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Short communication

Pharmacological characterization of α-adrenoceptors in the bovine median caudal artery

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Abstract

This study was initiated to characterize α-adrenoceptors in the isolated bovine median caudal (tail) artery preparation for use as a model of blood vessels in the extremities of cattle. Prazosin shifted the concentration–response relationship for noradrenaline and phenylephrine to the right with pA_2 values of 8.74 and 9.04, respectively. Against noradrenaline, phentolamine yielded a pA_2 of 7.36. The concentration–response relationship for medetomidine was not inhibited by rauwolscine or idazoxan. The noradrenaline K_A was 3.11 μM. These results suggest that catecholamines elicit α-adrenoceptor-mediated contractions primarily through α_1-adrenoceptors and not α_2-adrenoceptors. © 1997 Elsevier Science B.V.

Keywords: α-Adrenoceptors; Dissociation constant; (Bovine); Caudal artery; Tail artery

1. Introduction

Little information exists concerning vasoactivity in the extremities of cattle in response to physiologic/pharmacologic factors. Investigators have previously used the isolated ear artery, dorsal pedal vein, and tail artery to study vascular mechanisms in the extremities of cattle (Ashida and Blaustein, 1987; Solomons et al., 1989; Eghianruwa and Eyre, 1991). The use of the tail artery preparation has been limited. Previously, the bovine tail artery has been used to study the sodium/calcium exchanger (Ashida and Blaustein, 1987) and the sarcoplasmic reticulum calcium store (Ashida et al., 1988). Due to this lack of knowledge we initiated a study to investigate α-adrenoceptors in the isolated median caudal (tail) artery with the intention of developing this preparation as a model for studies on blood vessels found in the extremities of cattle. The purpose of this study was to determine the apparent dissociation constant for noradrenaline and several α-adrenoceptor antagonists.

2. Materials and methods

2.1. Tissue preparation

Adult bovine tails were collected at a local abattoir. The median caudal artery was dissected from the tail and cleaned of adhering connective tissue. The tissue was keep in a modified Krebs' solution with the following composition (mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $NaHCO_3$, 22.14; dextrose, 7.88; and disodium ethylenediamine tetracetic acid, 0.03. Arteries were cut into 4 mm ring segments and suspended in 10 ml isolated tissue baths maintained at 37°C and aerated with a 95% O_2–5% CO_2 mixture. Tissue responses were recorded isometrically by Grass FT-03 force transducers which were connected to a Grass or Beckman polygraph (model 7 or model R611, respectively). Tissues were permitted to relax under 3 g tension for 60 min and then maintained at 2 g tension for 30 min prior to drug administration.

2.2. Analysis of responses to agonists and antagonists

In all experiments, ring segments were primed with 3 μM noradrenaline for 10 min after the equilibration pe-

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riod. Agonists were administered cumulatively in approximately half-log increments. Data measurements were obtained at the effective agonist concentration required to elicit 50% of its maximal response (EC_{50}), as indicated by Schild (1949). Time-dependent shifts of the agonist concentration–response relationship during the course of an experiment were monitored using a paired 'time control' tissue, as recommended by Furchgott (1972), and as used in our laboratory (Zhang and Dyer, 1990).

2.3. Determination of dissociation constants

The methods and statistical analysis for determining the dissociation constant (K_A) of noradrenaline follow those described by Furchgott and Bursztyn (1967). Before the generation of a concentration–response relationship to noradrenaline (0.01–100 μM), tissues were incubated with 0.36 mM iproniazid (monoamine oxidase inhibitor) for 60 min and then washed every 10 min for 40 min. Tissue were also pretreated with 1 μM propranolol for 30 min to block β-adrenoceptors, and with cocaine (3 μM), corticosterone (10 μM), and tropolone (10 μM) for 20 min to block uptake$_1$ uptake$_2$, and catecholine-*O*-methyltransferase respectively. These enzymatic and uptake inhibitors have been extensively reviewed and used to specifically inhibit their respective uptake sites and enzymatic pathways by other investigators (Furchgott and Garcia, 1968; Levin and Furchgott, 1970; Zhang and Dyer, 1990; Costa et al., 1992). A concentration–response relationship to noradrenaline was initially determined. Dibenamine (0.3 μM) was incubated with the tissues for 20 min, and then washed 4 or 5 times over 30 min to inactivate a fraction of the α-adrenoceptors before repeating the concentration–response relationship to noradrenaline. After correcting for time-dependent changes in sensitivity, the noradrenaline concentration–response relationship before and after dibenamine treatment were plotted. A double reciprocal plot of equi-effective concentrations of noradrenaline before ($1/[A]$) and after ($1/[A']$) dibenamine treatment was made. The K_A value was calculated from this plot using the equation $K_A = \text{slope} - 1/\text{intercept}$. The receptor reserve was determined from the relationship: K_A/EC_{50} (Ruffolo, 1982).

