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Original Article

Roles for α_1-adrenoceptors during contractions by electrical field stimulation in mouse vas deferens

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ABSTRACT We have investigated the relative roles of α_1-adrenoceptors and purinoceptors in contractions to low and high frequency stimulation of the mouse vas deferens, in terms of the time course of responses. In separate experiments, isometric contractile responses were obtained to 10 pulses at 1 Hz and 40 pulses at 10 Hz. Responses to 1 Hz stimulation consisted of a series of discrete peaks. The α_{1A}-adrenoceptor antagonist RS100329 (10^{-9}M–10^{-7}M) significantly reduced the response to the first pulse, the α_{1D}-adrenoceptor antagonist BMY7378 (10^{-7}M–10^{-6}M) significantly reduced the response to the first two pulses, and the non-selective α_1-adrenoceptor antagonist prazosin (10^{-8}M) reduced the response to the first 4 pulses at 1 Hz. Responses to 10 Hz stimulation consisted of an early peak response and a maintained plateau response. RS100329 significantly reduced the peak response but did not significantly affect the plateau response. Prazosin, significantly reduced both the peak and plateau responses. The α_{1A}-adrenoceptor antagonist RS17053 in high concentrations reduced mainly the plateau response leaving a clear early peak response. The plateau response of contraction was almost abolished by the purinoceptor antagonist suramin. These results suggest that there is a relatively minor early α_{1D}-adrenoceptor and a larger early α_{1A}-adrenoceptor component to stimulation-evoked contractions of mouse vas deferens, but the major α_1-adrenoceptor component is revealed by prazosin to be α_{1B}-adrenoceptor mediated. α_{1B}-Adrenoceptor activation probably facilitates contractions mediated by other α_1-adrenoceptors and by purinoceptors. These results suggest that combined non-selective α_1-adrenoceptor blockade, particularly α_{1B}-adrenoceptor blockade, in addition to P2X1-purinoceptor blockade is useful in reducing male fertility.

INTRODUCTION

The rodent vas deferens is innervated by adrenergic nerves that release two major neurotransmitters, noradrenaline (NA) and ATP, to act on α-adrenoceptors and purinoceptors, respectively [1]. Pharmacological and gene knock-out studies have confirmed that major receptors involved in neurotransmission in the mouse vas deferens include α_{1A}-adrenoceptors and P2X1 receptors, with a lesser role for α_{1D}-adrenoceptors [2-4]. α_{1A}-Adrenoceptor knockout (α_{1A}-KO) reduces the maintained contraction to high frequency stimulation in mouse vas deferens, but even after combined α_{1A}-adrenoceptor and P2X1 KO, a small portion of the initial spike and later maintained response remains [5], and α_{1D}-adrenoceptor KO (α_{1D}-KO) reduced responses to low frequency stimulation and low concentrations of NA [2]. There was no evidence for involvement of α_{1B}-adrenoceptors in contractions of vas deferens in these studies.

It has been proposed that P2X1-purinoceptor blockade

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combined with α_{1A}-adrenoceptor blockade could prove useful as a male contraceptive [5]. In this study we have chosen the α_{1A}-adrenoceptor antagonist RS100329, the α_{1D}-adrenoceptor antagonist BMY7378, the non-selective α_1-adrenoceptor antagonist prazosin and the antagonist RS17053 to study components of the nerve stimulation evoked contraction in mouse vas deferens. We wished to investigate whether α_{1B}-adrenoceptors have an important role in contractions, particularly in response to high frequency stimulation, of mouse vas deferens and to confirm whether α_{1B}-adrenoceptor blockade may also be useful in reducing male fertility.

METHODS

Animals

Male C57 mice (18–25 g) were obtained from the Royal College of Surgeons in Ireland (RCSI) Biomedical Facility. All studies have been approved by the Health Products Regulatory Agency (HPRA) in Ireland and by the RCSI Research Ethics Committee. The animals were housed in a controlled environment with a 12-h light, 12-h dark cycle and were fed a standard rat diet.

Preparation of the isolated mouse vas deferens

Animals were killed by overdose of CO_2. For removal of vas deferens, a midline incision was made in the abdomen and the testis and epididymis exposed. Blunt forceps were placed to separate the vas deferens from the connective tissue. The whole vas deferens was tied with a long thread at the epididymal end and a short thread at the prostatic end, removed and carefully cleared of connective tissue. By convention, the vas was always placed in organ baths between platinum electrodes with the epididymal end attached to a transducer (Grass FT03) under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the following composition: (mM): NaCI 119; $NaHC0_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2P0_4 1.2; $MgS0_4$ 1.0.

