References

ANTONACCIO, M.J., HALLEY, J. & KERWIN, L. (1974). Functional significance of α-stimulation and α-blockade on responses to cardiac nerve stimulation in anaesthetized dogs. *Life Sci.*, **15**, 765–777.

CAVERO, I., LEFÈVRE, F. & ROACH, A.G. (1977). Differential effects of prazosin on the pre- and post-synaptic α-adrenoceptors in the rat and dog. *Br. J. Pharmac.*, **61**, 469P.

DOXEY, J.C. (1977). Effect of clonidine on cardiac acceleration in pithed rats. *J. Pharm. Pharmac.*, **29**, 173–174.

IDOWU, O.A. & ZAR, M.A. (1977). The use of rat atria as a simple and sensitive *in vitro* preparation for detecting presynaptic actions of drugs on adrenergic transmission. *Br. J. Pharmac.*, **61**, 157P.

LANGER, S.Z., ADLER-GRASCHINSKY, E. & GIORGI, O. (1977). Physiological significance of α-adrenoceptor-mediated negative feedback mechanism regulating noradrenaline release during nerve stimulation. *Nature, Lond.*, **265**, 648–650.

LOKHANDWALA, M.F. & BUCKLEY, J.P. (1976). Effect of presynaptic α-adrenoceptor blockade on responses to cardiac nerve stimulation in anaesthetized dogs. *Eur. J. Pharmac.*, **40**, 183–186.

Presynaptic α-adrenoceptors and [^{3}H]-noradrenaline overflow from the 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

The twitch response of the field stimulated mouse vas deferens is inhibited by clonidine, a selective presynaptic α-adrenoceptor agonist, and potentiated by yohimbine, a selective presynaptic α-adrenoceptor antagonist. (Marshall, Nasmyth, Nicholl & Shepperson, 1978).

We have now investigated the effects of these drugs upon the overflow of tritium, [^{3}H]-noradrenaline and its metabolites from the mouse vas deferens previously loaded with [^{3}H]-noradrenaline. Six vasa deferentia were suspended in a 1.0ml or 2.0ml organ bath containing magnesium free Krebs with 10mg/l EDTA, 20mg/l ascorbic acid, and 3.7μM oestradiol. Preparations were stimulated for 2 min at 1 Hz, 2 ms, 256 mA and the bath fluid was collected for 2 min periods before (basal), during and for 4 min after stimulation. Total tritium, and [^{3}H]-noradrenaline released upon stimulation (stimulated value minus basal), were expressed as a fraction of that remaining in the tissue at the time of stimulation (Dubocovich & Langer, 1976). Noradrenaline and its metabolites were separated on alumina and Dowex columns. (Graefe, Stefano & Langer, 1973).

Stjärne (1975) reported that clonidine (10 nM) did not alter the fractional release of tritium in the guinea pig vas deferens. In agreement with this, fractional release of tritium in the present experiments was not altered by 2.8 nM or 11.2 nM clonidine in the mouse vas deferens (t test, $P>0.05$).

The fractional release of [^{3}H]-noradrenaline (mean ± s.e. mean) in 3 successive stimulation periods was $5.94 \times 10^{-4} \pm 1.37$; $5.17 \times 10^{-4} \pm 1.75$; $3.99 \times 10^{-4} \pm 1.3$. This did not represent a significant fall off in [^{3}H]-noradrenaline over the 3 periods (t test $P>0.05$). Clonidine 2.8 nM was added 30 s before the second stimulation, and 11.2 nM 30 s before the third stimulation. Fractional release of [^{3}H]-noradrenaline in the control was $4.60 \times 10^{-4} \pm 0.8$, and clonidine 2.8 nM and 11.2 nM reduced this to $1.31 \times 10^{-4} \pm 0.34$ and $1.27 \times 10^{-4} \pm 0.31$ respectively. Unlike clonidine and in agreement with Starke, Borowski & Endo (1975) yohimbine increased tritium overflow from a control value of $3.80 \times 10^{-3} \pm 0.54$ to $5.59 \times 10^{-3} \pm 0.7$. When this is split into [^{3}H]-noradrenaline and metabolites, fractional [^{3}H]-noradrenaline increased from $4.05 \times 10^{-4} \pm 1.24$ to $10.11 \times 10^{-4} \pm 1.54$.

