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

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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).

References

ADLER-GRASCHINSKY, E. & LANGER, S.Z. (1975). Possible role of a ß-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feed-back mechanism. *Br. J. Pharmac.*, **53**, 43–50.

CELUCH, S.M., DUBOCOVICH, M.L. & LANGER, S.Z. (1977). Stimulation of presynaptic beta-adrenoceptors enhances [^{3}H]-noradrenaline release during nerve stimulation in the perfused cat spleen. *Br. J. Pharmac.* (in press).

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

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. Sixth Gaddum Memorial Lecture, *Br. J. Pharmac.*, **60**, 481–497.

O'DEA, R.F. & ZATZ, M. (1976). Catecholamine stimulated cyclic GMP accumulation in the rat pineal: Apparent presynaptic site of action. *Proc. Nat. Acad. Sci. U.S.A.*, **73**, 3398–3402.

PELAYO, F., DUBOCOVICH, M.L. & LANGER, S.Z. (1977). Regulation of noradrenaline release in the rat pineal through a negative feed-back mechanism mediated by presynaptic alpha-adrenoceptors. *Eur. J. Pharmac.* **45**, 317–318.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptors systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

WELLER, M. (1977). Evidence for the presynaptic location of adenylate cyclase and the cyclic AMP stimulated protein kinase which is bound to synaptic membranes. *Biochim. Biophys. Acta*, **469**, 350–354.

The effect of sympathetic nerve stimulation on [^{3}H]-prazosin release in rabbit pulmonary arteries

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It was initially suggested by Story (personal communication, 1976) that prazosin might be taken up into adrenergic nerve endings, displace noradrenaline from its neuronal storage sites and subsequently be released along with transmitter noradrenaline. However, in preliminary experiments employing, 2-[^{14}C] prazosin (4.47 μCi/mg; 10^{-4}M) inconclusive evidence was obtained of the release of prazosin from neuronal storage sites in response to nerve stimulation (Cambridge, Davey & Massingham, 1977). Since concentrations of prazosin in excess of 10^{-6}M cause a calcium independent, apparently intraneuronal, release of noradrenaline and its metabolites (Cambridge *et al*, 1977), it was decided to repeat these experiments with [^{3}H]-labelled prazosin of higher specific activity.

Spirally cut strips of rabbit pulmonary arteries were weighed and incubated for 1 h in Krebs bicarbonate solution at 37°C containing 10^{-8} or 10^{-6}M [^{3}H]-prazosin (300 mCi/mmol). The strips were then mounted between platinum electrodes for transmural stimulation after the method of Su & Bevan (1970). The superfusate was collected in sequential 3 min fractions and counted for [^{3}H]. Strips were stimulated on 6 occasions during the superfusion, which lasted 126 min, after which they were solubilized and counted for total [^{3}H].

Initial stimulations did not evoke any contraction in the tissues incubated with prazosin (10^{-6}M) but contractions to nerve stimulations became progressively larger with time in those tissues pretreated with prazosin (10^{-8}M), presumably as the superfusion removed prazosin from the tissues.

Transmural stimulation was not accompanied by a simultaneous enhanced release of [^{3}H] above basal levels into the superfusate. Similarly when tissues were exposed to tyramine (10^{-5}M for 3 min) instead of electrical stimulation there was again no associated increase in the rate of [^{3}H] lost from the tissue. Cooling (incubating at 0°C) caused a small (approximately 10%) reduction in the accumulation of [^{3}H] in the tissues. However whereas it had previously been shown that desipramine reduced the accumulation of [^{14}C] when strips were incubated with 2[^{14}C]-prazosin (10^{-4}M) (Cambridge *et al*, 1977), in the present series of experiments the accumulation of [^{3}H] during 60 min incubation in [^{3}H]-prazosin (10^{-6}M) was not modified by addition of cocaine (10^{-4}M) or desipramine (6×10^{-6}M) to the Krebs solution.

These results provide direct evidence that prazosin at concentrations up to 10^{-6}M does not enter the sympathetic neurones in vascular smooth muscle via Uptake-1. Furthermore they militate against the idea that prazosin is taken up in the storage granules and released exocytotically along with transmitter