The procedures for the determination of dissociation constants (K_B) and pA_2 values for adrenoceptor antagonists follow those as described by Furchgott (1972), and as used in our laboratory (Zhang and Dyer, 1990). As previously described, rings were pretreated with uptake and enzyme inhibitors and propranolol before generating concentration–response relationships (0.01–100 μM) to noradrenaline and phenylephrine. A control agonist concentration–response relationship was then attained. One concentration of either prazosin (3–100 nM), phentolamine (30–1000 nM), or idazoxan (3–1000 nM) was then allowed to equilibrate for 60 min with each tissue before repeating the agonist concentration–response relationship.

Idazoxan, rauwolscine, and prazosin were also evaluated against the selective α_2-adrenoceptor agonist, medetomidine. Since the tissue remained contracted after the initial exposure to medetomidine despite repeated washings with fresh Krebs' solution, the following procedure was used to evaluate the antagonism of this agonist. A control concentration–response relationship to KCl was initially determined on all tissues. One tissue served as the control and received no antagonist. The other tissues were each equilibrated with prazosin (3–300 nM), rauwolscine (3–100 nM), or idazoxan (10–1000 nM) for 60 min. This was followed by determining a medetomidine concentration–response relationship (0.01–100 μM). The selective α_2-adrenoceptor agonist, B-HT 920 (0.01–100 μM), was also going to be evaluated, but it did not elicit a contraction.

The effect of the endothelium on the noradrenaline response was determined on intact rings containing endothelium which were paired with either rings denuded of endothelium by rubbing with a toothpick, or intact endothelial rings incubated with the nitric oxide synthase inhibitor, $^{\omega}$*N*-nitro-L-arginine methyl ester HCl (L-NAME; 100 μM), for 30 min. As previously described, all rings were also pretreated with uptake and enzyme inhibitors and propranolol before generating a concentration–response relationship to noradrenaline.

The viability of the endothelium was evaluated using acetylcholine in the absence and presence of L-NAME (100 μM) for 30 min. Rings were initially precontracted with 45 mM KCl followed by the generation of an acetylcholine concentration–response relationship (0.01–100 μM), and then a subsequent application of 10 μM sodium nitroprusside.

2.4. Statistical analysis

Results are presented as mean ± S.E. The number, *n*, of animals used in each experiment is indicated. Agonist EC_{50} values were calculated using least squares linear regression on the steepest part of the concentration–response relationship. Significant differences between two means were tested using the two-tailed paired *t*-test (Neter et al., 1990). In the Schild plot, the *t*-test (null hypothesis is that slope = unity) was used to determine if the slope was significantly different from unity (Neter et al., 1990). Differences were taken as significant when $p < 0.05$.

2.5. Drugs

The following drugs were used: (−)-noradrenaline bitartrate, cocaine HCl, corticosterone 21-acetate, iproniazid phosphate, $^{\omega}$*N*-nitro-L-arginine methyl ester HCl, sodium nitroprusside, acetylcholine chloride (Sigma Chemical, St. Louis, MO); dibenamine HCl (Smith, Kline and French, Philadelphia, PA); medetomidine HCl (Farmos Group, Turku); prazosin HCl (Pfizer, Brooklyn, NY); pro-

pranolol HCl (Ayerst Laboratories, New York, NY); tropolone (Aldrich Chemical, Milwaukee, WI); phentolamine mesylate and rauwolscine HCl (Research Biochemicals, Natick, MA); phenylephrine HCl (Winthrop Laboratories, New York, NY); idazoxan (Kingston-Upon-Hull, UK); 5,6,7,8-tetrahydro-6-(2-propenyl)-4H-thiazolo [4,5-d] azepin-2-amine dihydrochloride (B-HT 920; Boehringer, Indelheim). All drugs were dissolved in 0.9% saline except for corticosterone 21-acetate and dibenamine which were dissolved in ethanol.

