ELSEVIER

European Journal of Pharmacology 280 (1995) 231–234

ejp

Short communication

4-Aminopyridine-induced phasic contractions in rat caudal epididymis are mediated through release of noradrenaline

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Received 19 January 1995; revised 25 April 1995; accepted 28 April 1995

Abstract

4-Aminopyridine, a K^+ channel blocker, evoked phasic contractions in the caudal duct of the rat epididymis. The 4-aminopyridine-induced contractile response was either inhibited or prevented by the α_1-adrenoceptor antagonists, prazosin ($IC_{50} = 2.7$ nM) and benoxathian ($IC_{50} = 14.6$ nM). Blockers (1 μM) of α_2-adrenoceptors and purinoceptors but not of β-adrenoceptors or muscarinic receptors caused a small but statistically significant reduction of the 4-aminopyridine-induced response. 4-Aminopyridine lost its ability to induce contractions after noradrenergic nerves had been destroyed by 6-hydroxydopamine. In addition, protriptyline and xylamine, blockers of noradrenaline uptake, also inhibited the 4-aminopyridine-induced contractile response. However, other putative K^+ channel blockers (tetraethylammonium ion, quinine, quinidine and glibenclamide) did not cause the muscle to contract. These findings demonstrate that the 4-aminopyridine-induced release of noradrenaline and adenosine 5′-triphosphate as co-transmitters results from membrane depolarization due to 4-aminopyridine blockade of K^+ channels in noradrenergic nerve terminals. The 4-aminopyridine-sensitive K^+ channels might thus play a physiological role in regulating the nerve membrane potential and neurotransmission in the rat caudal epididymis.

Keywords: 4-Aminopyridine; α-Adrenoceptor; Contraction, phasic; K^+ channel; Caudal epididymis, rat

1. Introduction

The caudal segment of the epididymis serves an important storage site for sperm transported from the testes to the vas deferens and is the only section in the epididymis where adrenergic innervation of smooth muscle is detected (Mitchell, 1935; Baumgarten et al., 1968). It is well known that the contractile response of the mammalian vas deferens and cauda epididymis to field nerve stimulation is primarily mediated by noradrenaline and adenosine 5′-triphosphate as co-transmitters released from the sympathetic nerve endings (Sneddon et al., 1984; Leedham and Pennefather, 1986; Allcorn et al., 1986; Ventura and Pennefather, 1991). The nerve stimulation-induced contraction can be attenuated by either prazosin, an α_1-adrenoceptor antagonist, or α,β-methylene ATP, a purinoceptor antagonist (Allcorn et al., 1986; Ventura and Pennefather, 1991). On the other hand, the release of noradrenaline is regulated by prejunctional α_2-adrenoceptors. There appear to be regional differences in prejunctional α_2-adrenoceptor distribution along the length of the male reproductive duct with caudal epididymis containing fewer receptor binding sites (Ventura and Pennefather, 1994). Spontaneous electrical and mechanical activity observed in many types of smooth muscle has been considered to be of myogenic or neurogenic origin (Janssen and Daniel, 1991; Suzuki et al., 1993). In contrast, the vas or epididymal smooth muscle is usually quiescent but contracts periodically under certain experimental conditions. The purpose of the present study was to investigate the possible role of K^+ channels in sympathetic neurotransmission in the rat caudal epididymis.

2. Materials and methods

2.1. Preparation

The Sprague-Dawley rats (~ 300 g) were killed by cervical dislocation and bled. The caudal portion of the

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0014-2999/95/$09.50
SSDI 0014-2999(95)00253-7

epididymis from each side was dissected out and placed on a dissecting dish. Segments of 4 mm in length were taken and mounted in an organ bath with one end attached to a Grass force transducer. The tissue was then incubated at $32 \pm 1°$ C in oxygenated (95% O_2 + 5% CO_2) Krebs-Henseleit solution of the following composition (mM): NaCl 119, KCl 4.2, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25, KH_2PO_4 1.2, EDTA 0.03, *d*-glucose 11.1, ascorbic acid 0.2. The preparation was equilibrated for 1 h under 300 mg resting tension.

