ELSEVIER

European Journal of Pharmacology 287 (1995) 287–293

ejp

Prejunctional actions of K^+ channel blockers in rat vas deferens

James R. Docherty *, Garrett Brady

Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland

Received 8 June 1995; revised 2 August 1995; accepted 8 August 1995

Abstract

In studies of isometric contractions in prostatic portions of rat vas deferens evoked by single pulse electrical stimulation, the K^+ channel blockers 4-aminopyridine, tetraethylammonium and charybdotoxin, but not apamin, significantly reduced the prejunctional inhibitory potency and the maximum inhibitory effect of the α_2-adrenoceptor agonist xylazine. The protein kinase C activator phorbol dibutyrate had similar effects to 4-aminopyridine against xylazine. However, 4-aminopyridine, tetraethylammonium, charybdotoxin and phorbol dibutyrate, but not apamin, significantly increased the magnitude of the isometric contraction to a single stimulus. 4-Aminopyridine and phorbol dibutyrate significantly reduced, while tetraethylammonium did not affect, isometric contractions to noradrenaline, and 4-aminopyridine failed to affect contractions to α,β-methylene-ATP, so that the effects of these agents on the isometric contraction to a single stimulus were presumably by a prejunctional action. The Ca^{2+} entry facilitator Bay K 8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methylester) increased stimulation-evoked contractions by a postjunctional action and reduced the inhibitory effects of xylazine. When the isometric contraction following 4-aminopyridine was reduced by decreasing the stimulation voltage or by reducing the Ca^{2+} concentration from 2.5 to 0.9 mM, 4-aminopyridine significantly reduced the potency of xylazine. However, tetraethylammonium and Bay K 8644 failed to affect the inhibitory potency of xylazine in low Ca^{2+}. It is concluded that the K^+ channel blocker 4-aminopyridine reduces the prejunctional inhibitory potency of xylazine, and this action is independent of increased neurotransmitter release. These results suggest that prejunctional α_2-adrenoceptor-mediated inhibition in rat vas deferens involves K^+ channels sensitive to block by 4-aminopyridine.

Keywords: Vas deferens, rat; α_2-Adrenoceptor; Xylazine; 4-Aminopyridine; Tetraethylammonium; Charybdotoxin; Phorbol dibutyrate; Bay K 8644

1. Introduction

α_2-Adrenoceptors mediate a number of physiological functions including prejunctional control of neurotransmission. α_2-Adrenoceptors have been subdivided into at least four subtypes, α_{2A}-, α_{2B}-, α_{2C}- and α_{2D}-adrenoceptors, based on ligand binding and molecular cloning studies (Bylund, 1992; Lorenz et al., 1990), although the α_{2D}-adrenoceptor of rat submandibular gland may be a species homologue of the human α_{2A}-adrenoceptor, reducing the number of genes coding for α_2-adrenoceptors to three (Lanier et al., 1991; Harrison et al., 1991; Smith and Docherty, 1992), so that the term $\alpha_{2A/D}$-adrenoceptor could be used to describe these homologues.

The functional prejunctional α_2-adrenoceptors in rat vas deferens closely resemble the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland (Connaughton and Docherty, 1990; Smith et al., 1992; Smith and Docherty, 1992; see also Smith et al., 1995), and is coupled to a G-protein which is sensitive to alkylation by *N*-ethyl-maleimide (Browne et al., 1994), but insensitive to pertussis toxin (Docherty, 1988). The signal transduction steps involved in this inhibition have not been fully elucidated. Recent evidence suggests that opening of K^+ channels to hyperpolarise the nerve ending may be the mechanism of action (Allgaier et al., 1993).

The object of this study is to look at the influence of K^+ channel blockers and other prejunctional modulators on α_2-adrenoceptor-mediated inhibition using a

* Corresponding author. Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland. Tel.: (353) 1-4780 200; fax: (353) 1-4780 018.

0014-2999/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0014-2999(95)00495-5

convenient model system: the rat vas deferens isometric twitch response. The agents employed in this study were: tetraethylammonium and 4-aminopyridine (non-selective K^+ channel blockers), apamin (K^+_{ATP} blocker), charybdotoxin (K^+_{Ca} channel blocker) (see Castle et al., 1989), phorbol dibutyrate (stimulates protein kinase C), forskolin (stimulates adenylate cyclase), isobutylmethylxanthine (inhibits phosphodiesterase), Bay K 8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methylester) (facilitates Ca^{2+} entry into smooth muscle via dihydropyridine-sensitive channels; see Hyland et al., 1984).

