Effect of denervation and cocaine on the response of isolated rat vas deferens to noradrenaline and methoxamine

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In the rat vas deferens, cocaine increases the maximum response and shifts the dose-response curve for noradrenaline (NA) to the left (Barnett, Greenhouse & Taber, 1968). The former has been ascribed to a postjunctional effect and the latter to the prevention of neuronal NA uptake by cocaine (Trendelenburg, 1972). To provide evidence for the site of action we used methoxamine, an alpha-adrenoceptor agonist, whose neuronal uptake is negligible (Trendelenburg, Maxwell & Pluchino, 1970).

Vasa deferentia of rats were placed in oxygenated Tyrode solution at 37°C, and the isotonic contractions were recorded on a kymograph and developed tension on Grass polygraph. Denervation of tissue was carried out by desheathing (Birmingham, 1970) 24 h before experiment. Reserpinization was achieved by reserpine (5 mg/kg and 2.5 mg/kg, 48 and 24 h before experiment respectively).

Cocaine (2.94 μM), potentiated the effect of NA 12-fold and increased the maximum response by $75 \pm 12.4\%$. Denervation potentiated the response to NA ten-fold. Cocaine had no significant effect on the dose-response curve and maximum response to NA in denervated vasa deferentia.

ED_{50} of methoxamine was 1.49 ± 0.36 μM in intact tissue and 2.83 ± 0.81 μM in reserpinized tissue. Devervation did not alter ED_{50} of methoxamine significantly (4.32 ± 0.77 μM in reserpinized tissue) and phentolamine shifted the dose-response curve to the right in a parallel fashion.

ED_{50} for methoxamine after pretreatment with cocaine was 1.49 ± 0.40 μM in intact tissue and 4.36 ± 1.01 μM in reserpinized tissue. Cocaine increased the maximum response to methoxamine by $3.5 \pm 5.3\%$ and $13 \pm 2.7\%$ in intact and reserpinized tissue respectively. This was significantly lower than that observed for NA.

These results support the idea that cocaine potentiates the response to NA by inhibiting the neuronal uptake of NA. They also suggest that the increase in maximum response to NA in intact rat vasa deferentia is at least partly due to prejunctional effects of cocaine.

References

BARNETT, A., GREENHOUSE, D.D. & TABER, R.I. (1968). A new type of drug enhancement: increased maximum response to cumulative noradrenaline in the isolated rat vas deferens. *Br. J. Pharmac.*, **33**, 171–176.

BIRMINGHAM, A.T. (1970). Sympathetic denervation of the smooth muscle of the vas deferens. *J. Physiol (Lond.)*, **206**, 645–661.

TRENDELENBURG, U. (1972). Factors influencing the concentration of catecholamines at the receptors. In *Handbook of Experimental Pharmacology*, Vol. **33**, eds. Blaschko, H. & Muscholl, E., pp. 726–761. Springer-Verlag: Berlin.

TRENDELENBURG, U., MAXWELL, R.A. & PLUCHINO, S. (1970). Methoxamine as a tool to assess the importance of intraneuronal uptake of l-norepinephrine in the cat's nictitating membrane. *J. Pharmac. exp. Ther.*, **172**, 91–99.

The effects of AH 5158 on metabolism of ^{3}H(–)noradrenaline released from the cat spleen by nerve stimulation

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In the isolated blood perfused cat spleen AH 5158, 5-(1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl) salicylamide, acts as a selective post-synaptic α-adrenoceptor antagonist. The drug elevates transmitter overflow but the mechanism involves inhibition of uptake rather than inhibition of pre-synaptic α-adrenoceptors (Blakeley & Summers, 1976). The present experiments investigate whether the site of uptake inhibition is neuronal or extraneuronal and whether AH 5158 affects degradative enzymes.

Isolated blood perfused cat spleens were labelled with [^{3}H]-(–)-noradrenaline (0.5 nM/min for 10 min; blood flow 6.0 ± 0.3 ml/min, $n = 9$). Loosely bound label was washed out by perfusion with Krebs bicarbonate saline for 30 minutes. Blood perfusion was re-established and the spleen perfused for 30 minutes. After stimulation of the splenic nerves with 200 impulses at 10 Hz, venous blood was collected in four 1 min fractions, chilled and centrifuged. One aliquot of plasma was counted directly and another was taken for the separation of [^{3}H]-(–)-noradrenaline

and its metabolites by column chromatography (Graefe, Stefano & Langer, 1973).

