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Expression of functional postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery—a permissive role for angiotensin II?

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In the rabbit isolated distal saphenous artery, the population of postjunctional adrenoceptors is of the α_1 variety under normal *in vitro* experimental conditions, based on the potency order of selective agonists and on the effects of the antagonists prazosin and rauwolscine against responses to UK-14304. Angiotensin II (A II, 0.05 μM) however, without affecting resting baseline tension, markedly enhanced responses to UK-14304, particularly at low concentrations. This previously unseen component of the response to UK-14304 was resistant to prazosin (0.1 μM) but susceptible to rauwolscine (1 μM). A II would therefore appear to have a permissive role for the expression of a quiescent population of postjunctional α_2-adrenoceptors in the rabbit distal saphenous artery.

Introduction The demonstration of postjunctional α_2-adrenoceptors in isolated vascular smooth muscle preparations is difficult, particularly in arteries (McGrath, 1982). Recently Sulpizio & Hieble (1987) and Furuta (1988) have demonstrated an enhancement of responses to α_2-adrenoceptor agonists in isolated preparations in the presence of pharmacological stimulants. The presence of Bay K 8644 or inducing tone with prostaglandin $F_{2\alpha}$, enhanced responses to BHT-920 which were prazosin-resistant and rauwolscine-sensitive, in canine isolated saphenous artery and portal vein respectively. Furthermore, the physiological stimulant angiotensin II (A II), enhances postjunctional α_2-adrenoceptor function in some venous preparations (Schumann & Lües, 1983; Daly *et al.*, 1988a). In this study we have examined the influence of A II on responses to the selective α_2-adrenoceptor agonist UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) in the rabbit distal saphenous artery.

Methods Male albino rabbits (2.5–2.7 kg) were killed by stunning followed by exsanguination. A length of distal saphenous artery from either leg was placed in physiological salt solution (for composition: see Daly *et al.*, 1988a) containing propranolol (1 μM) and cocaine (10 μM). The artery was then divided into six 'ring' segments (3–4 mm long) and prepared for recording of isometric tension.

After equilibration, each preparation was exposed to 3 μM (−)-noradrenaline (NA) and 10 min later washed until complete relaxation was effected. After a further 45 min cumulative concentration-response curves (CCRC) were obtained to either NA, phenylephrine (PE) or UK-14304. In experiments involving UK-14304, following complete washout, tissues were exposed to either saline, prazosin (0.1 μM) or rauwolscine (1 μM) 45 min before a second CCRC. In some tissues A II (0.05 μM) was added 15 min before UK-14304. Results are expressed as the percentage (mean ± s.e.mean) of the maximum response to NA or as the percentage of the maximum response of the first CCRC to UK-14304. Differences between means were considered statistically significant if $P < 0.05$: Student's t test (paired or unpaired).

Results All three agonists produced concentration-dependent contractions in the distal saphenous artery. Both NA and PE were full agonists while UK-14304 was a partial agonist producing approximately 65% of the NA maximum response. The relative potency of the three agonists was, NA > PE > UK-14304 (Figure 1a). The responses to UK-14304 were antagonized by both 1 μM rauwolscine (3 fold rightward displacement) and 0.1 μM prazosin (100 fold parallel rightward displacement) (Figure 1b).

A II (0.05 μM) produced a transient contraction (duration 10–12 min) which relaxed to baseline before the CCRC to UK-14304 was started. A II produced a marked increase in sensitivity to low concentrations of UK-14304, with no increase in the maximum response, resulting in a change in the slope of the CCRC to UK-14304 (Figure 1c). The magnitude of the displacement produced by A II for the threshold concentration of UK-14304 was