2. Materials and methods

Male Wistar rats (200–300 g) were obtained from Trinity College Dublin, and vas deferens was employed as outlined below.

2.1. Rat vas deferens: electrically evoked contractions

Prostatic portions of rat vas deferens were placed between platinum electrodes and attached to myograph transducers under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119; $NaHCO_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.0; EDTA 0.03, ascorbic acid 0.28. In experiments carried out in low Ca^{2+}, Ca^{2+} was reduced to 0.9 mM, replaced by Mg^{2+}. Tissues were stimulated every 5 min with a single stimulus (0.5 ms pulses, 70 V supramaximal voltage, or 10 V submaximal voltage) to produce isometric contractions, and, once consistent control responses had been obtained in the presence of test drugs or vehicle, a cumulative concentration-response curve was obtained to the α_2-adrenoceptor agonist xylazine, administered in 0.5 log unit increments beginning with 10 nM. In some experiments, control isometric responses were obtained to supramaximal stimulation in normal Ca^{2+} before reducing stimulation voltage or Ca^{2+} concentration.

2.2. Exposure to K^+ channel blockers

In experiments examining the actions of K^+ channel blockers and other agents, tissues were exposed to 4-aminopyridine, tetraethylammonium, charybdotoxin, apamin, phorbol dibutyrate, forskolin, isobutylmethylxanthine, Bay K 8644 or vehicle for 15 min before beginning the addition of xylazine.

2.3. Rat vas deferens: exogenous noradrenaline and α,β-methylene-ATP

In investigations of effects of test antagonists on contractions produced by exogenous noradrenaline, tissues were pre-exposed to noradrenaline (10 μM), and following 60 min washout, for the last 15 min of which the tissue was exposed to antagonist or vehicle, a single noradrenaline concentration response-curve was obtained per tissue.

In investigations of effects of 4-aminopyridine on contractions produced by exogenous α,β-methylene-ATP, following 60 min equilibration, for the last 15 min of which the tissue was exposed to 4-aminopyridine or vehicle, tissues were exposed to a single concentration of α,β-methylene-ATP (10 μM). This protocol was employed to prevent desensitization.

2.4. Drugs

4-Aminopyridine (Sigma, Poole, UK); apamin (Research Biochemicals International, USA); ATP (Sigma); Bay K 8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methylester: Research Biochemicals); charybdotoxin (Sigma); isobutylmethylxanthine (IBMX: Sigma); forskolin (Sigma); α,β-methylene-ATP (Sigma); noradrenaline bitartrate (RBI); phorbol 12,13-dibutyrate (Sigma); tetraethylammonium (Sigma); xylazine hydrochloride (gift: Bayer, Ireland).

Drugs were dissolved in distilled water, except for phorbol dibutyrate and Bay K 8644 (100% ethanol), and dilutions were made up in distilled water.

2.5. Statistics

Values are arithmetic means ± S.E.M. Xylazine or noradrenaline pD_2, maximum inhibition, or maximum

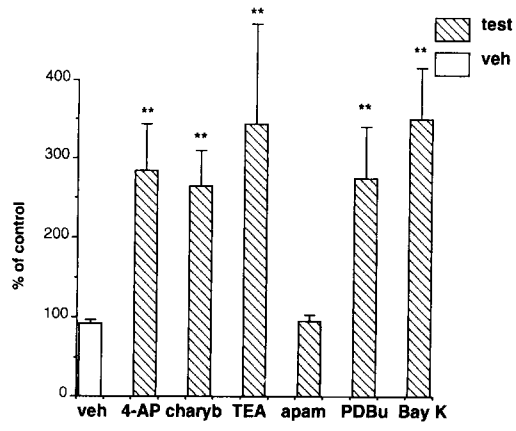

Fig. 1. Effects of vehicle, 4-aminopyridine (100 μM), charybdotoxin (0.1 μM), tetraethylammonium (1 mM), apamin (0.1 μM), phorbol dibutyrate (2 μM) and Bay K 8644 (10 μM) on the isometric contraction produced by single pulse electrical stimulation in prostatic portions of rat vas deferens. Responses in the presence of test agent are expressed as a percentage of the response prior to test agent (% of control). For convenience, all vehicle experiments are combined ($n = 37$). Vertical bars represent S.E.M. from at least four experiments.

contraction in the presence of test agents were compared with responses in the presence of vehicle using a Student's *t*-test for unpaired or paired data, where appropriate, and by Analysis of Variance (ANOVAR).