AH 5158 ($10^{-7}-3\times10^{-4}$ M) produced a dose dependent increase in the overflow of [^{3}H] following nerve stimulation ($r=0.83$; $P<0.02$; $n=8$). The dose required to produce a 50% increase in overflow was 2.3×10^{-5}M. These results are similar to those obtained by measurement of the overflow of endogenous noradrenaline (Blakeley & Summers, 1976).

AH 5158 had no apparent effect on the production of [^{3}H]-DOMA [3,4-dihydroxy mandelic acid] or the 0-methylated deaminated metabolites, [^{3}H]-MOPEG [3-methoxy 4-hydroxy phenyl glycol and [^{3}H]-VMA [3-methoxy 4-hydroxy mandelic acid], indicating that the drug does not directly inhibit MAO, COMT or extraneuronal uptake. [^{3}H]-DOPEG (3:4 dihydroxy phenyl ethylene glycol) production was inhibited, particularly during the last 3 min of collection when, under normal conditions, a high proportion of [^{3}H] overflowed as metabolites. The inhibition was dose-dependent and the doses required to reduce [^{3}H]-DOPEG production by 50% in the last 3 min of collection were 1.2×10^{-5} M, 2.3×10^{-5} M and 2.4×10^{-5} M. There was a significant ($r>0.885$, $P<0.001$, $n>6$) inverse relationship between the change in proportion of [^{3}H]-(−)-noradrenaline and [^{3}H]-DOPEG recovered for each of the last three 1 min collection periods. DOPEG is thought to be derived from the fraction of transmitter taken back into the nerves which is metabolized by MAO and aldehyde reductase (Kopin, 1972), and the amount formed has been taken as an index of neuronal uptake (Cubeddu, Barnes, Langer & Weiner, 1974). Since both the neuronal uptake inhibitor cocaine (Cubeddu *et al.*, 1974) and AH 5158 inhibit the production of [^{3}H]-DOPEG following nerve stimulation it is likely that the site of uptake inhibition by AH 5158 is neuronal.

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References

BLAKELEY, A.G.H. & SUMMERS, R.J. (1976). The effects of AH 5158 on the overflow of transmitter and the uptake of [^{3}H]-(−)-noradrenaline in the cat spleen. *Br. J. Pharmac.*, **56**, 364–365P.

CUBEDDU, L.X., BARNES, E., LANGER, S.Z. & WEINER, N. (1974). Release of norepinephrine and dopamine-β-hydroxylase by nerve stimulation, I. Role of neuronal and extraneuronal uptake and of alpha presynaptic receptors. *J. Pharmac. èxp. Ther.*, **190**, 431–450.

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

KOPIN, I.J. (1972). Metabolic degradation of Catecholamines. The relative importance of different pathways under physiological conditions and after administration of drugs. In: *Handbook Exper. Pharm. XXXIII*—Catecholamines, eds. Blaschko, H. & Muscholl, E. Springer-Verlag: Berlin and Heidelberg, New York.

The receptors involved in catecholamine mediated growth and secretion in rat parotid gland

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Catecholamines increase the rate of growth and produce a protein-rich secretion in rat salivary glands (Schneyer, 1974; Muir, Pollock & Turner, 1975). These effects are inhibited by propranolol and are presumably mediated via β-adrenoceptors. The increase in growth may arise from a depletion of secretory material (especially amylase) following catecholamine stimulation (Schneyer, 1974). This hypothesis was investigated by comparing the ability of sympathetic nerve stimulation and several adrenergic agonists to enhance growth and deplete amylase and total protein stores. A classification of the β-adrenoceptor involved in terms of β_1 or β_2 activity (Lands, Arnold, McAuliff, Luduena & Brown, 1967) was also made.

In anaesthetized (halothane 3%, N_2O/O_2 2:1) rats the cervical sympathetic trunk or the parasympathetic nerves to the parotid were stimulated (20 Hz, 1 ms, 30 s/min supramaximal for up to 90 min) unilaterally or drugs given i.p. and saliva collected from the cannulated duct. Pieces of gland (10–20 mg) were removed before and again after (90 min) treatment and the total protein (Lowry, Rosebrough, Farr & Randall, 1951) and amylase levels (Phadebas commercial kit) compared. Saliva was also assayed for total protein and amylase content. Total protein and amylase content of unstimulated contralateral glands measured before and after nerve stimulation were not significantly different. Increased growth was measured, as an increased DNA synthesis by the uptake of [^{3}H]-thymidine and as an increase in the