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**α₂-Adrenoceptor mediated inhibition of forskolin-stimulated cyclic AMP accumulation in isolated porcine palmar lateral veins**

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**Abstract** The aim of this study was to use a ³H-adenine pre-labelling technique to characterise the effect of α₂-adrenoceptor activation on forskolin-stimulated cyclic AMP accumulation in the isolated porcine palmar lateral vein. Forskolin (10⁻⁷–10⁻⁴ M) stimulated ³H-cyclic AMP accumulation in the isolated porcine palmar lateral vein in a biphasic and concentration-dependent manner. In the absence of the cyclic AMP-selective phosphodiesterase inhibitor rolipram, forskolin stimulated ³H-cyclic AMP accumulation approximately 7–8 fold. The response reached a peak after 5 min. In the presence of rolipram (10⁻⁵ M), basal ³H-cyclic AMP levels were approximately 70% higher than in its absence (basal: 1823 ± 57 dpm; rolipram: 3088 ± 229, n = 3) and forskolin (3 × 10⁻⁵ M) stimulated ³H-cyclic AMP accumulation approximately 8 fold. The latter response reached a plateau 10 min after the addition of forskolin. In all subsequent studies, the tissues were incubated with forskolin (3 × 10⁻⁵ M) for 5 min in the absence of rolipram. Noradrenaline (NA; 10⁻⁹–10⁻⁴ M) and UK14304 (10⁻⁹–10⁻⁴ M) inhibited forskolin-stimulated ³H-cyclic AMP accumulation in a concentration-dependent manner with mean pIC₅₀ values of 7.61 ± 0.37 (n = 4) and 7.76 ± 0.23 (n = 5), respectively. With either NA or UK14304, the maximal inhibition of the forskolin response obtained was approximately 75%. Neither NA (10⁻⁴ M) nor UK14304 (10⁻⁴ M) altered basal ³H-cyclic AMP levels. Phenylephrine (10⁻⁴ M) had no effect on basal ³H-cyclic AMP levels and produced a 25.4 ± 7.1% inhibition of the forskolin-stimulated response, an effect that was reversed by 10⁻⁶ M rauwolscine. Rauwolscine (10⁻⁹–10⁻⁶ M) produced a concentration-dependent reversal of the inhibitory effect of UK14304 (10⁻⁶ M) on forskolin-stimulated ³H-cyclic AMP accumulation with a mean pKᵢ of 8.35 ± 0.39 (n = 3), but had no effect on basal or on forskolin-stimulated ³H-cyclic AMP levels. Similarly, prazosin (3 × 10⁻⁸–3 × 10⁻⁵ M) or imiloxan (3 × 10⁻⁸–3 × 10⁻⁵ M) produced a concentration-dependent reversal of the UK14304 (10⁻⁷ M)-induced inhibition of forskolin-stimulated ³H-cyclic AMP accumulation, with mean pKᵢ values of 6.32 ± 0.22 (n = 4) and 6.01 ± 0.30 (n = 3), respectively; neither drug had any effect on basal or on forskolin-stimulated ³H-cyclic AMP levels. This suggests that the receptor is of the α₂ₐ-adrenoceptor subtype. It can be seen from these studies that it is possible to measure changes in cyclic AMP accumulation in porcine vascular smooth muscle using a pre-labelling technique, and it has been possible to demonstrate the presence of functional α₂-adrenoceptors, stimulation of which results in inhibition of forskolin-stimulated cyclic AMP formation.

**Key words** α₂-Adrenoceptors · forskolin · rolipram · cyclic AMP · porcine vascular tissue

**Introduction**

The existence of post-junctional α₁- and α₂-adrenoceptors has frequently been demonstrated by functional studies of arterial resistance in vivo, but the presence of post-junctional α₂-adrenoceptors has been more difficult to demonstrate in vitro (see Agrawal et al. 1987; McGrath et al. 1991), functionality often only being observed in the presence of an ancillary spasmogen (Daly et al. 1988; Dunn et al. 1991). However, overt α₂-adrenoceptor mediated contractions have been observed to occur in a range of porcine distal veins and non-conducting arteries not generally used for routine experimentation (Wilson et al. 1993; Blaylock and Wilson 1995; Wright et al. 1995a). We have previously reported that porcine vessels possess different densities of α₂-adrenoceptors (Wright et al. 1993a; Wright et al. 1995a). One of these vessels which displayed an overt
α2-adrenoceptor mediated functional response and a high density of α2-adrenoceptor sites was the porcine palmar lateral vein. It is well established that α2-adrenoceptors are negatively coupled to adenyl cyclase and that their activation results in an inhibition of cyclic adenosine monophosphate (cyclic AMP) formation in non-vascular preparations (Duman and Enna 1986; Bylund 1988). Since β-adrenoceptor-mediated relaxation appears to involve an increase in cellular cyclic AMP, it is possible that inhibition of adenyl cyclase could be one of the mechanisms through which vascular α2-adrenoceptors produce contractile responses. To date, only two studies have successfully demonstrated that vascular α2-adrenoceptors have the potential to modulate cellular cyclic AMP levels (Fredholm et al. 1985; Stubbs et al. 1988). Thus, the precise role of this pathway in contractile events has remained obscure.

We have recently shown that α2-adrenoceptor-mediated contraction of the isolated porcine palmar lateral vein is less sensitive to the inhibitor effects of forskolin (a known activator of adenyl cyclase (Seamon and Daly 1986)) than α1-adrenoceptor-mediated responses (Wright et al. 1995b). This observation raises the possibility that, under the appropriate conditions, inhibition of adenyl cyclase may be of physiological importance. In the present study, we have examined the suitability of the 3H-adrenaline pre-labelling method used to label cyclic AMP in brain slices (Duman and Enna 1986) for studying cellular cyclic AMP metabolism in porcine vascular smooth muscle. In addition, we have made a preliminary pharmacological characterisation of the α2-adrenoceptor subtype present in this tissue.