3. Results

3.1. Prostatic portions of rat vas deferens: nerve-mediated responses

In prostatic portions, single pulse electrical stimulation produced a contraction of 0.81 ± 0.09 g ($n = 37$), consisting mainly of the first non-noradrenergic phase. Vehicle administration had no significant effect on the contractile response to a single stimulus (92.5 ± 5.0% of control, $n = 37$) (Fig. 1). Pre-exposure to 4-aminopyridine (100 μM), tetraethylammonium (1 mM), charybdotoxin (0.1 μM) or phorbol dibutyrate (2 μM) significantly increased the electrically evoked isometric contraction (Fig. 1). Apamin (0.1 μM) produced no significant effect, but forskolin and isobutylmethylxanthine abolished contractions to nerve stimulation and were not examined further. The action to increase stimulation-evoked contractions was prejunctional in origin (see below). The Ca^{2+} entry facilitator Bay K 8644 (10 μM) significantly increased the electrical stimulation-evoked isometric contraction by a postjunctional action, as demonstrated by the spontaneous contractions occurring in the presence of Bay K 8644.

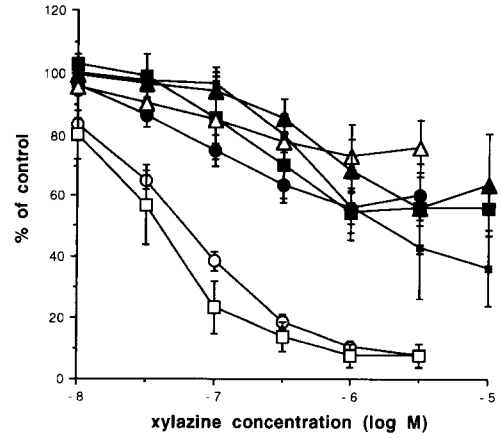

Fig. 2. Inhibitory effects of the α_2-adrenoceptor agonist xylazine following pre-exposure to vehicle (○), 4-aminopyridine (100 μM) (●), tetraethylammonium (1 mM) (△), charybdotoxin (0.1 μM) (■), apamin (0.1 μM) (□), phorbol dibutyrate (2 μM) (▲) and Bay K 8644 (×) on the isometric contraction produced by single pulse electrical stimulation in prostatic portions of rat vas deferens. Responses in the presence of xylazine are expressed as a percentage of the response in the absence of xylazine. Vertical bars represent S.E.M. from at least four experiments.

Xylazine produced a concentration-dependent inhibition of the stimulation-evoked contraction, with an pD_2 ($-\log IC_{50}$) of 7.34 ± 0.06, and a maximum inhibition of 92.2 ± 2.0% ($n = 37$) (see Table 1 and Fig. 2). 4-Aminopyridine, charybdotoxin, tetraethylammonium, phorbol dibutyrate and Bay K 8644, but not apamin, significantly reduced the prejunctional inhibitory effects of the α_2-adrenoceptor agonist xylazine, both in terms of potency and maximum inhibitory response (Table 1 and Fig. 2).

In further experiments, the contractile response to a single stimulus was reduced by reducing the stimulation voltage to 10 V, or by reducing the extracellular Ca^{2+} to 0.9 mM. When the stimulation voltage was reduced to 10 V, the contractile response in the presence of 4-aminopyridine was reduced to that of vehicle experiments at 70 V (106.7 ± 14.2% of response at 70 V stimulation voltage in the absence of 4-aminopyridine, $n = 5$; in absolute terms: 0.91 ± 0.32 g). When the Ca^{2+} concentration was reduced to 0.9 mM, the response to a single electrical stimulus was reduced to a very small contraction or abolished (mean of 0.10 ± 0.03 g, $n = 18$), making it difficult to examine the effects of xylazine. However, 4-aminopyridine (100 μM), tetraethylammonium (1 mM) or Bay K 8644 (10 μM) significantly increased the stimulation-evoked contractions as shown in absolute terms as g tension in Fig. 3 (percentage increases would be meaningless given the small or absent contraction prior to these agents). Phorbol dibutyrate produced variable effects on the isometric

Table 1
Effects of the test agents on the potency and maximum effect of xylazine at inhibiting isometric contraction to a single stimulus in prostatic portions of rat vas deferens in normal or low Ca^{2+}