3. Results

3.1. Determination of the dissociation constant (K_A) for noradrenaline

The fraction of α-adrenoceptors remaining, q, after irreversible α-adrenoceptor inactivation with dibenamine was calculated from the slope of the double reciprocal plot of equi-effective concentrations of noradrenaline before and after dibenamine treatment ($n = 5$). The calculated q

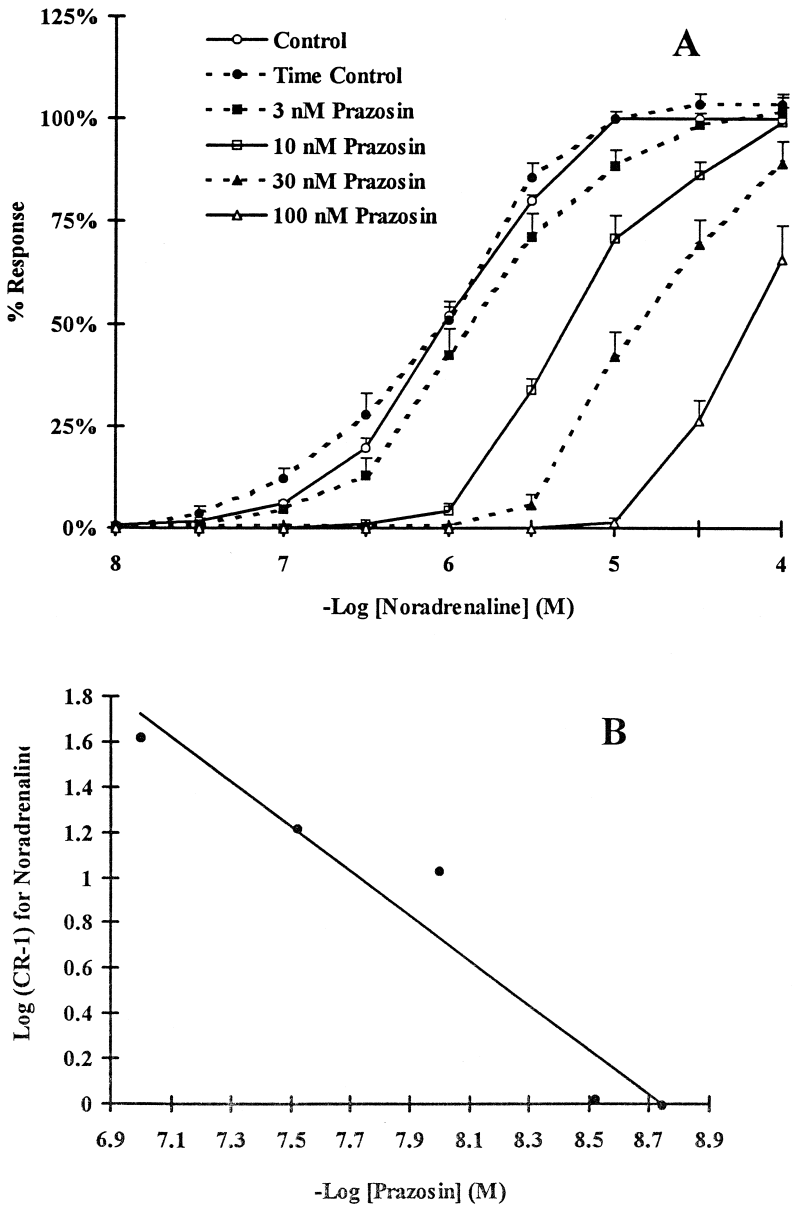

Fig. 1. Antagonism of contractions to noradrenaline by prazosin. (A) Concentration–response relationship to noradrenaline in the presence of several prazosin concentrations. Data are expressed as a percentage of the response to 10 μM noradrenaline. (B) Schild plot analysis of the concentration ratios (CR) for noradrenaline. The concentration ratio represents a ratio at the noradrenaline EC_{50} after and before antagonism by various concentrations of prazosin (Furchgott, 1972). Data were corrected for time-dependent changes before being subjected to Schild plot analysis. Each data point represents the mean concentration ratio from 5 animals. The pA_2 value for prazosin is represented by the abscissa intercept ($y = -0.99x + 8.67$; $r = 0.95$; $pA_2 = 8.74$).

value was 0.26, indicating that 26% of the α-adrenoceptors remained after dibenamine treatment. The calculated noradrenaline dissociation constant ($K_A = 3.11$ μM) was higher than the noradrenaline EC_{50} (1.14 μM). The ratio of K_A/EC_{50} (2.73) indicated that an α-adrenoceptor reserve exists in the bovine median caudal artery. The percentage of adrenoceptors that need to be occupied to generate a half-maximal response was calculated using the ratio EC_{50}/K_A, which is equal to 37% (Ruffolo, 1982). It can be concluded that 3.11 μM noradrenaline occupied 50% of the α-adrenoceptors, but noradrenaline needed to occupy 37% of the α-adrenoceptors to achieve a half maximal response. This 37% receptor occupancy was achieved at 1.14 μM noradrenaline.

3.2. Determination of the dissociation constants (K_B) for α-adrenoceptor antagonists