The regulation of twitch height by presynaptic α-adrenoceptors is potentiated by reducing the concentration of calcium in the Krebs from 2.54 to 1.27 mM (Marshall, Nasmyth & Shepperson, 1977). In low calcium, Krebs yohimbine potentiates the twitch but the overflow of tritium and [^{3}H]-noradrenaline was not increased above controls where no antagonist was present.

Low doses of selective presynaptic α-adrenoceptor agonists decrease the twitch height and decrease the overflow of [^{3}H]-noradrenaline although these were not dose related. Conversely a selective presynaptic α-adrenoceptor antagonist increases twitch height, tritium and [^{3}H]-noradrenaline overflow. This relationship is not observed with tissues in half calcium concentration Krebs.

N.B.S. is an MRC student.

References

DUBOCOVICH, M.L. & LANGER, S.Z. (1976). Influence of the frequency of nerve stimulation on the metabolism of [^{3}H]-norapinephrine released from the perfused cat spleen: Differences observed during and after the period of stimulation. *J. Pharmac. exp. Ther.*, **198**, 83–101.

GRAEFE, K.H., STEFANO, F.J.E. & LANGER, S.Z. (1973). Preferential metabolism of (-)-[^{3}H]-norepinephrine through the deaminated glycol in the rat vas deferens. *Biochem Pharmac.*, **22**, 1147–1160.

MARSHALL, I., NASMYTH, P.A. & SHEPPERSON, N.B. (1977). The relationship between presynaptic α-adrenoceptors, stimulation frequency and calcium. *Br. J. Pharmac.*, **61**, 128P.

MARSHALL, I., NASMYTH, P.A., NICHOLL, C.G. & SHEPPERSON, N.B. (1978). α-Adrenoceptors in the mouse vas deferens and the effects on its response to electrical stimulation. *Br. J. Pharmac.* (In press).

STARKE, K., BOROWSKI, E. & ENDO, E. (1975). Preferential blockade of presynaptic α-adrenoceptors by yohimbine. *Eur. J. Pharmac.*, **34**, 385–388.

STJÄRNE, L. (1975). Clonidine enhances the secretion of sympathetic neurotransmitter from isolated guinea pig tissues. *Acta Physiol. scand.*, **93**, 142–144.

Effect of cyclic nucleotides on [^{3}H]-neurotransmitter release induced by potassium stimulation in the rat pineal gland

MARGARITA L. DUBOCOVICH, S.Z. LANGER & F. PELAYO

Synthelabo (L.E.R.S.), Biology Department, Paris, France.

Presynaptic α-adrenoceptors mediate a negative feedback mechanism which leads to inhibition of transmitter release during depolarization induced by nerve stimulation or potassium (Langer, 1974, 1977; Starke, 1977). In addition, presynaptic β-adrenoceptors have been described in noradrenergic nerve terminals (Adler-Graschinsky & Langer, 1975). These receptors when activated by low concentrations of ß-agonists lead to an enhancement in transmitter release which appears to be mediated through an increase in the levels of cyclic AMP in noradrenergic nerve endings (Celuch, Dubocovich & Langer, 1977). Our experiments were designed to determine whether α and β presynaptic adrenoceptors are present in the noradrenergic nerve endings of the rat pineal gland. Release of the [^{3}H]-neurotransmitter was induced by exposure to potassium and to tyramine. Male rats (160–200g) were killed by decapitation and their pineal glands were immediately removed. The endogenous noradrenaline stores were labelled *in vitro* by incubating the pineals in Krebs solution with 0.5μM (±)-7-[^{3}H]-noradrenaline for 30 minutes. In the controls, the fraction of the total tissue radioactivity released by the first exposure to 60mM potassium for one minute (S_1) was 17.13 ± 1.05 (× 10^{-3}) (n=74), and the ratio obtained between two consecutive stimulation periods (S_2/S_1) was: 1.15 ± 0.10 (n=10). Under these experimental conditions, [^{3}H]-noradrenaline release by potassium was found to be entirely calcium dependent. Denervated pineal glands (7 days after bilateral superior cervical ganglionectomy) failed to retain [^{3}H]-noradrenaline and did not release tritium when exposed to 60mM potassium.