Test agent	Xylazine potency (pD2, −logM)	Xylazine maximum (% inhibition)
Normal Ca^{2+}		
Vehicle ($n = 37$)	7.34 ± 0.06	92.2 ± 2.0%
Apamin ($n = 4$)	7.46 ± 0.16	92.4 ± 3.8
4-Aminopyridine ($n = 11$)	6.96 ± 0.11 [b]	45.3 ± 4.2 [c]
4-Aminopyridine low V. ($n = 7$)	6.72 ± 0.08 [b]	60.7 ± 5.0 [b]
Charybdotoxin ($n = 6$)	6.72 ± 0.20 [a]	50.8 ± 5.8 [b]
Tetraethylammonium ($n = 9$)	(7.18 ± 0.29)	31.7 ± 11.3 [b]
Phorbol dibutyrate ($n = 5$)	6.28 ± 0.26 [c]	51.5 ± 11.2 [c]
Bay K 8644 ($n = 6$)	6.09 ± 0.18 [c]	73.9 ± 8.1 [c]
Low Ca^{2+}		
4-Aminopyridine ($n = 15$)	6.84 ± 0.06 [c]	88.4 ± 2.6
Tetraethylammonium ($n = 13$)	7.32 ± 0.12	91.1 ± 2.6
Phorbol dibutyrate ($n = 7$)	6.83 ± 0.12 [a]	87.8 ± 4.1
Bay K 8644 ($n = 8$)	7.21 ± 0.14	80.8 ± 5.3 [a]

Values are xylazine pD_2 ($-\log IC_{50}$) and maximum inhibition of stimulation evoked contraction, mean ± S.E.M., with n, the number of experiments. Xylazine pD_2 values were not calculated when the xylazine maximum inhibition was too small to allow meaningful calculation. For tetraethylammonium in normal Ca^{2+}, a pD_2 value is presented for the five from nine experiments in which xylazine produced an inhibition. [a,b,c] Denote responses significantly different from effects of vehicle (Student's *t*-test; [a] $P < 0.05$; [b] $P < 0.01$, [c] $P < 0.001$).

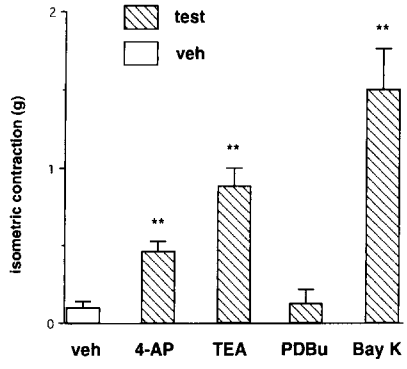

Fig. 3. Effects of vehicle, 4-aminopyridine (100 μM), tetraethylammonium (1 mM), phorbol dibutyrate (2 μM) and Bay K 8644 (10 μM) on the isometric contraction produced by single pulse electrical stimulation in prostatic portions of rat vas deferens with low Ca^{2+}. Responses in the presence of test agent or vehicle are expressed as absolute tension in g. For convenience, all vehicle experiments are combined ($n = 25$). Vertical bars represent S.E.M. from at least seven experiments.

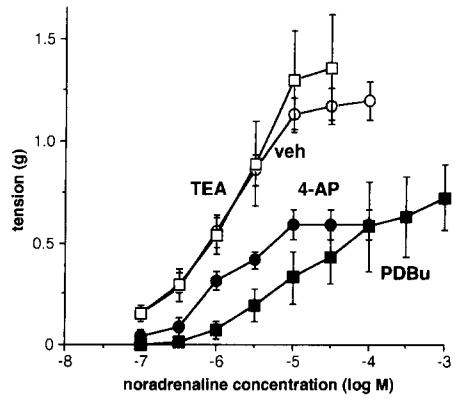

Fig. 5. Effects of vehicle (○), 4-aminopyridine (100 μM) (●), tetraethylammonium (1 mM) (□) and phorbol dibutyrate (2 μM) (■) on isometric contractions produced by noradrenaline in prostatic portions of rat vas deferens. Responses in the presence of test agent or vehicle are expressed as the absolute tension in g. Vertical bars represent S.E.M. from at least four experiments.

response to a single stimulus, sometimes failing to increase the response (Fig. 3): effects of phorbol dibutyrate on the response to xylazine are calculated from those experiments where it increased the response to a single stimulus (7 from 12 experiments). The response to a single stimulus in low Ca^{2+} in the presence of Bay K 8644 was significantly greater than the response in the presence of tetraethylammonium, which was significantly greater than in the presence of 4-aminopyridine (Fig. 3).

