tetrabenazine-treated rats. Incubation with noradrenaline had no effect on the vesicles of terminals in reserpine-treated irides. Electron-density of vesicle cores in iris nerve terminals was also induced by incubation of tetrabenazine-treated tissues with 5-hydroxydopamine (1×10^{-4} M). This did not occur in reserpine-treated irides.

Hepatic portal veins were also removed from the treated rats and suspended between platinum-wire electrodes in Krebs' solution gassed with 95% O_2/5% CO_2 at 37°C. Log frequency/response curves to nerve stimulation (140 V, 200 μs, 10 s trains; 1–20 Hz) were recorded. The responses of veins, from tetrabenazine- or reserpine-treated rats to each frequency of stimulation were much lower than those obtained from veins of untreated animals. The veins were then incubated with noradrenaline (5×10^{-6} M) for 15 min and log frequency/response curves repeated. This treatment increased the slope of the curve for tetrabenazine-treated veins but had no effect on that obtained from reserpine-treated veins.

These findings are consistent with the suggestion that tetrabenazine causes a reversible impairment of vesicular uptake and storage of noradrenaline but that the effect of reserpine on a particular population of vesicles is irreversible.

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References

McLELLAN, D.L., CHALMERS, R.J. & JOHNSON, R.H. (1974). A double-blind trial of tetrabenazine, thiopropazate and placebo in patients with chorea. *Lancet*, **1**, 104–107.

QUINN, G.P., SHORE, P.A. & BRODIE, B.B. (1959). Biochemical and pharmacological studies of Ro1-9569 (tetrabenazine), a non-indole tranquillizing agent with reserpine-like effects. *J. Pharmacol. exp. Ther.*, **127**, 103–109.

The influence of pre-synaptic α-adrenoceptors on the overflow of noradrenaline in the stimulated mouse vas deferens

I. MARSHALL, P.A. NASMYTH & N.B. SHEPPERSON

Department of Biochemical and Experimental Pharmacology, St. Mary's Hospital Medical School, London W2 1PG

In sympathetically innervated tissues a negative feedback system regulates noradrenaline (NA) output via a pre-junctional α-adrenoceptor (Langer, 1974). This receptor was implicated in the control of the twitch response of the mouse vas deferens (Marshall, Nasmyth, Nicholl & Shepperson, 1977). The present experiments provide direct evidence for the existence of this α-adrenoceptor.

Eight vasa deferentia were tied together and stimulated at 256 mA, 1 ms for 120 pulses at 1, 10 or 16 Hz. Contractions were recorded isometrically. The bath was emptied 3.5 min (or 5 min when drugs were present) after stimulation and the Krebs solution was assayed for NA by the specific radio-enzymatic method of Henry, Starman, Johnson & Williams, 1975.

Although the tension developed by the vasa was greater with increasing stimulation frequency, NA overflow did not increase proportionately. Addition of cocaine (10 μM) and oestradiol (3.7 μM)to the Krebs significantly inhibited the twitch (52.7%) at 1 Hz ($P < 0.05$) but not at 10 Hz or 16 Hz. However, at all three rates of stimulation NA overflow was approximately doubled. When phentolamine (10 μM) was added to the Krebs containing cocaine and oestradiol, the inhibition seen at 1 Hz was reversed and NA overflow was now increased a further 5-fold, a 12-fold increase over controls.

These results show that inhibition of the twitch response by cocaine and oestradiol (Marshall, Nasmyth & Shepperson, 1977) at 1.0 Hz correlates with increased NA overflow. In other tissues regulation of NA output is mediated by pre-junctional α-adrenoceptors only at low rates of stimulation (Starke, Endo & Taube, 1975). In agreement with this, phentolamine produced a much greater increase in NA overflow at low rates of stimulation.

In other experiments vasa deferentia were pre-incubated for 45 min with 100 ng/ml of [7-^{3}H]-(−)-noradrenaline (specific activity 5.8 Ci/mmol). The [^{3}H]-catechol overflow from 4 vasa after 120 stimuli at 0.2, 1.0 or 10 Hz, 2 ms, 256 mA was absorbed onto alumina and counted.

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.

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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).