Phentolamine, the nonselective α-adrenoceptor antagonist, shifted the noradrenaline concentration–response curve to the right in a concentration-dependent fashion. The calculated pA_2 and K_B values were 7.36 and 43.65 nM, respectively ($n = 6$). Prazosin, a selective α_1-adrenoceptor antagonist, also shifted the noradrenaline concentration–response curve to the right (Fig. 1). The calculated pA_2 and K_B values for prazosin against noradrenaline were 8.74 and 1.82 nM, respectively ($n = 5$). The slope of the Schild plots for both phentolamine and prazosin indicated that the antagonism was competitive (Table 1). The potency of noradrenaline, as indicated by the pD_2 value ($-\log EC_{50}$), was 6.16 ($n = 5$).

Prazosin was a potent antagonist against the selective α_1-adrenoceptor agonist, phenylephrine. At higher concentrations prazosin depressed the maximum response to phenylephrine ($n = 5$). The data were analyzed at the EC_{30} instead of the EC_{50} since the response to phenylephrine in the presence of prazosin (30 nM) did not reach the 50% level. The contraction was almost completely inhibited by 100 nM prazosin. The Schild plot yielded pA_2 and K_B values of 9.04 and 0.91 nM, respectively (Table 1)

Inhibition of contractions to medetomidine (selective α_2-adrenoceptor agonist) by prazosin were highly variable and only at the 300 nM concentration was the inhibition significant ($n = 6$). The selective α_2-adrenoceptor antagonists, rauwolscine and idazoxan, did not inhibit contractions to medetomidine ($n = 6$). Idazoxan also did not inhibit contractions to phenylephrine ($n = 6$). The potency of medetomidine yielded a pD_2 value of 5.61 ($n = 5$). Another selective α_2-adrenoceptor agonist, B-HT 920, failed to produce a contraction up to a concentration of 100 μM ($n = 5$).

Both inhibition of nitric oxide synthase with L-NAME and endothelium removal did not significantly alter the maximum tension developed to noradrenaline or significantly shift the noradrenaline EC_{50} to the left ($n = 4$; data not shown). The endothelium in the ring preparations were intact and functional since both acetylcholine and sodium nitroprusside significantly relaxed the precontracted rings ($n = 4$; data not shown). These relaxations were inhibited by L-NAME.

