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Pharmacological characterization of postsynaptic α-adrenoceptor subtypes in five different dog arteries in-vitro

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The responses of helically cut strips of arteries isolated from five different sites in the body of dogs to relatively selective α_1- and α_2-adrenoceptor agonists and the antagonism exerted on these responses by relatively selective α_1- and α_2-adrenoceptor blockers have been studied. On all arteries (renal, splenic, cranial mesenteric, jejunal and femoral) phenylephrine was a full agonist whereas UK-14,304 was a partial agonist causing maximal contractions of 49, 30, 27, 27 and 10% of the maximum, respectively. Phenylephrine was more potent than UK-14,304, being 9 times more potent in the renal artery and up to 42 times more potent in the cranial mesenteric artery. In the dog saphenous vein, where there are both α_1- and α_2-adrenoceptors, it has been previously shown that UK-14,304 is 530 times more potent than phenylephrine. Prazosin in low concentrations displaced concentration-response curves for both phenylephrine and UK-14,304 (pA_2 values of 8·16–8·43 and 8·13–8·79, respectively) whereas yohimbine was much less potent (pA_2 values of 6·53–6·88 and 6·50–7·20, respectively). The results suggest that the α-adrenoceptors of all arteries studied are predominantly, if not exclusively, of the α_1-subtype.

Much evidence supporting the existence of postsynaptic α_2-adrenoceptors on vascular smooth muscle has been obtained in in-vivo experiments (Drew & Whiting 1979; Docherty et al 1979; Timmermans et al 1979). There is some evidence from in-vitro studies with veins from the dog, of postsynaptic α_2-adrenoceptors: thus they have been demonstrated in the saphenous vein (De Mey & Vanhoutte 1981; Shepperson & Langer 1981) and in cephalic, femoral and external jugular veins (Shoji et al 1983). However, it is unclear whether postsynaptic α_2-adrenoceptors are demonstrable in artery preparations in-vitro.

In the present study a pharmacological characterization of α-adrenoceptor subtypes was made in five different arteries of the dog using phenylephrine and UK-14,304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) (Cambridge 1981) as agonists and prazosin and yohimbine as antagonists.

Materials and methods

Mongrel dogs, 7–15 kg, were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} i.v. injected in the forelimb) and segments of cranial mesenteric, jejunal, splenic, renal and femoral arteries were removed. Helically cut strips of about 2·5 × 30 mm were prepared and suspended in a 20 ml bath containing Krebs-Henseleit solution (Guimarães & Osswald 1969). The bath solution was kept at 37 °C and bubbled with 95% O_2 and 5% CO_2. The strips were connected to isotonic levers and adjusted to give an approximately 5-fold magnification and counterweighed to provide a load of 2 g. The responses were recorded with a frontal level on a smoked drum. Each strip was allowed to stabilize during an equilibrium period of 1 h.

Concentration-response curves to phenylephrine and UK-14,304 were determined in the presence of 0·5 μmol $litre^{-1}$ propranolol, 12 μmol $litre^{-1}$ cocaine and 50 μmol $litre^{-1}$ U-0521. Concentrations were increased by a factor of 3 until maximal responses were obtained. Propranolol, cocaine, and U-0521 were added to the bath 30 min before the addition of the agonists, and they were left in the bath throughout the experiment. Only two concentration-response curves were determined per strip. The pD_2 (negative logarithm of the concentration required for a 50% maximal response) values of the agonists were determined from the concentration-response curve by interpolation. To determine the maximal response, 200 μmol $litre^{-1}$ noradrenaline was added to the preparation at the end of each experiment.

To study the antagonism by prazosin or yohimbine, pA_2 values were calculated according to the method of Van Rossum (1963) from the equation: $pA_2 = pA_x + \log (x - 1)$ in which x represents the factor of the shift of the concentration-response curve to the right and pA_x the negative logarithm of the molar concentration of the antagonist which caused this shift.

The results are presented as arithmetic means with their standard errors or geometric means with 95% confidence limits. Differences between means were compared by Student's *t*-test and those with *P* value of 0·05 or less were considered significant.

