Evidence for a noradrenergic innervation to α_{1A} -adrenoceptors in rat kidney

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1 Experiments were undertaken to characterize the α_1 -adrenoceptor subtype mediating vasoconstrictor responses to periaxillary noradrenergic nerve stimulation (PNS) in the isolated perfused kidney of the rat.

2 Vasoconstrictor responses to nerve stimulation were inhibited by prazosin (10 nM), 5-methyl-urapidil (30 nM), and nitrendipine (1 μ M) but were resistant to inhibition by chloroethylclonidine (100 μ M).

3 5-Methyl-urapidil (30 nM), chloroethylclonidine (100 μ M) and nitrendipine (1 μ M) did not inhibit the neuronal release of tritium from nerves loaded with [³H]-noradrenaline.

4 The results suggest that renovascular α_{1A} -adrenoceptors receive a noradrenergic innervation and that the innervated receptors are coupled to dihydropyridine-sensitive calcium channels.

Keywords: α_{1A} -adrenoceptors; 5-methyl-urapidil; chloroethylclonidine; dihydropyridine-sensitive calcium channels; innervation; kidney

Introduction

Recently subtypes of α_1 -adrenoceptors have been proposed based upon radioligand binding studies in which two distinct binding sites have been resolved (Morrow & Creese, 1986). Binding sites with high affinity for WB 4101, phentolamine (Morrow & Creese, 1986), 5-methyl-urapidil (Gross *et al.*, 1988) and (+)-niguldipine (Boer *et al.*, 1989) have been designated α_{1A} -adrenoceptors, whereas binding sites with low affinity for these ligands have been designated α_{1B} -adrenoceptors. The α_{1B} -adrenoceptor binding site is further defined by its high sensitivity to alkylation by chloroethylclonidine (Johnson & Minneman, 1987). Functional studies provide support for such a subdivision of α_1 -adrenoceptors (Han *et al.*, 1987a,b; 1990; Clarke *et al.*, 1991).

α_1 -Adrenoceptor subtypes have also been shown to couple to distinct mechanisms of calcium mobilisation (Han *et al.*, 1987a). It is generally thought that α_{1A} -adrenoceptors are coupled to the influx of extracellular calcium via dihydropyridine (DHP)-sensitive calcium channels, whereas α_{1B} -adrenoceptors are coupled to inositol phosphate formation and the release of intracellular calcium (for review see Minneman, 1988). Apparent exceptions to these effector mechanisms for α_{1A} - and α_{1B} -adrenoceptors, however, have been reported (Reynolds & Dubyak, 1985; Wilson & Minneman, 1990; Clarke *et al.*, 1991; Blue *et al.*, 1991).

Few studies have addressed the issue as to whether α_{1A} - or α_{1B} -adrenoceptors receive a sympathetic noradrenergic innervation (Spriggs *et al.*, 1991; Sulpizio & Hieble, 1991). Thus, the present study was conducted to identify and characterize the α_1 -adrenoceptor mediating vasoconstrictor responses to periaxillary nerve stimulation (PNS) in the isolated perfused kidney of the rat. The rat kidney was chosen because vasoconstrictor responses to PNS are mediated by α_1 -adrenoceptors (Charlton *et al.*, 1984; Schwartz & Malik, 1989). Furthermore, recent studies in rat kidney have revealed that the α_{1A} -adrenoceptor subtype mediates vasoconstrictor responses to noradrenaline (NA) (Blue *et al.*, 1991; Clarke *et al.*, 1991; Munavvar & Johns, 1991; Eltz *et al.*, 1991; Elhawary *et al.*, 1992), although a limited involvement of α_{1B} -adrenoceptors has not been excluded (Clarke *et al.*, 1991). Finally α_{1A} -adrenoceptors in rat kidney appear to couple to either DHP-sensitive or DHP-insensitive calcium channels (Clarke *et al.*, 1991; Blue *et al.*, 1991) and it was of interest to

determine which of these two receptor-effector mechanisms is triggered by the renal sympathetic, noradrenergic innervation.