4-Aminopyridine (100 μM) still significantly reduced the potency of xylazine when the magnitude of the stimulation evoked contraction was reduced by reducing stimulation voltage or by reducing the extracellular Ca^{2+} (Fig. 4 and Table 1). In low Ca^{2+}, phorbol dibutyrate still significantly reduced the inhibitory potency of xylazine. When the extracellular Ca^{2+} was reduced, tetraethylammonium (1 mM) or Bay K 8644 (10 μM) did not significantly affect the potency of xylazine as compared to vehicle experiments in normal Ca^{2+} (Table 1). Bay K 8644 did significantly reduce the maximum inhibitory response to xylazine, presumably by a postjunctional action as spontaneous contractions occurred in the presence of Bay K 8644 even in low Ca^{2+} (Table 1).

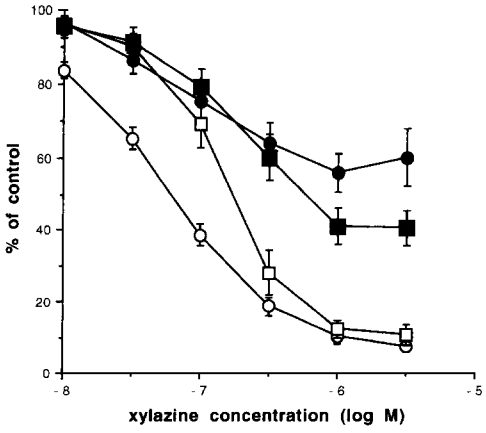

Fig. 4. Inhibitory effects of the α_2-adrenoceptor agonist xylazine following pre-exposure to vehicle (○), 4-aminopyridine (100 μM) (●), 4-aminopyridine (100 μM), low voltage (■) and 4-aminopyridine (100 μM), low Ca^{2+} (□), on the isometric contraction produced by single pulse electrical stimulation in prostatic portions of rat vas deferens. Responses in the presence of xylazine are expressed as a percentage of the response in the absence of xylazine. Vertical bars represent S.E.M. from at least seven experiments.

3.2. *Rat vas deferens: responses to exogenous noradrenaline and α,β-methylene-ATP*

In vehicle experiments, noradrenaline produced contractions consisting of intermittent spikes superimposed on a tonic contraction with a pD_2 of 5.91 ± 0.10 and a maximum contraction (measured as the maximum height of intermittent spikes) of 1.20 ± 0.10 g (mean ± S.E.M., $n = 10$). 4-Aminopyridine (100 μM) or phorbol dibutyrate (2 μM) significantly reduced the maximum contraction to noradrenaline ($P < 0.05$) (Fig. 5). Phorbol dibutyrate significantly reduced the potency of noradrenaline (pD_2 of 4.72 ± 0.42, $n = 4$, $P < 0.01$), but 4-aminopyridine failed to affect the potency of noradrenaline (5.76 ± 0.16). In tissues pretreated with tetraethylammonium (1 mM), there was no significant change in noradrenaline potency or maximum response (Fig. 5). In tissues treated with Bay K 8644 (10 μM), spontaneous contractions in the absence of noradrenaline were similar to contractions in the presence of noradrenaline (Bay K 8644 alone: 2.00 ± 0.56 g, $n = 6$).

ATP (up to 300 μM) produced very small contractions which were not affected by 4-aminopyridine, so that α,β-methylene-ATP was chosen as the agonist to test the effects of 4-aminopyridine on purinergic responses. α,β-Methylene-ATP (10 μM) produced a transient contraction of 0.57 ± 0.05 g ($n = 5$) following vehicle, and a contraction of 0.73 ± 0.15 g ($n = 5$) following 4-aminopyridine (100 μM) (no significant difference).

4. Discussion

We have investigated the effects of K^+ channel blockers on the prejunctional inhibitory potency of xylazine in rat vas deferens. Before discussing the main findings of this study, it is necessary to discuss the preparation employed: the rat vas deferens isometric contraction.

4.1. Electrical stimulation-evoked isometric contractions in rat vas deferens

In rat whole vas deferens, the electrical stimulation-evoked contraction to a single stimulus consists of a biphasic response, the first phase of which is non-adrenoceptor-mediated (probably ATP) and predominates in prostatic portions, and the second phase of which is α_1-adrenoceptor mediated, and predominates in the epididymal portion (see Brown et al., 1983; Aboud et al., 1993). In prostatic portions, the twitch response to a single stimulus is mainly non-noradrenergic, and can be inhibited by α_2-adrenoceptor agonists by a prejunctional action, whereas α_1-adrenoceptor agonists produce direct contractions and increase nerve stimulation-evoked contractions by a postjunctional action (see Brown et al., 1983).