Drugs used were: cocaine hydrochloride (Uquipa), (−)-noradrenaline bitartrate (Koch-Light), (−)-phenylephrine hydrochloride (Boehringer-Sohn), prazosin hydrochloride (Pfizer), propranolol hydrochloride (ICI), U-0521 (3,4-dihydroxy-2-methyl propiophenone(Upjohn), UK-14,304(5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline(Pfizer), yohimbine hydrochloride(Sigma).

Results

Contractile responses to phenylephrine and UK-14,304. Responses to the α-adrenoceptor agonists phenylephrine and UK-14,304 were studied on cranial, mesen-

* Correspondence.

Table 1. Potency of phenylephrine (PHE) and UK-14,304. The values represent geometric means and the 95% confidence limits.

Artery	Phenylephrine (pD_2)	n	UK-14,304* (pD_2)	n	Antilog pD_2PHE–pD_2-UK-14,304
Renal	6·66 (6·52–6·80)	14	5·70 (5·47–5·94)	12	9·1
Femoral	6·28 (6·10–6·46)	6	4·95 (4·50–5·40)	6	21·4
Splenic	6·55 (6·38–6·74)	12	4·93 (4·43–5·43)	8	41·8
Cranial mesenteric	6·49 (6·30–6·68)	8	5·02 (4·61–5·33)	8	29·8
Jejunal	6·31 (6·19–6·44)	13	5·21 (4·96–5·45)	9	12·6

* The values for UK-14,304 correspond to 50% of its own maximal response.

teric, jejunal, renal, splenic and femoral arteries in the presence of 0·5 μmol litre^{-1} propranolol (to block β-adrenoceptors), 12 μmol litre^{-1} cocaine (to inhibit neuronal uptake) and 50 μmol litre^{-1} U-0521 (to inhibit COMT).

Phenylephrine behaved as a full agonist in all arterial vessels in that it caused contractions with the same magnitude as that of the maximal contraction caused by noradrenaline.

The sensitivity of the strips to phenylephrine, as expressed by pD_2 values, did not differ appreciably among the various arteries (Table 1). Phenylephrine was more potent than UK-14,304 in all vessels studied. The pD_2 values for phenylephrine ranged from 6·28 to 6·66 while those for UK-14,304 ranged from 4·93 to 5·70. The pD_2 values for UK-14,304 varied between arteries to a greater extent than did the pD_2 values for phenylephrine.

UK-14,304 was a partial agonist in all five arterial vessels. The maximal contraction caused by UK-14,304, expressed as per cent of the maximal contraction caused by noradrenaline ranged between 10 and 49% (Table 2 and Fig. 1).

Antagonism by prazosin and yohimbine

Both prazosin and yohimbine were added to the bathing solution 45 min before the second concentration-response curve was determined.

Very low concentrations of prazosin (10 and 25 nmol litre^{-1}) caused similar shifts to the right of the concentration-response curve to phenylephrine and UK-14,304 without depressing the maximal response. The pA_2 values (Table 3) for the antagonism exerted by prazosin against the responses elicited by phenylephrine and UK-14,304 were quite similar in all five arteries, suggesting that the effects caused by these agonists were due to activation of the same receptors.

Yohimbine (200 nmol litre^{-1} and 1 μmol litre^{-1}) also shifted the concentration-response curves to phenylephrine and UK-14,304 to the right but yohimbine was 18–151 times less potent than prazosin (Table 3). The pA_2 values for the antagonism exerted by yohimbine against phenylephrine and UK-14,304 were also similar indicating that this antagonism results from the interaction of yohimbine with the same α-adrenoceptor.

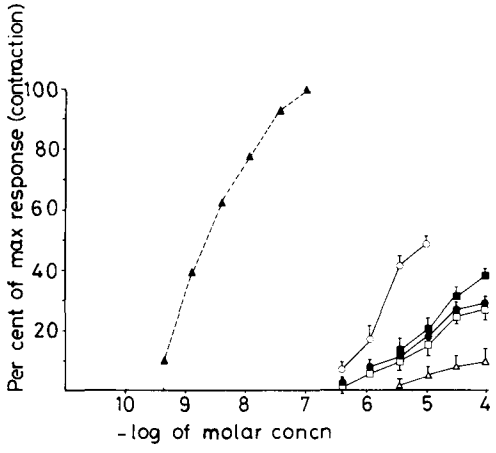

FIG. 1. Concentration-response curves for UK-14,304 obtained in different dog arteries: ○——○ renal; ■——■ splenic; ●——● jejunal; □——□ cranial mesenteric; △——△ femoral. The concentration-response curve for the dog saphenous vein (▲——▲) which is shown for comparison was taken from Polónia et al (1983).