Methods

Isolation and removal of kidneys

Male Sprague-Dawley (Charles River) rats (300–350 g) were anaesthetized with sodium pentobarbitone (55 mg kg⁻¹, i.p.) and the renal artery and right kidney were isolated as described previously (Charlton *et al.*, 1984; 1986). A cannula was placed in the mesenteric artery and advanced across the abdominal aorta into the renal artery. The kidney was removed and immediately perfused (6 ml min⁻¹) with Krebs bicarbonate solution (pH 7.4, 37°C) of the following composition (mM): NaCl 118.5; KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25 and dextrose 5. The Krebs solution was bubbled continually with a 95% O₂: 5% CO₂ mixture. Bipolar platinum electrodes were placed around the renal artery for PNS. Perfusion pressure was measured by a Spectramed physiological pressure transducer (model number: P23XL) positioned near the kidney and displayed on a Beckman physiograph (model R611).

Experimental protocol

The following procedures were carried out to obviate the potentially confounding influence of neuronally-released ATP (Schwartz & Malik, 1989). Kidneys were given a bolus dose of ATP (100 μ g in 0.1 ml of 0.9% saline). After perfusion pressure returned to baseline, α - β -methylene ATP (1 μ M) was added to the Krebs solution and perfused through the kidney to desensitize P_{2X}-purinoceptors (Schwartz & Malik, 1989). Desensitization of P_{2X}-purinoceptors was confirmed by injecting ATP (100 μ g) 10 min after starting the infusion of α - β -methylene ATP. The effect of α - β -methylene ATP (1 μ M) upon vasoconstrictor responses to infused noradrenaline (NA) and PNS was determined in a separate set of experiments. A 60 min infusion of α - β -methylene ATP (1 μ M) did not alter significantly the EC₅₀ or maximum response to NA (EC₅₀s to NA before α - β -methylene ATP and during infusion of α - β -methylene ATP were 0.20 μ M and 0.25 μ M respectively). Similarly, a 60 min infusion of α - β -methylene ATP

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did not alter significantly the maximum response to PNS (maximum responses to 3 and 10 Hz before α - β -methylene ATP were 31 ± 12.7 and 184 ± 7.3 and during infusion of α - β -methylene ATP were 38 ± 6.3 and 195 ± 20.9). Furthermore, the appearance of vasoconstrictor responses to PNS were not altered by α - β -methylene ATP. However, as a precaution against the potential involvement of ATP, α - β -methylene ATP was retained in the Krebs solution in subsequent experiments.

For PNS, priming stimulations were carried out at 10 Hz (1 ms pulse duration) to determine supramaximal voltage (≈ 70 V). Subsequently kidneys were allowed to equilibrate for 45 min to yield a stable baseline perfusion pressure (40–60 mmHg).

Vasoconstrictor responses to 3 and 10 Hz stimulations were elicited until steady-state, control vasoconstrictor responses were obtained. Perfusion pressure was allowed to return to baseline between stimulations. The kidneys were then perfused for 60 min with Krebs solution containing vehicle or antagonist, after which responses to 3 and 10 Hz stimulations were repeated in the presence of the antagonist or vehicle (time control). The protocol differed for experiments with chloroethylclonidine. After control responses were obtained, kidneys were perfused with chloroethylclonidine (100 μ M) for 20 min and then perfused for 40 min with Krebs solution free of chloroethylclonidine. Responses to 3 and 10 Hz stimulations were then repeated.