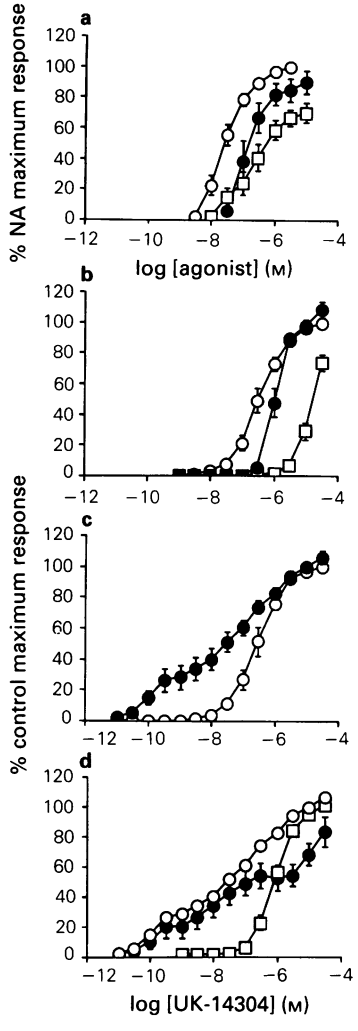

Figure 1 The effects of agonists and antagonists at α-adrenoceptors and the influence of angiotensin II (A II) in the rabbit isolated distal saphenous artery. (a) The contractile responses to the agonists (−)-noradrenaline (NA) (○), phenylephrine (PE) (●) and UK-14304 (□). (b) The effect of rauwolscine 1 μM (●) and prazosin 0.1 μM (□) on contractions elicited by UK-14304 (○). (c) The effect of A II 0.05 μM (●) on contractions elicited by UK-14304 (○). (d) The effect of prazosin 0.1 μM (●) and rauwolscine 1 μM (□) on contractions elicited to UK-14304 (○) in the presence of A II 0.05 μM. All responses are expressed as a percentage of either the maximum response to NA (a) or the maximum response in the control cumulative concentration-response curve to UK-14304 (b, c, d) and are the mean of 6–7 observations of different animals. The vertical lines indicate the s.e.mean.

approximately 300 fold: 10^{-8} M in the absence of A II compared to 10^{-11} M in the presence of A II. Prazosin (0.1 μM) in the presence of A II, was ineffective against the 'uncovered responses' to low concentrations of UK-14304 but displaced the upper portion of the CCRC. Rauwolscine (1 μM) prevented the potentiating effect of A II on responses to UK-14304 (Figure 1d).

Discussion Although pressor responses to postjunctional α_1- and α_2-adrenoceptor stimulation are easily demonstrated *in vivo* (McGrath, 1982), responses via postjunctional α_2-adrenoceptors in isolated vascular preparations have been difficult to show. This is particularly true in arterial vessels, the likely source of α_2-adrenoceptor-mediated pressor responses in whole animals, while even in veins only a few clear examples have been shown (e.g. Constantine *et al.*, 1982; Daly *et al.*, 1988b). There is, however, some evidence that the presence of tissue stimulants can enhance the expression of α_2-adrenoceptor-mediated vasoconstriction (see Introduction). We have now, for the first time in an isolated arterial preparation, shown that activation with a physiological stimulant, namely A II, reveals a quiescent population of α_2-adrenoceptors in a vessel whose response was mediated entirely by α_1-adrenoceptors in the absence of A II. This is associated with a marked increase in the sensitivity of the preparation (up to 300 fold) to the selective α_2-adrenoceptor agonist UK-14304.

The rabbit distal saphenous artery clearly responds to α-adrenoceptor agonists through α_1-adrenoceptors under normal, *in vitro*, experimental conditions. Firstly, the relative potency of agonists is NA > PE > UK-14304 and secondly, prazosin and rauwolscine produced antagonism against UK-14304 consistent with that shown previously at α_1-adrenoceptors in other rabbit blood vessels: prazosin (estimated $-\log K_b$ value 8.7), rauwolscine (estimated $-\log K_b$ value 6.3) (see Daly *et al.*, 1988c). A II, without altering the resting baseline tension, produced a marked increase in sensitivity to UK-14304. Thus, previously absent responses were 'uncovered' at low concentrations of UK-14304: these responses were unaffected by prazosin (0.1 μM: a concentration 50 times greater than its K_b value at α_1-adrenoceptors in this tissue). The CCRC at higher concentrations of UK-14304 however, was shifted to the right, indicating the continued expression of responses via α_1-adrenoceptors. In contrast, in the presence of A II, rauwolscine (1 μM) antagonized the response to UK-14304 more effectively than prazosin (0.1 μM), in effect preventing the increase in sensitivity produced by A II. This confirms that the 'uncovered' responses were mediated by postjunctional

α_2-adrenoceptors. The present study shows that a stimulating agent is required for expression of functional postjunctional α_2-adrenoceptors in the rabbit distal saphenous artery. The requirement for physiological stimulants, such as A II, could therefore explain the hitherto perplexing differences in the expression of postjunctional α_2-adrenoceptors between *in vivo* and *in vitro* preparations.

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