4.2. Effects of test agents on stimulation-evoked contractions

In this study, 4-aminopyridine, tetraethylammonium and charybdotoxin significantly increased the nerve stimulation-evoked contraction, which, at least in the case of tetraethylammonium and 4-aminopyridine, was not due to a postjunctional action, since contractions to exogenous noradrenaline were either unaffected or reduced by tetraethylammonium and 4-aminopyridine, respectively. Contractions to exogenous α,β-methylene-ATP were also unaffected by 4-aminopyridine, confirming that purinergic responses are not selectively increased by this agent. It is well known that K^+ channel blockers such as 4-aminopyridine, tetraethylammonium and charybdotoxin potentiate the stimulation-evoked release of neurotransmitters such as noradrenaline (Johns et al., 1976; Allgaier et al., 1993), acetylcholine (Molgo et al., 1977) and nitric oxide (De Man et al., 1993). This action may be by prolongation of the action potential duration by block of K^+ channels, delaying repolarisation, increasing the availability of Ca^{2+}. Phorbol dibutyrate also increased stimulation-evoked contractions by a prejunctional action, presumably due to stimulation of protein kinase C (see Musgrave and Majewski, 1989; Bucher et al., 1991). By comparison, Bay K 8644 increased stimulation-evoked contractions by a postjunctional action, as demonstrated by its action to produce spontaneous contractions and by its reported lack of prejunctional actions (Hyland et al., 1984). Hence, the actions of Bay K 8644 can be used to determine the influence of an increased postjunctional contraction on the observed prejunctional effects of xylazine.

4.3. Effects of test agents against the inhibitory actions of xylazine

4-Aminopyridine, tetraethylammonium, charybdotoxin and phorbol dibutyrate reduced the potency of xylazine by a factor of 3–5 in prostatic portions of rat vas deferens, and reduced the maximum inhibition. However, Bay K 8644, acting postjunctionally to increase stimulation-evoked contractions, also significantly reduced the potency and maximum inhibition by xylazine. Admittedly, in the presence of Bay K 8644, the response to a single stimulus following xylazine (0.1 μM and above) tended towards a slow contraction rather than the rapid contraction found in the absence of xylazine. Hence, even though responses in the presence of Bay K 8644 and subsequent xylazine clearly differed from other responses, it was still important to rule out an influence of the magnitude of the isometric contraction on the effects of xylazine in the case of 4-aminopyridine and tetraethylammonium. This was done in two ways, reduction of stimulation voltage and reduction of Ca^{2+} concentration.

4.4. Experiments in low Ca^{2+}

Interpretation of results is complicated by the fact that all K^+ channel blockers except apamin significantly increased the stimulation-evoked contraction and reduced the inhibitory actions of xylazine. Hence, the actions of 4-aminopyridine, tetraethylammonium and charybdotoxin in reducing the inhibitory effects of xylazine may also have been indirectly by prolonging the action potential duration to allow greater Ca^{2+} entry, making it more difficult to produce inhibition. In an attempt to rule out an influence of the increased magnitude of the isometric contraction, further experiments were carried out, in which the response to a single stimulus following 4-aminopyridine was reduced to a level found in vehicle experiments by reducing the

stimulus voltage or by reducing the extracellular Ca^{2+} to 0.9 mM. Reducing the stimulus voltage may simply reduce the number of nerve terminals depolarised and demonstrate whether the magnitude of the postjunctional contraction influence the response to xylazine. Reducing the extracellular Ca^{2+} is likely to mean that Ca^{2+} availability in the presence of K^+ channel blockers is reduced towards that in vehicle experiments in normal Ca^{2+}. Under circumstances of reduced stimulus voltage or low Ca^{2+}, 4-aminopyridine still significantly reduced the potency of xylazine but had little effect on the maximum inhibition. In contrast, even though the response to a single stimulus in low Ca^{2+} was significantly greater in the presence of tetraethylammonium or Bay K 8644 than in the presence of 4-aminopyridine, neither tetraethylammonium nor Bay K 8644 significantly altered the potency of xylazine in low Ca^{2+} although Bay K 8644 did slightly but significantly reduce the maximum inhibition, presumably by a postjunctional action since spontaneous activity occurred in the presence of Bay K 8644 even in low Ca^{2+}.

These results suggest that, independent of increasing stimulation-evoked contractions, 4-aminopyridine modulates α_2-adrenoceptor function, suggesting that α_2-adrenoceptor-mediated inhibition involves opening of K^+ channels sensitive to 4-aminopyridine.