Experimental protocol

Bathing fluid was changed every 15 min, except during cumulative antagonist administration. Following 60 min equilibration, changing bathing fluid every 15 min, tissues were stimulated electrically at 5 min intervals with 10 pulses at 1 Hz or 40 pulses at 10 Hz (0.5 ms pulses, supramaximal voltage) using a Grass S88 stimulator. When consistent responses in terms of magnitude and time course of isometric contraction had been obtained, antagonists were added in increasing cumulative concentrations in 1 log unit increments at 5 min intervals beginning immediately after a period of stimulation, and stimulation was repeated 5 min later in the presence of the antagonist concentration. Responses were obtained to 10 pulses at 1 Hz and 40 pulses at 10 Hz in separate experiments since high frequency stimulation reduces responses to particularly the first pulse at 1 Hz.

Following the control stimulation-evoked contraction (the response obtained immediately before the first addition of vehicle or antagonist), four cumulative additions of antagonist were carried out in 1 log unit increments for RS100329 (10^{-10}M–10^{-7}M), BMY7378 (10^{-8}M–10^{-5}M), prazosin (10^{-9}M–10^{-6}M) and RS17053 (10^{-7}M–10^{-5}M), and in vehicle experiments 4 cumulative additions of vehicle were given (veh 1–veh 4). Hence, the first vehicle (veh 1) is the vehicle that was compared with RS100329 (10^{-10}M), BMY7378 (10^{-8}M) and prazosin (10^{-9}M), etc. RS17053 was employed only in 10 Hz studies.

Contractions were calculated as absolute tension (g). Graphs showing the response to each pulse at 1 Hz and the time course of contractions at 10 Hz (at 0.2 sec intervals) were plotted from mean data obtained.

The effects of the four α_1-adrenoceptor antagonists on the response to 10 Hz were compared directly with the effects of vehicle by expressing responses in the presence of antagonist or vehicle as a percentage of the relevant control response at 4 time points: 0.8 sec (peak), 2.0, 3.0 & 4.0 sec (plateau).

Drugs

BMY7378 (8-[2-(4-(2-methoxyphenyl) piperazin-1-yl)ethyl]-8-azaspiro[4,5]decane-7,9-dione) (Tocris, Bristol, UK); RS17053 (*N*-[2-(2-Cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride) (Tocris); RS100329 (5-methyl-3-[3-[4-[2-(2,2,2,-trifluoroethoxy)phenyl]-1-piperazinyl]propyl]-2,4-(1H)-pyrimidinedione) (Tocris); nifedipine (Sigma, Dublin, Ireland); prazosin hydrochloride (Sigma); suramin hexasodium salt (Tocris).

Drug stocks were dissolved in distilled water, except for nifedipine and RS17053 which were dissolved in ethanol.

Statistics

Values are expressed as mean ± standard error of mean (SEM) from 6 experiments except for the studies with nifedipine and suramin, where n = 5, and with RS17053, where n = 4. The minimum level for statistical significance was $p < 0.05$. Differences between test antagonists and vehicle were compared using the GraphPad Prism program (GraphPad Software Inc., San Diego, CA, USA) for MacIntosh by two way ANOVA for multiple groups, and, only when ANOVA showed significance of $p < 0.05$, with Bonferroni test for comparison of effects with control or the vehicle group. Antagonist potency at producing 50% inhibition of contractions (pIC_{50} value, –log M) to a single pulse, 1 Hz or 10 Hz stimulation was calculated from individual experiments by non-linear regression using GraphPad Prism for MacIntosh.

RESULTS

Single pulse

At frequencies below 1 Hz, responses did not summate, so that the first pulse at 1 Hz can be considered the response to low frequencies of 0.1–0.2 Hz (see Fig. 1A). Vehicle had no significant effect on contractions when given in 4 additions at 5 min intervals (Figs. 1B and 2A). BMY7378 (10^{-7}M) (Figs. 1C and 2B), RS100329 (10^{-9}M) (Figs. 1D and 2C), and prazosin (10^{-8}M) (Figs. 1E and 2D) significantly reduced the response to a single stimulus. These were the threshold concentrations of each antagonist at causing significant inhibition.

1 Hz stimulation

Stimulation with 10 pulses at 1 Hz produced a series of 10 discrete peaks of contraction (Fig. 1A), the first of which was generally above 50% of the maximum response to 1 Hz (Fig. 1A). There was summation of response and maximum responses were usually reached by the 2nd, 3rd or 4th pulse in the train (Fig. 1A).

Vehicle had no significant effect on contractions when given in 4 additions at 5 min intervals (Figs. 1B and 2A). The effects of the α_{1D}-adrenoceptor antagonist BMY7378, the α_{1A}-adrenoceptor antagonist RS100329 and the non-selective antagonist prazosin on contractions to 1 Hz stimulation are also shown in Fig. 1C–E. To compare antagonists with vehicle statistically, responses in the presence of antagonist were expressed as a percentage of the relevant control. BMY7378 (10^{-7}M), RS100329 (10^{-9}M) and prazosin (10^{-8}M) were the threshold concentrations for producing inhibition of 1 Hz responses (Fig. 2B–D).