Table 2. Maximal contractile responses of the arteries to UK-14,304 expressed as per cent of the maximal response to noradrenaline (mean ± s.e.).

Artery	Maximal contraction	n
Renal	48·5 ± 4·1	14
Femoral	9·5 ± 2·8	6
Splenic	29·9 ± 4·8	9
Cranial mesenteric	27·3 ± 4·6	8
Jejunal	27·1 ± 4·1	11

Discussion

Adrenoceptors of the α_1-subtype are activated preferentially by phenylephrine (Starke et al 1975a) and blocked by prazosin (Cambridge et al 1977), whereas

Table 3. Shown are pA_2 values for the antagonism exerted by prazosin and yohimbine on the contractions caused by phenylephrine and UK-14,304 in the different arteries. Both prazosin and yohimbine were added to the bath 45 min before starting the determination of concentration-response curve for the agonist (second curve). The values represent geometric means with the confidence limits. The pA_2 for the antagonism exerted on contractions caused by UK-14,304 were not determined for the femoral artery because the maximal contraction caused by this agonist was very small (see Table 2).

Artery	Phenylephrine pA_2				UK-14,304 pA_2			
	Prazosin	n	Yohimbine	n	Prazosin	n	Yohimbine	n
Renal	8·43 (8·18–8·66)	7	6·88 (6·68–7·09)	4	8·45 (8·28–8·62)	7	7·20 (6·68–7·76)	4
Femoral	8·35 (8·26–8·43)	5	6·53 (6·33–6·68)	5	—		—	
Splenic	8·28 (8·11–8·44)	5	6·69 (6·17–7·25)	5	8·77 (8·34–9·22)	4	6·59 (6·14–7·06)	4
Cranial mesenteric	8·30 (7·82–8·81)	5	6·95 (6·81–7·23)	6	8·79 (8·44·9·15)	4	6·77 (6·01–7·64)	4

those of the α_2-subtype are activated preferentially by UK-14,304 (Cambridge 1981) and blocked by yohimbine and rauwolscine (Starke et al 1975b; Weitzell et al 1979).

In the present study phenylephrine was a full and potent agonist, inducing contractions in all arteries examined, while UK-14,304 was a partial agonist of low potency under the same conditions.

The ratios ED50 for UK-14,304/ED50 for phenylephrine obtained in this study were: 29·8 for the cranial mesenteric, 12·6 for the jejunal, 41·8 for the splenic, 9·1 for the renal and 21·4 for the femoral artery.

According to Cambridge (1981), UK-14,304 is about 300-fold more active on α_2- than on α_1-adrenoceptors and about 10 times more potent than noradrenaline in eliciting contractions induced by activating α_2-adrenoceptors. In the saphenous vein of the dog, a tissue in which both α_1- and α_2-adrenoceptors co-exist, UK-14,304 is an almost full agonist (with an ED50 of 3·4 nmol litre^{-1}): it is more potent than noradrenaline (ED50 = 100 nmol litre^{-1}) and much more potent than phenylephrine (ED50 = 1800 nmol litre^{-1}) (Polónia et al 1983). This means that in a tissue in which both phenylephrine and UK-14,304 have practically the same intrinsic activity, UK-14,304 is about 500 times more potent than phenylephrine. Comparison of this ratio with those obtained in the present study leads to the conclusion that there are virtually no postsynaptic α_2-adrenoceptors in the arteries examined in the present investigation.