Table 1
Dissociation constants (K_B) for α-adrenoceptor antagonists

Antagonist	Agonist	K_B (nM)	pA_2[a]	Slope[b]	r[c]	n
Phentolamine	noradrenaline	43.65	7.36	−1.05	0.95	6
Prazosin	noradrenaline	1.82	8.74	−0.99	0.95	5
Prazosin	phenylephrine	0.91	9.04	−0.83	0.89	5

n represents the number of animals used.
[a]Abscissa intercept of the Schild plot.
[b]Slopes are not significantly different from unity which indicate competitive antagonism.
[c]Correlation coefficient from the Schild plot.

4. Discussion

α-Adrenoceptor analysis indicated that 37% of the receptors needed to be occupied by noradrenaline to produce a half-maximal response. In arteries supplying the bovine oviduct 22% of the α-adrenoceptors need to be occupied (Costa et al., 1992), while in the rabbit aorta only 6% of the receptors need to be occupied to produce a half-maximal response (Besse and Furchgott, 1976). Therefore, the bovine median caudal artery has a limited α-adrenoceptor reserve. The K_A (3.11 μM) for noradrenaline on the bovine median caudal artery is similar to that reported (Costa et al., 1992) for arteries supplying the bovine oviduct (K_A, 3.95 μM). The K_A/EC_{50} ratio is an index for the receptor coupling efficiency and receptor reserve (Ruffolo, 1982). This ratio was 2.73 on the bovine median caudal artery and is similar to the 2.86 ratio found for the bovine oviductal arteries (Costa et al., 1992). These ratios are low when compared to the ratio of 7.9 found in the rat tail artery (Oriowo et al., 1989). However, all three of these ratios are low when compared to the aorta of the cat and major arteries of the rabbit and rat (Oriowo et al., 1987; Oriowo et al., 1989). As similarly concluded by Costa et al. (1992) for the bovine oviductal arteries, the α-adrenoceptors in the bovine median caudal artery may be inefficiently coupled to second messenger systems which trigger the biologic response.

A pA_2 value is unique for that antagonist acting on a specific receptor and is a measure of the affinity of the drug–receptor complex (Arunlakshana and Schild, 1959). The pA_2 value (7.36) for phentolamine acting on α-adrenoceptors in the bovine median caudal artery is similar to that reported for the rabbit pulmonary artery (7.48) and rabbit aorta (7.8) (Furchgott, 1972; Starke et al., 1975). Prazosin antagonized contractions induced by noradrenaline and phenylephrine, yielding pA_2 values of 8.74 and

9.11, respectively. On bovine oviductal arteries (Costa et al., 1992) and the rat caudal artery (Oriowo et al., 1989) which were contracted to noradrenaline, prazosin had pA_2 values of 9.38 and 8.8, respectively. It is unlikely that the presence of an intact endothelium altered the pA_2 values determined since neither removal of the endothelium or L-NAME significantly altered the EC_{50} for noradrenaline.

Some uncertainty exists concerning the existence of α_2-adrenoceptors since B-HT 920 did not elicit a response, while medetomidine did cause a significant contraction. However, medetomidine's potency was very low ($pD_2 = 5.61$) on the bovine median caudal artery when compared to its potency ($pD_2 = 9.0$) on the mouse vas deferens, an α_2-adrenoceptor-containing tissue (Virtanen et al., 1988). High concentrations of prazosin inhibited contractions to medetomidine in this study. However, the data were difficult to analyze since in the presence of prazosin, medetomidine did not generate a sigmoidal concentration response curve with a steep linear relationship. The selective α_2-adrenoceptor antagonists, idazoxan and rauwolscine, did not inhibit responses to medetomidine. On the rat anococcygeus muscle, high medetomidine concentrations caused contractions that were inhibited by both prazosin and idazoxan, thereby indicating that medetomidine acts as a partial α_1-agonist (Scheinin et al., 1989). Our results suggest that medetomidine may act as a partial α_1-agonist at high concentrations in the bovine median caudal artery since prazosin, but not idazoxan nor rauwolscine, inhibited the response to medetomidine. These data, plus the fact that B-HT 920 failed to produce a contraction, indicates that the bovine median caudal artery lacks functional α_2-adrenoceptors. In summary, our data suggests that the α-adrenoceptor reserve in this artery is limited. Vasoconstriction is primarily mediated by α_1-adrenoceptors since no evidence was obtained for the presence of functional α_2-adrenoceptors.

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