BMY7378 (10^{-8}M) had no effect, BMY7378 (10^{-7}M) significantly reduced the response to the first and second pulses at 1 Hz (Fig. 2B) but BMY7378 (10^{-6}M–10^{-5}M) had no further effect (see Fig. 2B). Although RS100329 (10^{-9}M) significantly reduced the response to the first pulse (Fig. 2C), even RS100329 (10^{-7}M) failed to significantly affect responses to the second and subsequent pulses (see Fig. 2C). Prazosin (10^{-8}M) significantly reduced the response to the first 4 pulses at 1 Hz and prazosin (10^{-6}M) significantly reduced the response to the first 6 pulses (Fig. 2D).

10 Hz stimulation

Stimulation with 40 pulses at 10 Hz produced a contractile response that consisted of an early peak and a maintained plateau response (see Fig. 3A). Vehicle had no significant effect on contractions when given in 4 additions at 5 min intervals

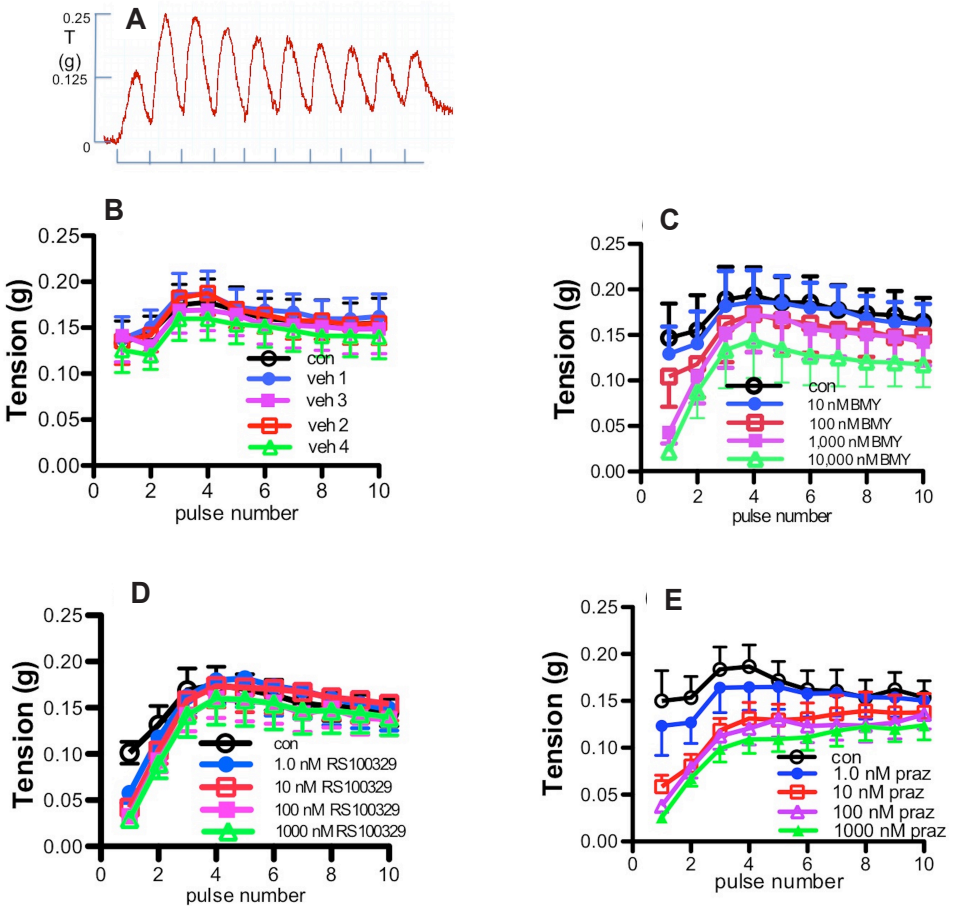

Fig. 1. Effects of vehicle and antagonists on isometric contractions of mouse vas deferens produced by 10 pulses at 1 Hz, expressed as tension in grams. (A) Original recording of isometric contractions obtained to field stimulation in mouse vas deferens with 10 pulses at 1 Hz. The vertical axis shows tension in grams. The horizontal axis shows pulse number at 1 Hz and the time between each pulse is 1 sec. The response to the first pulse is clearly shown. (B–E) The effects of (B) vehicle (veh) (4 additions), (C) BMY7378 (BMY) (10 nM–10 μM), (D) RS100329 (1 nM–1 μM) and (E) prazosin (praz) (1 nM–1 μM) on isometric contractions to each pulse of 10 pulse field stimulation at 1 Hz in mouse vas deferens, expressed as absolute tension in grams. Values are presented as mean ± SEM from 6 experiments. Statistical tests were carried out on data expressed as a percentage of control (see Fig. 2).

