 ml bath under isometric conditions at an initial resting tension of 0.5 g in Kreb's solution at 37°C with [^{3}H]-NA (NA, 0.5 μM; 5 μCi/ml h; 1-(7-^{3}H) NA acetate, Radiochemical Centre, Amersham) for 30 min (EDTA 1.3 μg/ml; ascorbic acid 20 μg/ml also present). The tissues were then held isometrically in air under an initial resting tension of 0.5 g and superfused with drug-free Krebs' solution at 37°C at a rate of 0.66 ml/min. Sequential 2 ml aliquots of superfusate (corresponding to 3 min periods) were collected, 1 ml of which was mixed with 10 ml Toluene-Triton X scintillation fluid and the radioactivity counted in a liquid scintillation counter (Packard Tri-Carb, Model 3390) for 5 minutes. The tissue concentration of ^{3}H measured at the end of several experiments divided by the ^{3}H content of the corresponding incubation medium gave a ratio of 4.5 ± 0.5 ($n=6$). In a further three experiments where the tissue was digested immediately after incubation with [^{3}H]-NA, mean tissue/medium ratio was 5.5 ± 1.3 ($n=3$). Isometric tension in the tissue was measured throughout.

Following incubation with [^{3}H]-NA the quantity of ^{3}H in the superfusate from the anococcygeus muscles showed an exponential decay with time which could be separated into two log-linear components with half-lives respectively of 25 ± 5 and 116 ± 10 min ($n=6$). Test drugs were therefore added 75 min after starting perfusion when the decay was essentially log-linear.

LSD (5 μM) or tyramine (5 μM) increased this spontaneous overflow whereas barium chloride (8 mg/ml) or carbachol (3 μM) did not although each substance contracted the tissue to a similar degree.

Field stimulation of the tissue with 1 ms pulses, supramaximal voltage, 150–300 pulses, at 5–20 Hz produced reproducible increases in the basal efflux of ^{3}H in the 6 min period following stimulation. LSD (5 μM) inhibited both this latter nerve induced efflux and the accompanying motor tension response.

This confirms, as previously shown indirectly in this tissue, (Gillespie & McGrath, 1975), that LSD possesses the dual properties of indirect sympathomimetic and pre-synaptic inhibitor of NA release from adrenergic nerve terminals and illustrates that the preparation can be used to study the release of NA by nerve stimulation or by drugs.

Preliminary experiments indicate that nerve-induced ^{3}H overflow can be potentiated by blockade of neuronal NA uptake (cocaine 1 μM) or by a pre-synaptic α-receptor antagonist (piperoxan 3–30 μM).

References

GILLESPIE, J.S. & McGRATH, J.C. (1975). The effects of lysergic acid diethylamide on the response to field stimulation of the rat vas deferens and the rat and cat anococcygeus muscles. *Br. J. Pharmac.*, **54**, 481–488.

NASH, C.W., GILLESPIE, J.S. & ROBERTSON, E.N. (1974). Noradrenaline uptake properties of the anococcygeus muscle of the rat. *Canad. J. Physiol. Pharmacol.*, **52**, 430–440.

Monoamine oxidation in tissues of the developing chick

B.A. CALLINGHAM & C.J. FOWLER

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

Monoamine oxidase (MAO) activity in several animal tissues is related to growth (see Gripois, 1975). For example, in the rat heart, its specific activity increases with age due to a slowing of the rate of degradation of the enzyme (Callingham & Della Corte, 1972). Changes in the thyroid status modify this relationship by changing the rate of synthesis (Lyles & Callingham, 1974). The domestic fowl, which, at hatching is an independent but immature animal, has been used in this present study.

The hearts and livers of male chicks were homogenized in 1 mM potassium phosphate buffer, pH 7.4. MAO activity was assayed radiochemically using [^{3}H]-tyramine and [^{14}C]-benzylamine, and expressed as nmol substrate consumed (mg protein)$^{-1}$ h^{-1}. MAO specific activities were determined in the hearts and livers of groups of 4 chicks, at 6, 21, and 40 days after hatching. In both organs there was an increase in enzyme activity with age using either substrate.

Chicks were made hypothyroid by the addition of 0.2% (w/w) of 2-thiouracil in the feed, beginning the day after hatching. At 40 days after hatching, the treated chicks were about 70% of the control weight, and their hearts 60%. In the liver there were significant increases in MAO specific activity, 2.4 and 2.1 times control for tyramine and benzylamine respectively. In the heart there was a significant decrease in activity towards benzylamine (56% of control) but no significant change in tyramine oxidizing activity.

Chicks were made hyperthyroid by daily injections of (–)-thyroxine (1 mg/kg, s.c.) for 11 days, beginning on the second day after hatching, and killed 24 h after the last injection. At this time, the hyperthyroid birds had an increased temperature, a body weight 80% of that of the controls, but no change in heart weight. In the heart there were significant increases in MAO specific activity to 1.4 and 1.5 times control for tyramine and benzylamine respectively. No significant changes in activity were seen in the liver.

Experiments using clorgyline showed that tyramine oxidation in the heart was brought about by MAO-A and -B together with a clorgyline-resistant enzyme. Benzylamine oxidation in the heart was brought about wholly by this clorgyline-resistant enzyme, which was, however, inhibited by semicarbazide. In the liver, MAO-A and -B were responsible for all the tyramine oxidation, but MAO-B and the clorgyline-resistant enzyme oxidized benzylamine.

These results indicate that the enzymes responsible for the oxidation of tyramine and benzylamine in chick heart and liver can be resolved into MAO-A and -B (Johnston, 1968) and a clorgyline-resistant enzyme (Lyles & Callingham, 1975) that does not appear to be flavine-dependent. The activities of these enzymes are influenced by the development and thyroid status of the bird.

Supported by a grant from The British Heart Foundation. C.J.F. is a Medical Research Council Scholar. We thank May & Baker Ltd., for a gift of clorgyline (M & B 9302).

References

CALLINGHAM, B.A. & DELLA CORTE, L. (1972). The influence of growth and of adrenalectomy upon some rat heart enzymes. *Br. J. Pharmac.*, **46**, 530–531P.

GRIPOIS, D. (1975). Review: developmental characteristics of monoamine oxidase. *Comp. Biochem. Physiol.*, **51C**, 143–151.

JOHNSTON, J.P. (1968). Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmac.*, **17**, 1285–1297.

LYLES, G.A. & CALLINGHAM, B.A. (1974). The effects of thyroid hormones on monoamine oxidase activity in the rat heart. *J. Pharm. Pharmac.*, **26**, 921–930.

LYLES, G.A. & CALLINGHAM, B.A. (1975). Evidence for a clorgyline-resistant monoamine metabolizing activity in the rat heart. *J. Pharm. Pharmac.*, **27**, 682–691.