The results obtained with prazosin and yohimbine fully confirm this conclusion. The pA_2 values of prazosin for interaction with α_1-adrenoceptors have been reported to range between 7·9 and 9·7 in several vessels, such as canine splenic and femoral arteries (De Mey & Vanhoutte 1981), rabbit portal vein and pulmonary artery (Docherty & Starke 1981), rabbit aorta (Cavero et al 1978), and human omental arteries and veins (Steen et al 1984). In our study, the pA_2 values found for phenylephrine and UK-14,304 ranged between 8·13 (in the cranial mesenteric against UK-14,304) and 8·79 (in the jejunal artery against UK-14,304). This means that prazosin in low concentrations antagonized not only the responses produced by phenylephrine but also those caused by UK-14,304, and suggests that phenylephrine and UK-14,304 produced their effects by activation of α_1-adrenoceptors.

The low pA_2 values obtained for yohimbine antagonism further support this conclusion. Like prazosin, yohimbine caused rightward and essentially parallel shifts in the concentration-response curves for phenylephrine and UK-14,304. However, to inhibit the contractile responses to UK-14,304, the concentrations of yohimbine required were much higher than those needed to block α_2-adrenoceptors (Sullivan & Drew 1980; De Mey & Vanhoutte 1981; Guimarães et al 1983).

It is very unlikely that any component of the contractile effects of phenylephrine and UK-14,304 is due to activation of α_2-adrenoceptors in any of the arteries studied. The pD_2 values for UK-14,304 were very low and the responses to UK-14,304 were highly susceptible to the antagonistic action of prazosin and very resistant to that of yohimbine. These three factors together make it exceedingly unlikely that α_2-adrenoceptors were involved in the responses to UK-14,304.

The role played by α_1-adrenoceptors in the regulation of blood flow in dog vascular beds in-vivo (Drew & Whiting 1979; Gardiner & Peters 1982; Guimarães & Garrett 1982; Horn et al 1982; Shepperson et al 1982) cannot be explained by the α-adrenoceptor subtypes found in-vitro in arteries in the present study and veins (Shoji et al 1983). The postjunctional α-adrenoceptors found in mesenteric, renal, splenic and femoral arteries of the dog in the present investigation are predominantly or totally of α_1-subtype, whereas in the circuits to which these arteries belong the role played by α_2-adrenoceptors seems to be important. This discrepancy seems to indicate a predominance of α_2-adrenoceptors in the arterioles controlling blood flow, and these vessels have not yet been studied in-vitro.

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Effect of verapamil and diltiazem on isolated gastro-oesophageal sphincter of the rat

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The effect of verapamil and diltiazem on the contraction induced by agonists on the rat lower oesophageal sphincter in-vitro has been studied. Both calcium entry blockers inhibited the contractile response to acetylcholine, carbachol and KCl. The potency of the inhibitory action was diltiazem > verapamil. The results give substance to the use of calcium entry blockers in the treatment of oesophageal spasm.

Calcium antagonists such as verapamil and diltiazem were originally described as coronary vasodilators but it is now well known that these drugs have effects on other organs and tissues, such as smooth muscle of blood vessels and airways. Weiser et al (1978) have indicated that nifedipine (a calcium entry blocker) also exerts a relaxant effect on oesophageal smooth muscle and they have suggested the possibility of a role for this drug in the treatment of oesophageal spasm. Several investigators (Blackwell et al 1981; Bortolotti & Labo 1981; Nasrallah 1982) have demonstrated clinically, the usefulness of drugs therapy with calcium-antagonists in relieving the dysphagia of patients with achalasia. However little attention is paid to in-vitro studies on action of calcium antagonists on gastrointestinal smooth muscle.

In common with other smooth muscle, the contraction of the lower oesophageal sphincter (LES) have been shown to be dependent on calcium. Cohen & Green (1973) showed that the peak force and maximal velocity of shortening were diminished by reducing the external calcium concentration. De Carle et al (1977) demonstrated that LES tone was also dependent on Ca^{2+}.

In this communication we describe the effect of verapamil and diltiazem on contraction induced by acetylcholine, carbachol and KCl in the LES of the rat in-vitro.

Methods

Albino rats (200–250 g) of either sex were killed by a blow to the head and exsanguinated. The abdomen was opened and the stomach, together with 1·5 cm of oesophagus, was removed and immersed in a room-temperature aerated Krebs solution (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 10. According to Takayanagi & Kasuya (1977), transverse strips (approximately 7 mm × 3·5 mm) were taken from the gastro-oesophageal junc-