In a separate series of experiments, the effect of chloroethylclonidine (100 μ M), 5-methyl-urapidil (30 μ M) and nitrendipine (1 μ M) was determined upon the stimulus-induced release of [3 H]-NA from postganglionic sympathetic nerves to the kidney. Postganglionic sympathetic nerves were loaded with (–)-[7,8- 3 H]-NA (specific activity: 8–15 Ci mmol $^{-1}$) by perfusing kidneys for 30 min with Krebs solution containing [3 H]-NA (3.7 nM), followed by a 60 min perfusion with Krebs solution free of [3 H]-NA. Three sets of stimulations with 3 and 10 Hz were conducted at 10 min intervals. Following the third set of control stimulations, kidneys were perfused for 60 min with Krebs solution containing an antagonist or vehicle. Stimulations were then obtained in the presence of the antagonist or vehicle, the latter serving as a time control. With chloroethylclonidine, kidneys were perfused with Krebs solution containing chloroethylclonidine (100 μ M) or vehicle for 20 min and then perfused with chloroethylclonidine-free Krebs solution for 40 min. Stimulations were then repeated.

Basal (non-stimulated) release of tritium was obtained by collecting the venous effluent for 20 s before each stimulation. The venous effluent was then collected during a 20 s stimulation and for an additional 20 s following stimulation (i.e. a total of 40 s). Stimulus-induced release was calculated by subtracting basal release from that due to stimulation. The basal efflux of tritium, and that due to stimulation, was counted in 0.5 ml samples of the venous effluent plus 4.5 ml of Ready Safe by liquid scintillation spectrometry with quench correction.

The effect of each treatment on stimulus-induced release of tritium is expressed as a percentage of the third set of control stimulations. This procedure was employed to obviate the decreases in tritium release with the first two stimulations.

Statistics

Data are expressed as the mean $\pm$ the standard error of the mean (s.e.mean) with 95% confidence limits. Each experiment was compared to its own control (100%) by a paired *t* test (two tailed). The effect of each antagonist was compared to the time control by an unpaired *t* test (two tailed). A probability of less than 0.05 was considered significant.

Drugs

Drugs were obtained from the following sources: prazosin hydrochloride (Pfizer Inc., Groton, CT, U.S.A.), 5-methyl-

urapidil and chloroethylclonidine (Research Biochemicals Inc., Natick, MA, U.S.A.), ATP and α - β -methylene ATP (Sigma Chemical Co., St. Louis, MO, U.S.A.), nitrendipine (Syntex Research, Palo Alto, CA, U.S.A.) and [3 H]-noradrenaline (New England Nuclear, Boston, MA, U.S.A.).

Stock solutions were prepared in deionized water with the exception of nitrendipine which was dissolved in 100% ethanol and 5-methyl-urapidil which was dissolved in hydrochloric acid (1 N) and then made up to the desired concentration by adding deionized water.

Results

Periarterial nerve stimulation

PNS produced frequency-dependent vasoconstrictor responses in the rat isolated perfused kidney (Figure 1). Mean vasoconstrictor responses ($\pm$ s.e.mean) to 3 and 10 Hz stimulations were 28.8 ± 5.5 mmHg and 185 ± 5.6 mmHg ($n = 23$) respectively. The mean stimulus-induced release of tritium in response to 3 and 10 Hz stimulations was 1839 ± 177 d.p.m. and 6850 ± 716 d.p.m. ($n = 16$) respectively. Basal, non-stimulated release of tritium was approximately 350 d.p.m. and was not affected by any of the drugs used.

Effects of antagonists on vasoconstrictor responses

Figure 2 shows the effect of α_1 -adrenoceptor antagonists and nitrendipine on PNS-induced vasoconstrictor responses. Vasoconstrictor responses did not vary significantly with time. However, vasoconstrictor responses were reduced significantly by the non-selective α_1 -adrenoceptor antagonist, prazosin (10 nM), the selective α_{1A} -adrenoceptor antagonist, 5-methyl-urapidil (30 nM), and the DHP calcium channel antagonist, nitrendipine (1 μ M). In contrast, vasoconstrictor responses were not altered by chloroethylclonidine (100 μ M).