2.2. Drugs

The following drugs and chemicals were used: 4-aminopyridine, prazosin, benoxathian, noradrenaline, propranolol, 6-hydroxydopamine, glibenclamide, clonidine (Sigma), α,β-methylene ATP (Calbiochem), tetraethylammonium ion, quinine, quinidine (Merck). Prazosin, clonidine and glibenclamide were dissolved in dimethyl sulfoxide (0.2%) and the rest in distilled water. 6-Hydroxydopamine was made up in 0.2 μM ascorbic acid immediately before use and kept on ice to minimize oxidation. Dimethyl sulfoxide alone did not cause muscle contraction and had no effect on the 4-aminopyridine-induced phasic activity in caudal epididymis.

2.3. Statistics

The phasic contraction of the rat caudal epididymis induced by 4-aminopyridine was expressed as the total number of twitches measured at 20-min intervals upon addition of the drug. The results were represented as the means ± S.E.M. of n experiments. A probability level of $P < 0.05$, using the Student t-test, was regarded as statistically significant.

3. Results

4-Aminopyridine evoked phasic contractions of the rat caudal epididymis in a concentration-related manner (0.5–5 mM). The 4-aminopyridine (> 2 mM)-induced contractile response normally consists of two components, a phasic component superimposed on an initial tonic component, and phasic activity declined with time. Removal of extracellular calcium completely prevented the muscle response to 4-aminopyridine. Other putative K^+ channel blockers such as tetraethylammonium ions (< 3 mM), quinine (1 mM), quinidine (3 mM) and glibenclamide (0.1 mM) did not evoke any contractile activity (data not shown). Fig. 1A shows that prazosin, a selective α_1-adrenoceptor antagonist, concentration-dependently decreased the effect of 4-aminopyridine. It can be seen that the inhibitory effect of prazosin is due to a reduction in both the number of

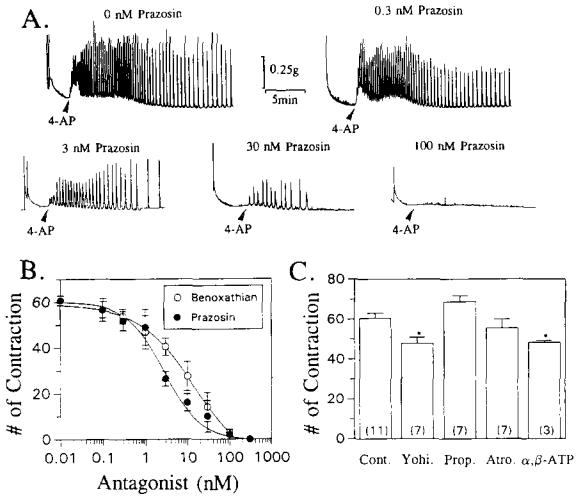

Fig. 1. (A) 4-Aminopyridine (4-AP, 3 mM) evoked phasic contractions in the isolated rat caudal epididymis. Prazosin inhibited the 4-aminopyridine-induced response in a concentration-dependent manner (0.1–100 nM) when added to the bath solution 10 min before application of 4-aminopyridine. (B) Mean logarithmic concentration-dependent inhibition of the 4-aminopyridine-induced phasic contractions by α_1-adrenoceptor antagonists: prazosin (●) and benoxathian (○). Points represent the mean of 8–10 experiments for prazosin and 6–8 experiments for benoxathian. Vertical bars represent the S.E.M. (C) Effects of the other antagonists on the 4-aminopyridine-induced response. Each antagonist at 1 μM was applied to the bath for 10 min before addition of 4-aminopyridine. The following antagonistic agents were used: yohimbine (Yohi., $n = 7$), propranolol (Prop., $n = 7$), atropine (Atro., $n = 7$) and α,β-methylene ATP (α,β-ATP, $n = 3$). The results were expressed as the means ± S.E.M. of the number of experiments indicated in parentheses at the foot of each column. *Significant difference ($P < 0.05$) from the control value.

contractions in a total of 20 min of measurement and in mean amplitude of each twitch. Benoxathian, another α_1-adrenoceptor antagonist for smooth muscle cells (Aboud et al., 1993) also inhibited the 4-aminopyridine-induced phasic contractions. Benoxathian was slightly less potent than prazosin. Fig. 2B summarizes the concentration-dependent inhibition of the 4-aminopyridine-induced contractile response by prazosin ($IC_{50} = 2.7$ nM) and benoxathian ($IC_{50} = 14.6$ nM). The present findings indirectly support a previous observation that 4-aminopyridine enhances the stimulus-evoked transmitter release in sympathetic nerve endings of guinea-pig and mouse vas deferens (Stjarne et al., 1990).

