SENSITIZATION OF NORADRENALINE RESPONSES BY INHIBITORS OF EXTRANEURONAL UPTAKE IN A CORONARY ARTERY PREPARATION

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Inhibition of neuronal uptake with cocaine had no significant effect on the dose-response curve to noradrenaline in strips of beef coronary artery. However, responses were enhanced about 3-fold by 17β-oestradiol and the haloalkylamine GD-131, two known inhibitors of extraneuronal uptake.

The coronary blood vessels of diverse species generally respond to the sympathetic nerve transmitter, noradrenaline, with β-adrenoceptor mediated relaxation, although under *in vivo* conditions the intensity of the overall effect may be related to the metabolic state of the heart (Gaal, Kattus, Kolin & Ross, 1966; Parratt, 1967). The mechanisms terminating the dilator action of noradrenaline on the coronary vasculature which thereby influence the magnitude and the duration of response have not been studied. In the present experiments the effects of inhibitors of neuronal and extraneuronal uptake on the responses to noradrenaline in a coronary artery of beef cattle were examined *in vitro*.

Methods Hearts were obtained immediately after slaughter of the cattle, immersed in oxygenated Krebs solution and transported to the laboratory (total time about 20 minutes). The circumflex coronary artery was dissected out, cleaned of visible fat and cut into spiral strips of about 23 x 2.5 mm. The strips were suspended under 2 g tension in 15 ml muscle chambers containing Krebs-Henseleit solution at 37° C and after a 90 min period of equilibration responses to drugs were recorded isotonically.

(−)-Noradrenaline bitartrate concentrations are expressed as the base, cocaine hydrochloride as the salt and potassium chloride in terms of molarity. 17β-Oestradiol was dissolved in propylene glycol to give a stock concentration of 10 mg/ml. The volume of propylene glycol usually added to the muscle chambers was 0.015 ml and when used alone it had no effect on the basal tone of strips or on their responses to potassium or noradrenaline. Strips were exposed to cocaine (10 μg/ml) or to 17β-oestradiol (10 μg/ml) for 15 min and responses to agonists were then obtained without washout of the muscle chambers.

The haloalkylamine compound, GD-131 (*N*-cyclohexylmethyl-*N*-ethyl-β-chloroethylamine) (Smith, Kline & French) was added to the chambers in a concentration of 3 or 10 μg/ml for 15 min followed by an additional 15 min period with frequent washes of the chambers before agonist testing. In a majority of experiments strips cut from the same preparation were used for all treatment conditions to reduce variability. In addition, only one dose-response curve to noradrenaline was obtained on each strip which was then discarded.

Results The untreated circumflex coronary artery of cattle usually does not constrict in response to noradrenaline (100 pg to 30 μg/ml), reflecting the absence in this preparation of any significant population of α-adrenoceptors. However, if a state of partial tone is induced with potassium 30 to 60 mM (usually 50 mM) the artery relaxes in stepwise fashion in response to cumulative additions of noradrenaline (1 ng to 3 μg/ml). Confirmation that these responses were mediated by β-adrenoceptor activation is the finding that they were blocked completely by propranolol (3 μg/ml).

The mean cumulative dose-response curve to noradrenaline obtained in 18 control strips is presented in Figure 1. The maximal inhibitory effect of noradrenaline on potassium-induced tone was a mean of 86% of the initial plateau amplitude of contraction of 52.6 ± 3.6 mm and presumably reflected saturation of the β-adrenoceptor population. The capacity of the strips to relax further is not exhausted after the maximal response to noradrenaline is achieved as the addition of $NaNO_2$ (1 mg/ml), a non-competitive inhibitor of smooth muscle tone, returned the strips to their pre-potassium level of tone or slightly below.