Effect of antagonists on the stimulus-induced release of tritium

Figure 3 shows the effect of α_1 -adrenoceptor subtype selective antagonists and nitrendipine upon stimulus-induced release of tritium. Control experiments showed that the release of tritium in response to stimulations of 3 and 10 Hz was reduced significantly compared with control responses when elicited after a 60 min perfusion with Krebs solution alone. However, 5-methyl-urapidil (30 nM), chloroethylclonidine (100 μ M) and nitrendipine (1 μ M) failed to inhibit stimulus-induced release of tritium. In fact, stimulus-induced release of tritium in response to 10 Hz was increased significantly over the time control by both 5-methyl-urapidil and nitrendipine.

Figure 1 Representative vasoconstrictor responses to 3 and 10 Hz periaxillary nerve stimulations in rat isolated perfused kidney. Kidneys were stimulated at supramaximal voltage (≈ 70 V) until steady-state vasoconstrictor responses were obtained (1 ms pulse duration). Baseline perfusion pressure was approximately 50 mmHg.

Figure 2 The effect of α_1 -adrenoceptor antagonists and nitrendipine upon periarterial nerve stimulation-induced vasoconstrictor responses in the rat isolated perfused kidneys. Kidneys were perfused with antagonists for 60 min before testing. For chloroethylclonidine, kidneys were perfused with chloroethylclonidine for 20 min and then washed with Krebs solution free of chloroethylclonidine for 40 min before testing. Column (A), responses to 3 and 10 Hz before perfusion with vehicle or antagonist (set to 100%); column (B), vehicle; column (C), chloroethylclonidine (100 μ M); column (D), prazosin (10 nM); column (E), 5-methyl-urapidil (30 nM) and column (F), nitrendipine (1 μ M). Significantly different from column A (*) and B (†) at $P < 0.05$. Columns B to F represent the mean value ($\pm$ s.e.mean, vertical bars) obtained from 4 kidneys.

Figure 3 The effect of 5-methyl-urapidil, chloroethylclonidine and nitrendipine upon periarterial nerve stimulation-induced tritium release to 3 and 10 Hz in the rat isolated perfused kidney. Kidneys were perfused with antagonists for 60 min before testing. For chloroethylclonidine, kidneys were perfused with chloroethylclonidine for 20 min and then washed with Krebs solution free of chloroethylclonidine for 40 min before testing. Column (A), responses to 3 and 10 Hz before perfusion with vehicle or antagonist (set to 100%); column (B), vehicle; column (C), chloroethylclonidine (100 μ M); column (D), 5-methyl-urapidil (30 nM) and column (E), nitrendipine (1 μ M). Significantly different from column A (*) and B (†) at $P < 0.05$. Columns B to E represent the mean value ($\pm$ s.e.mean, vertical bar) obtained from 4 kidneys.

Discussion

The present study is the first to show innervation of α_{1A} -adrenoceptors. Furthermore, these receptors appear to be coupled to DHP-sensitive calcium channels.

Vasoconstrictor responses to PNS were inhibited by the non-selective α_1 -adrenoceptor antagonist, prazosin, at a concentration, 10 nM, which is approximately 30 times its equilibrium dissociation constant for α_1 -adrenoceptors in rat kidney (Clarke *et al.*, 1990; Blue *et al.*, 1991). This result

confirms previous reports that α_1 -adrenoceptors mediate responses to PNS in rat kidney (Charlton *et al.*, 1984; Schwartz & Malik, 1989).