In the present study, phorbol dibutyrate reduced the prejunctional inhibitory actions of xylazine even when the Ca^{2+} levels were reduced, suggesting that phorbol dibutyrate interferes with α_2-adrenoceptor-mediated inhibition even when the effect on transmitter release is taken into account. Admittedly, stimulation-evoked contractions in low Ca^{2+} remained small after addition of phorbol dibutyrate, presumably due to postjunctional inhibitory actions of phorbol dibutyrate. These results contrast with previous reports that stimulation of protein kinase C does not affect α_2-adrenoceptor-mediated inhibition in rat tail artery (Bucher et al., 1991).

4.5. Comparison with other studies

In previous studies of rat vas deferens investigating G-proteins involved in prejunctional α_2-adrenoceptor function, *N*-ethyl-maleimide reduced the potency of xylazine by a factor of 3–5, presumably by inactivation of G-proteins involved in prejunctional α_2-adrenoceptor function (Browne et al., 1994), but pertussis toxin failed to modulate α_2-adrenoceptor function (Docherty, 1988; see also Weber, 1989; Murphy et al., 1992). Hence, these responses can be described as *N*-ethyl-maleimide-sensitive, pertussis toxin-insensitive, and this may reflect the sensitivity of a G-protein involved (in contrast, note that prejunctional α_2-adrenoceptor-mediated responses in rabbit hippocampus are *N*-ethyl-maleimide-sensitive and pertussis toxin-sensitive: Allgaier et al., 1985,1986). However, assuming that *N*-ethyl-maleimide acts at a G-protein, the fact that *N*-ethyl-maleimide shifted the potency of xylazine without reducing the maximum inhibition might suggest that there is spare G-protein capacity allowing xylazine to produce a maximum effect even though a proportion of the G-proteins has been inactivated. These findings may be relevant to the present investigation of the involvement of K^+ channels in that they demonstrate that there is spare capacity in the α_2-adrenoceptor system beyond the receptor level: hence, 4-aminopyridine shifted potency but not maximum response to xyazine in low Ca^{2+}. Previous studies have suggested species differences in the susceptibility of prejunctional α_2-adrenoceptor-mediated inhibition to K^+ channel blockers: interaction in rat but not rabbit hippocampus (Allgaier et al., 1993). tetraethylammonium has also been reported not to affect the prejunctional inhibitory actions of histamine in low Ca^{2+} (Schlicker et al., 1994).

In conclusion, certain K^+ channel blockers increased neurotransmitter release and decreased prejunctional α_2-adrenoceptor-mediated inhibition. When the influence of increased neurotransmitter release was eliminated by lowering the Ca^{2+} concentration, 4-aminopyridine and phorbol dibutyrate but not tetraethylammonium decreased the prejunctional α_2-adrenoceptor-mediated inhibition. This may suggest that α_2-adrenoceptor mediated inhibition in rat vas deferens involves opening of K^+ channels sensitive to 4-aminopyridine.

Acknowledgements

Supported by The Health Research Board (Ireland), the Irish Heart Foundation, Royal College of Surgeons in Ireland and EureCa (EU project BMH1-CT-94-7274).

References

Aboud, R., M. Shafii and J.R. Docherty, 1993, Investigation of the subtypes of α_1-adrenoceptor mediating contraction of rat aorta, vas deferens and spleen, Br. J. Pharmacol. 109, 80.

Allgaier, C., T.J. Feuerstein, R. Jackisch and G. Hertting, 1985, Islet-activating protein (pertussis toxin) diminishes α_2-adrenoceptor mediated effects on noradrenaline release, Naunyn-Schmied. Arch. Pharmacol. 331, 235.

Allgaier, C., T.J. Feuerstein and G. Hertting, 1986, *N*-Ethyl-maleimide (NEM) diminishes α_2-adrenoceptor mediated effects on noradrenaline release, Naunyn-Schmied. Arch. Pharmacol. 333, 104.

Allgaier, C., H. Repp and G. Hertting, 1993, Effect of K^+ channel blockers on the α_2-adrenoceptor-coupled regulaton of electrically evoked noradrenaline release in hippocampus, Naunyn-Schmied. Arch. Pharmacol. 347, 14.

Brown, D.A., J.R. Docherty, A.M. French, A. MacDonald, J.C. McGrath and N.C. Scott, 1983, Separation of noradrenergic and non-noradrenergic contractions to field stimulation in the rat vas deferens, Br. J. Pharmacol. 79, 379.