Some of the data were presented in a preliminary form at the Bradford Meeting of the British Pharmacological Society (Wright et al. 1993b).

### Methods

**Tissue preparation.** Fore-trotters from male pigs were obtained from a local abattoir, shortly after the animals had been killed, and were transported to our laboratory in ice-cold Krebs' solution. The palmar lateral vein was dissected from the trotters (the location of this vessel has been reported previously by Wright et al. (1995a)). Tissues were stored overnight in Krebs' solution containing 2% Ficoll (to prevent osmotic swelling of the smooth muscle). On the following day, the vessels were cut into 5 mm lengths (approximately 4 mg wet weight) and then incubated for 60 min at 37°C in a shaking waterbath. The segments of vessel were then incubated with 3H-adrenaline (specific activity = 851 GBq.mmol⁻¹; 74 kBq.ml⁻¹) for 60 min at 37°C in a shaking waterbath. After incubation, the segments of vessel were washed 3 times by re-suspension and then one segment was transferred into each of an appropriate number of flat-bottomed plastic incubation vials containing Krebs' solution accordant with a final assay volume of 300 µl. The segments were allowed to equilibrate for 20 min. Agonists (noradrenaline (NA), UK14304, or phenylephrine (PE)), each at concentrations of 10⁻⁹–10⁻⁴ M, were added 10 min prior to the addition of forskolin, and antagonists (rauwolscine (10⁻⁹–10⁻⁶ M), prazosin (3 x 10⁻⁸–3 x 10⁻⁵ M), imiloxan (3 x 10⁻⁸–3 x 10⁻⁵ M)) were added 10 min before the agonists. Forskolin (3 x 10⁻⁵ M) was allowed to act for 5 min. The incubation tubes were re-sealed under O₂-CO₂ (95%: 5%) after each addition. At the end of the incubation, 200 µl 1 M HCl followed by 750 µl of distilled water were added to stop the reaction. All experiments were carried out in quadruplicate.

3H-cyclic AMP was separated from 3H-adrenaline and other 3H-products by sequential Dowex/alumina chromatography (Salomon et al. 1974). In order to correct the results for variations in recovery, 1C-cyclic AMP (ca. 30 Bq tube) was added and variations in the amount of tissue were taken into consideration by measuring the total 3H taken up into individual tissue samples. 3H-cyclic AMP, 1C-cyclic AMP, and total 3H in each tissue sample were determined using liquid scintillation counting.

Preliminary experiments were made to examine the effects of various concentrations of forskolin on cyclic AMP accumulation. Then, using one concentration (3 x 10⁻⁵ M), a time course was constructed and the effects of the cyclic AMP selective phosphodiesterase inhibitor rolipram (10⁻⁵ M) (Beavo and Reifsnyder 1990; Nicholson et al. 1991) on the time course were studied. Also, as most contractile studies are carried out in the presence of cocaine (10⁻⁵ M) and propranolol (10⁻⁵ M) in order to inhibit uptake, and β-adrenoceptors, respectively, preliminary experiments were made to determine whether these agents had any effect on the response to noradrenaline (NA).

#### Data analysis

The curves for NA and UK14304 inhibition of forskolin-stimulated cyclic AMP accumulation were analysed, using iterative fitting to a least squares non-linear equation (Kaleidagraph, Macintosh), to generate IC₅₀ values (concentration of drug at the 50% value on the curve for inhibition of forskolin-stimulated cyclic AMP accumulation).

\[
Y = \frac{m0 - (m0 - m6)(m0 + m2)}{m0 + m2} \quad \text{where} \quad m0 = \text{an independent variable} \\
m1 = \text{the asymptote} \\
m2 = \text{the slope of the curve} \\
m3 = IC_{50} \\
m6 = \text{the basal value} \\
y = \text{the response}
\]

None of these values were restrained. It was not possible to determine IC₅₀ values for the agonists as the interaction of forskolin with the catalytic C subunit of the enzyme does not follow mass action drug-receptor kinetics. Thus, the results are expressed as pIC₅₀ values to allow the relative potencies of the agonists to be determined. In the studies of reversal by (rauwolscine, prazosin, and imiloxan, the IC₅₀ values were converted to inhibitory constants (Ki) by the modified equation of Cheng and Prusoff (1973), where

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### Drugs

The composition of the Krebs' solution was (mM): NaCl 118.4, KCl 4.7, CaCl₂ 1.3, MgSO₄ 7H₂O 1.2, NaHCO₃ 24.9, KH₂PO₄ 1.2 and glucose 11.1.

The following compounds were used: 3H-adrenaline and 1C-cyclic AMP (Amersham International, Amersham); (±)-noradrenaline bitartrate (Sigma); UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinazolinedine bitartrate; Pfizer); phenylephrine HCl (Sigma); rauwolscine HCl (Roth); propranolol HCl (Sigma); cocaine HCl (MacCathy); imiloxan hydrochloride (Syntex); prazosin HCl (Pfizer); forskolin (Sigma); rolipram (Schering). Solutions of noradrenaline, UK14304, phenylephrine, rauwolscine, imiloxan hydrochloride (Syntex), and propranolol were prepared fresh each day in Krebs' solution; prazosin (10⁻³ M) was dissolved in 0.1 M lactic acid and