α_1 -Adrenoceptor-mediated vasoconstriction was further characterized by use of the selective α_{1A} -adrenoceptor antagonist, 5-methyl-urapidil (Gross *et al.*, 1988) and the preferential α_{1B} -adrenoceptor alkylating agent, chloroethylclonidine (Johnson & Minneman, 1987). 5-Methyl-urapidil, at a concentration of 30 nM (approximately 30 times its equilibrium dissociation constant for α_{1A} -adrenoceptors in rat kidney; Clarke *et al.*, 1991), inhibited responses to PNS. This result suggests that α_{1A} -adrenoceptors mediate the response to PNS as 5-methyl-urapidil is approximately 70-fold selective for the α_{1A} -adrenoceptor subtype (Gross *et al.*, 1988; Boer *et al.*, 1989; Hanft & Gross, 1989). In addition, it should be noted that the concentrations of 5-methyl-urapidil and prazosin were selected to produce equal occupancy of α_{1A} -adrenoceptors (see above). Therefore, equal antagonism toward vasoconstrictor responses to PNS by 5-methyl-urapidil and prazosin again suggests the singular involvement of the α_{1A} -adrenoceptor subtype. Furthermore, the irreversible α_{1B} -adrenoceptor antagonist, chloroethylclonidine, failed to inhibit vasoconstrictor responses to PNS. Kidneys were exposed to chloroethylclonidine for 20 min. This concentration and treatment protocol for chloroethylclonidine has been shown previously to inactivate irreversibly α_{1B} -adrenoceptors in several tissues (Johnson & Minneman, 1987; Han *et al.*, 1987b; 1990; Minneman *et al.*, 1988), including rat kidney (Sharif *et al.*, 1991). Taken together, the results with 5-methyl-urapidil and chloroethylclonidine demonstrate that vasoconstrictor responses to PNS are mediated by α_{1A} -adrenoceptors.

The inhibition of PNS-induced vasoconstriction by 5-methyl-urapidil cannot be explained by prejunctional inhibition of noradrenaline release. Indeed, 5-methyl-urapidil increased tritium release at the 10 Hz stimulation. In this regard, evidence exists for inhibitory prejunctional α_1 -adrenoceptors in rat kidney (Rump & Majewski, 1987; Murphy & Majewski, 1991). The lack of an effect of chloroethylclonidine on PNS-induced release of tritium suggests that prejunctional α_1 -adrenoceptors may be of the α_{1A} -subtype.

Previous attempts to characterize α_1 -adrenoceptor-mediated responses to nerve stimulation have met with limited success. In field stimulated rat vas deferens, contractile responses to single stimuli have been reported to be mediated by α_{1B} -adrenoceptors (Mallard *et al.*, 1990; 1992) whereas both α_{1A} - and α_{1B} -adrenoceptors may be activated by trains of stimuli (Spriggs *et al.*, 1991; Mallard *et al.*, 1992). In these studies, however, α_{1A} - and α_{1B} -adrenoceptor-mediated responses were defined by sensitivity to inhibition by nifedipine and chloroethylclonidine respectively. Characterization of α_{1A} -adrenoceptors on the basis of nifedipine is dubious as α_{1A} -adrenoceptors have been shown to couple to calcium influx via both dihydropyridine-sensitive and dihydropyridine-insensitive calcium channels (Wilson & Minneman, 1990; Clarke *et al.*, 1991). Furthermore, α_{1B} -adrenoceptors may couple to DHP-sensitive channels (Reynolds & Dubyak, 1985; Clarke *et al.*, 1991).

In contrast to studies in rat vas deferens, Sulpizio & Hieble (1991) used the selective α_{1A} -adrenoceptor antagonists 5-methyl-urapidil and WB 4101 in addition to chloroethylclonidine to characterize biphasic contractile responses to field stimulation in the rat perfused caudal artery. However, these authors were unable to show selective inhibition of either phase. It was concluded that the α_1 -adrenoceptor involved did not fit the current classification scheme.

Previous studies using infused NA suggest that α_{1A} -adrenoceptors in rat kidney are coupled to DHP-sensitive and DHP-insensitive calcium channels (Clarke *et al.*, 1991; Blue *et al.*, 1991). The present study demonstrates that sympathetic nerve stimulation activates only those α_{1A} -adrenoceptors which are coupled to DHP-sensitive channels. This selectivity of the innervation suggests that the α_{1A} -adreno-

ceptors linked to DHP-insensitive calcium channels may lie outside vascular neuro-effector junctions and, as such, may subserve a role for circulating adrenaline. Diverse ways of gating calcium may underlie important differences in the physiological roles of neuronal versus hormonal regulation of renal vascular resistance.

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