It has been demonstrated that the caudal epididymis also receives a cholinergic input in addition to noradrenergic innervation (El-Badawi and Schenk, 1967). It is likely that 4-aminopyridine might non-selectively block the K^+ channel and cause membrane depolarization in nerves supplying the caudal epididymal smooth muscle since both vas and caudal epididymis might have the same source of nerve supply. It was, therefore, worth testing this possibility by examining the

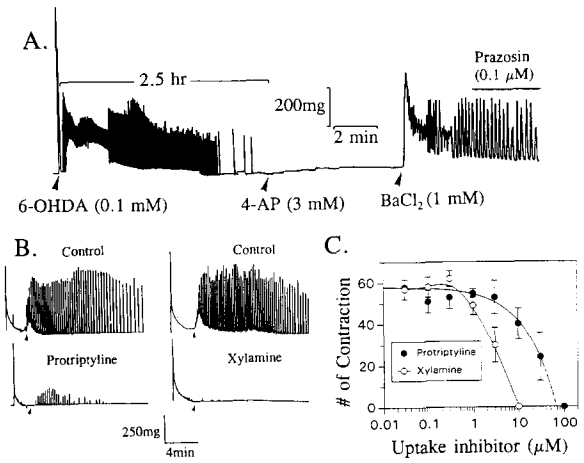

Fig. 2. (A) Effect of 6-hydroxydopamine on the 4-aminopyridine-induced phasic contractions. The preparation was incubated with 0.1 mM 6-hydroxydopamine for 2.5 h to impair sympathetic activity. 4-Aminopyridine failed to cause any muscle contraction after this treatment. In contrast, $BaCl_2$ at 1 mM evoked phasic activity which was resistant to prazosin (0.1 μM). (B) Protriptyline (30 μM) and xylamine (10 μM) inhibited the 4-aminopyridine-induced phasic contractions. Noradrenaline uptake inhibitors were incubated 10 min before the addition of 4-aminopyridine (3 mM) indicated by arrows. (C) Concentration-dependent inhibition of the number of contractions induced by 4-aminopyridine (3 mM) in the presence of protriptyline (●) and xylamine (○). Each point is mean of 4–7 experiments.

effects of antagonists for other postjunctional receptors. Atropine (1 μM) and propranolol (1 μM) did not alter the 4-aminopyridine-induced response. This rules out the involvement of cholinergic nerve and β-adrenoceptor in the 4-aminopyridine-induced contraction. On the other hand, yohimbine (1 μM) and α,β-methylene ATP (1 μM) produced a small but significant decrease in the total number of contractions evoked by 4-aminopyridine (Fig. 1C). These results indicate that 4-aminopyridine mainly targets the sympathetic nerve terminals and noradrenaline is the major transmitter causing contractions in the caudal epididymis.

Further supporting evidence comes from the experiments using 6-hydroxydopamine to impair intrinsic sympathetic nerve fibers. 6-Hydroxydopamine itself, at 0.1 mM, induced phasic activity which lasted for about 2 h. These contractions were markedly suppressed by 0.1 μM prazosin (data not shown), suggesting that 6-hydroxydopamine could initially release noradrenaline in the rat caudal epididymis. Cessation of contractile activity indicated that 6-hydroxydopamine had effectively impaired noradrenergic transmission. After 6-hydroxydopamine treatment, 4-aminopyridine failed to evoke any contractions. Fig. 2A shows a representative record of seven similar experiments. In contrast, $BaCl_2$, a non-selective K^+ channel blocker, was still able to induce phasic activity in the same preparation. However, the $BaCl_2$-induced response was totally insensitive to either prazosin (0.1 μM) or benoxathian (0.1 μM, data not shown).

Both protriptyline and xylamine have been found to inhibit noradrenaline uptake into central and peripheral noradrenergic neurones (Ransom et al., 1985). Fig. 2B shows the original traces in the absence and presence of noradrenaline uptake blockers. Fig. 2C shows the concentration-dependent inhibition of the 4-aminopyridine-induced phasic contractions. These results indicate that junctional noradrenaline might have been increased due to blockade of its uptake and this increased noradrenaline might act on prejunctional α_2-adrenoceptors to inhibit the 4-aminopyridine-induced noradrenaline release. However, low concentrations of noradrenaline or clonidine (10 nM) did not affect the 4-aminopyridine-induced response (data not shown). This is in agreement with the recent finding that nerve stimulation-induced contractions of rat caudal epididymis are unaffected by the α_2-adrenoceptor agonists, clonidine and xylazine (Ventura and Pennefather, 1992). It is possible that some noradrenaline uptake blockers might have an additional effect distal to inhibition of noradrenaline uptake. Indeed, protriptyline (30 μM) and xylamine (10 μM) completely prevented the noradrenaline-induced contractions (n = 4, data not shown), indicating that they might antagonize the effect of noradrenaline on smooth muscle.