Browne, I., G. Thomas, K. Gavin and J.R. Docherty, 1994, Prejunctional actions of *N*-ethyl-maleimide and phenoxybenzamine, Eur. J. Pharmacol. 265, 125.

Bucher, B., J. Neuburger and P. Illes, 1991, Protein kinase C and α_2-adrenoceptor-mediated inhibition of noradrenaline release from the rat tail artery, J. Cardiovasc. Pharmacol. 17, 913.

Bylund, D.B., 1992, Heterogeneity of α_2-adrenergic receptors, Pharmacol. Biochem. Behav. 22, 835.

Castle, N.A., D.G. Haylett and D.H. Jenkinson, 1989, Toxins in the characterization of potassium channels, Trends Neurosci. 12, 59.

Connaughton, S. and J. R. Docherty, 1990, Functional evidence for heterogeneity of peripheral prejunctional α_2-adrenoceptors, Br. J. Pharmacol. 101, 285.

De Man, J.G., G.E. Boeckxstaens, P.P. Pelckmans, B.Y. De Winter, A.G. Herman and Y.M. Van Maercke, 1993, Prejunctional modulation of the nitrergic innervation of the canine ileocolonic junction via potassium channels, Br. J. Pharmacol. 110, 559.

Docherty, J.R., 1988, Pertussis toxin and prejunctional α_2-adrenoceptors in rat heart and vas deferens, J. Auton. Pharmacol. 8, 197.

Harrison, J.K., W.R. Pearson and K.R. Lynch, 1991, Molecular characterization of α_1- and α_2-adrenoceptors, Trends Pharmacol. Sci. 12, 62.

Hyland, L., P. Warnock and J.R. Docherty, 1984, An investigation of the actions of the Ca^{2+} entry facilitator Bay K 8644 on the rat vas deferens, Eur. J. Pharmacol. 104, 363.

Johns, A., D.S. Golko, P.A. Lauzon and D.M. Paton, 1976, The potentiating effects of 4-aminopyridine on adrenergic transmission in the rabbit vas deferens, Eur. J. Pharmacol. 38, 71.

Lanier, S.M., S. Downing, E. Duzie and C.J. Homey, 1991, Isolation of rat genomic clones encoding subtypes of the α_2-adrenergic receptor, J. Biol. Chem. 266, 10470.

Lorenz, W., J.W. Lomasney, S. Collins, J.W. Regan, M.G. Caron and R.J. Lefkowitz, 1990, Expression of three α_2-adrenergic receptor subtypes in rat tissues: implications for α_2-receptor cassification, Mol. Pharmacol. 38, 599.

Molgo, J., M. Lemeignan and P. Lechat, 1977, Effects of 4-aminopyridine at the frog neuromuscular junction, J. Pharmacol. Exp. Ther. 203, 653.

Murphy, T.V., S. Foucart and H. Majewski, 1992, Prejunctional α_2-adrenoceptors in mouse atria function through G-proteins which are sensitive to *N*-ethylmaleimide, but not pertussis toxin, Br. J. Pharmacol. 106, 871.

Musgrave, I.F., H. Majewski, 1989, Effect of phorbol ester and pertussis toxin on the enhancement of noradrenaline release by angiotensin II in mouse atria, Br. J. Pharmacol. 96, 609.

Schlicker, E., M. Kathmann, M. Detzner, H.J. Exner and M. Gothert, 1994, H_3 receptor-mediated inhibition of noradrenaline release: an investigation into the involvement of Ca^{2+} and K^+ ions, G protein and adenylate cyclase, Naunyn-Schmied. Arch. Pharmacol. 350, 34.

Smith, K. and J.R. Docherty, 1992, Are the prejunctional α_2-adrenoceptors of the rat vas deferens and submandibular gland of the α_{2A}- or α_{2D}-subtype?, Eur. J. Pharmacol. 219, 203.

Smith, K., S. Connaughton and J. R. Docherty, 1992, Investigations of prejunctional α_2-adrenoceptors in rat atrium, vas deferens and submandibular gland, Eur. J. Pharmacol. 211, 251.

Smith, K., K. Gavin and J.R. Docherty, 1995, Investigation of the subtype of alpha$_2$-adrenoceptor mediating prejunctional inhibition of cardio-acceleration in the pithed rat heart, Br. J. Pharmacol. 115, 316.

Weber, H.D., 1989, Presynaptic α_2-adrenoceptors at the terminals of perivascular sympathetic nerves are coupled to an *N*-ethylmaleimide-sensitive G-protein, Naunyn-Schmied. Arch. Pharmacol. 339, R35.