Release of [^{3}H]-noradrenaline induced by potassium was reduced in the presence of the α-adrenoceptor agonist oxymetazoline (10 μM):S_2/S_1 = 0.60 ± 0.03, n=6; P<0.001. On the other hand the α blocking agent yohimbine (10 μM) increased transmitter overflow more than 2 fold S_2/S_1:2.32 ± 0.27, n=5; P<0.005). When [^{3}H]-transmitter release was elicited by exposure to tyramine (3 μM), the fraction of total tissue radioactivity released by S_1 was: 36.75 ± 2.64 × 10^{-3} (n=18) and the ratio between two consecutive stimulation periods, was S_2/S_1 = 1.11 ± 0.12 (n=5). Under these conditions neither oxymetazoline (10 μM) nor yohimbine (10 μM) when added before the second exposure to tyramine were able to modify [^{3}H]-neurotransmitter release.

Recently O'Dea & Zatz (1976) have demonstrated the existence in the rat pineal gland of a calcium-dependent presynaptic mechanism for the generation of cGMP which may be mediated by an α-adrenoceptor-like receptor. In our experiments when dibutyryl 3′5′ cyclic guanosine monophosphate (dbcGMP) 0.1 and 0.5 mM was added before S_2, the ratios S_2/S_1 were: 0.88 ± 0.14, n=4 and 0.61 ± 0.10, n=5 P<0.005) respectively. The reduction in potassium-induced transmitter release obtained in the presence of dbcGMP is compatible with the view that cyclic GMP might be involved in the sequence of events leading to a reduction in transmitter release after activation of presynaptic α-receptors (Pelayo, Dubocovich & Langer, 1977).

The β-adrenoceptor agonists isoprenaline, 14 nM and terbutaline, 80 nM were found to enhance significantly [^{3}H]-noradrenaline release induced by potassium. The ratios S_2/S_1 were 1.86 ± 0.22 (n=6; P<0.02), and 2.14 ± 0.29 (n=4; P<0.01) respectively. The facilitating effect of isoprenaline on [^{3}H]-noradrenaline release was prevented by pre incubation with (±)-propranolol, 0.1 μM (S_2/S_1 = 0.95 ± 0.15; n=4).

Exposure to dibutyryl cyclic adenosine monophosphate (dbcAMP) during the second potassium stimulation increased [^{3}H]-noradrenaline release. The ratios S_2/S_1 were 1.49 ± 0.20 (n=4), 2.12 ± 0.36 (n=4, P<0.05) and 2.37 ± 0.28, (n=6, P<0.005) for 0.05, 0.1 and 0.5 mM of the drug respectively. On the other hand 0.5 mM of dbcAMP failed to modify [^{3}H]-transmitter release induced by tyramine.

The increase in potassium-evoked [^{3}H]-noradrenaline release obtained in the presence of β-adrenoceptor agonists and dbcAMP indicates the presence of presynaptic β-adrenoceptors in the rat pineal gland. Our results suggest that these presynaptic β-adrenoceptors might be linked to adenylate cyclase activation in noradrenergic nerve terminals (Langer, 1977; Celuch *et al*, 1977, Weller, 1977).