4. Discussion

The present results clearly demonstrate that the K^+ channels in sympathetic nerve fibers supplying the caudal epididymis are the primary sites for the action of 4-aminopyridine. These channels are apparently open at the resting membrane potential and their blockade induced membrane depolarization which in turn probably activated the voltage-sensitive calcium channel. Noradrenaline as well as adenosine 5′-triphosphate were consequently secreted from nerve terminals and acted on postjunctional α-adrenoceptors and purinoceptors to cause phasic contractions. Firstly, selective α_1-adrenoceptor antagonists, prazosin and benoxathian, potently inhibited the 4-aminopyridine-induced contractions. In addition, an α_2-adrenoceptor antagonist, yohimbine, and a purinoceptor antagonist, α,β-methylene ATP, attenuated the 4-aminopyridine-induced response in the caudal epididymis. Secondly, 4-aminopyridine failed to initiate any contractions after the sympathetic nerve terminals had been chemically destroyed with 6-hydroxydopamine. However, atropine, a muscarinic receptor antagonist, did not affect the 4-aminopyridine-induced phasic contraction. These findings indicate that 4-aminopyridine mainly interacts with noradrenergic nerves but not with cholinergic nerves that have been reported to also innervate the

smooth muscle of the rat caudal epididymis (El-Badawi and Schenk, 1967). Similarly, atropine was found to cause only a slight inhibition of the nerve stimulation-induced contraction in the rat caudal epididymis (Ventura and Pennefather, 1991).

The smooth muscle fibers of the rat caudal epididymis and vas deferens are densely innervated by noradrenergic nerve terminals. Nerve stimulation usually causes biphasic contraction in the mammalian vas deferens, the adrenergic component inhibited by prazosin and the purinergic component inhibited by α,β-methylene ATP or suramin (McGrath, 1978). However, a monophasic contraction is seen in the rat caudal epididymis in response to nerve stimulation, but it is only partially inhibited by prazosin and α,β-methylene ATP (Ventura and Pennefather, 1991). These results provide pharmacological evidence that noradrenaline and adenosine 5′-triphosphate can be released from the sympathetic nerve fibers as co-transmitters in these preparations. A similar conclusion can be drawn from the present work, that noradrenaline and adenosine 5′-triphosphate might not be secreted independently from the nerve terminals in response to 4-aminopyridine in the rat caudal epididymis. However, the 4-aminopyridine-induced contraction seems to differ from the nerve stimulation-induced contraction in one major aspect, i.e. the relative proportion of noradrenaline and adenosine 5′-triphosphate co-released might be different with these two types of stimuli. Prazosin at a maximal concentration of 0.1 μM caused 45% of inhibition of the mean height of monophasic contraction in the rat caudal epididymis in response to nerve stimulation whereas α,β-methylene ATP caused a greater reduction (64%) of contraction (Ventura and Pennefather, 1991). However, prazosin at 0.1 μM completely prevented the 4-aminopyridine-induced phasic contraction of the same preparation in the present experiments, and α,β-methylene ATP only reduced the mean number of contractions measured at 20-min intervals to 8% of the control. These results indicate that co-transmission can be modulated to control the relative amount of co-transmitters released from the sympathetic nerve, depending on the nature of the stimulation.

In summary, this study has provided novel pharmacological evidence to demonstrate that 4-aminopyridine blocks the K^+ channel in the sympathetic nerve terminals. Calcium entry through the depolarized nerve membrane might trigger secretion of noradrenaline as the principal transmitter which activates the postjunctional α-adrenoceptor to induce phasic contractions. The 4-aminopyridine-sensitive K^+ channels might be active at rest and have a physiological role in regulation of nerve membrane potential and neurotransmission in the rat caudal epididymis.

Acknowledgements

The author wishes to thank Professor P.Y.D. Wong for critically reading the manuscript and Mr. C.-W. Lau for his excellent technical assistance. This work was supported by a CUHK Direct Grant (A/C 220403490).

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