Coronary vessel strips taken from the same preparations as the controls were contracted by potassium and exposed to noradrenaline after treatment with cocaine to inhibit neuronal uptake or to 17β-oestradiol or GD-131 to inhibit extraneuronal uptake. Cocaine had no statistically significant effect on the magnitude of responses to noradrenaline at any point along the curve but responses were enhanced significantly by each of the known inhibitors of extraneuronal uptake

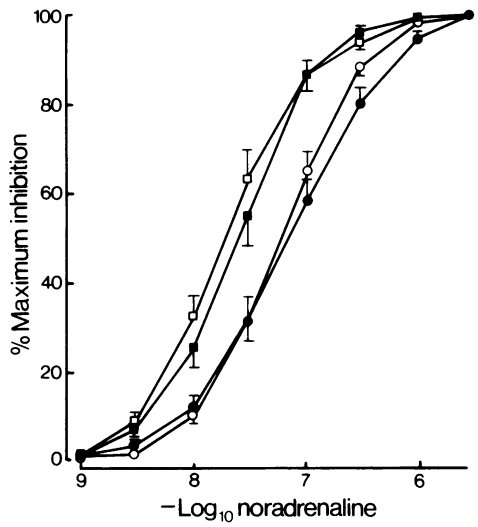

Fig. 1 Effects of inhibitors of extraneuronal and neuronal uptake on the cumulative dose-response curve to noradrenaline. Control (●); cocaine (○); GD-131 (■); and 17β-oestradiol (□). Number of values for each group are 18, 12, 16 and 13.

(Figure 1). At the level of the ED_{50} the sensitization is 3.7 and 2.8-fold by 17β-oestradiol and GD-131. The maximum inhibition of the amplitude of the potassium response did not differ significantly from that of controls in any of the treatment groups as would be anticipated if potentiation is related to increased concentrations of the agonist at the receptors rather than to an alteration in the mechanics of the responding tissue. The specificity of the enhancing effects of 17β-oestradiol and GD-131 was confirmed by the finding that these agents did not alter detectably the inhibitory responses to $NaNO_2$ (10 ng to 10 μg/ml) on strips contracted by potassium.

Discussion The uptake of catecholamines into effector cells of blood vessels such as the ear artery, pulmonary artery and aorta has now been demonstrated by several workers using a variety of techniques (Kalsner & Nickerson, 1969; Gillespie, Hamilton & Hosie, 1970; Burnstock, McCulloch, Story & Wright, 1972). There is also considerable evidence now accumulated to indicate that uptake and metabolism in non-neuronal tissue is a major mechanism terminating the action of noradrenaline in vascular tissue (Kalsner & Nickerson, 1969; Kalsner, 1969a, b; Berkowitz, Tarver & Spector, 1971; Bevan & Su, 1973). However, no information is available on the relative importance of neuronal and extraneuronal mechanisms in terminating responses to noradrenaline in the coronary vasculature.

The overall effect of noradrenaline on coronary vessel blood flow *in vivo* is dilator but the mechanisms involved have been controversial due to the multiplicity of factors such as extravascular compression, heart rate and myocardial metabolism which can override the direct effect (Parratt, 1967). It now appears likely that the direct effect is in most cases inhibitory, mediated by β-adrenoceptor activation and antagonized by β-adrenoceptor blocking agents (Gaal *et al.*, 1966; Bohr, 1967; Parratt, 1967). The population of α-adrenoceptors appears small to negligible in most preparations of coronary artery strips tested *in vitro* (Bohr, 1967).

In the present experiments on the circumflex coronary artery of cattle only inhibitory responses to noradrenaline mediated by β-adrenoceptor activation were observed. Inhibition of neuronal uptake with cocaine had no significant effect on their magnitude but they were potentiated consistently by 17β-oestradiol and the haloalkylamine GD-131, two known inhibitors of extraneuronal metabolism (Kalsner & Nickerson, 1969; Kalsner, 1969a, b). The magnitude of the sensitization, if due solely to block of uptake, indicates that approximately 70% of the noradrenaline at the ED_{50} is inactivated by extraneuronal mechanisms even in the presence of the competing pathway of agonist diffusion into the muscle chambers. Recent evidence indicates that uptake in vascular tissue involves a specific carrier system which obeys Michaelis-Menten type kinetics and is inhibited by cold (Gillespie *et al.*, 1970). The magnitude of the sensitization achieved by inhibitors of extraneuronal uptake indicates that the possibility of a derangement of the uptake process in experimental and clinical conditions of altered coronary blood flow should be explored.

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