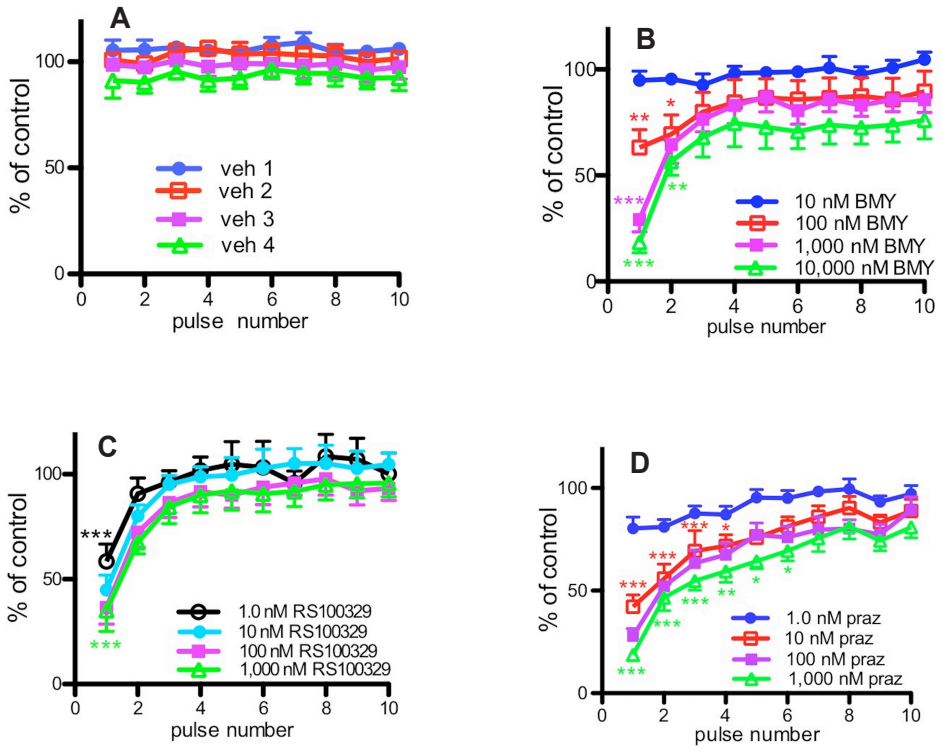

Fig. 2. Effects of vehicle and antagonists on isometric contractions of mouse vas deferens produced by 10 pulses at 1 Hz, expressed as % of control. The effects of (A) vehicle (veh) (4 additions), (B) BMY7378 (BMY) (10 nM–10 μM), (C) RS100329 (1 nM–1 μM) and (D) prazosin (praz) (1 nM–1 μM) on isometric contractions to each pulse of 10 pulse field stimulation at 1 Hz in mouse vas deferens, expressed as % of control response (see Fig. 1 for responses expressed as absolute tension). Values are presented as mean ± SEM from 6 experiments. Responses in the presence of antagonist were compared with the effects of respective vehicle by 2 way analysis of variance and Bonferroni *post-hoc* test. Asterisks denote effects of antagonist significantly different from the effects of vehicle in response to a given pulse, and are colour coded to match the antagonist concentration symbol colour: *p < 0.05; **p < 0.01; ***p < 0.001.

(Fig. 3B). The α_{1D}-adrenoceptor antagonist BMY7378 (10^{-6}M) had only small effects on the peak response (Fig. 3C). The α_{1A}-adrenoceptor antagonist RS100329 (10^{-9}M–10^{-8}M) reduced the peak response but had less effect on the plateau response (Fig. 3D), whereas prazosin (10^{-8}M–10^{-7}M) antagonized both peak and plateau components markedly (Fig. 3E). RS17053 (10^{-5}M) markedly reduced the plateau component of the response but the early peak was still very prominent (Fig. 3F).

To compare antagonists with vehicle statistically, responses in the presence of antagonist were expressed as a percentage of the relevant control at the time points of 0.8 (peak response) and 2.0, 3.0, and 4.0 sec (plateau response) after beginning stimulation at 10 Hz (Fig. 4). Vehicle had no significant effect on contractions when given in 4 additions at 5 min intervals (Fig. 4A).

BMY7378 (10^{-6}M) did not significantly affect the peak or plateau response (Fig. 4B). RS100329 (10^{-8}M) significantly reduced the peak response, but RS100329 (10^{-7}M) had no further effect (Fig. 4C). Prazosin (10^{-8}M) significantly reduced both the peak and plateau response, and prazosin (10^{-7}M) produced further inhibition (Fig. 4D). Concentrations of prazosin at 10^{-6}M and above produced no further inhibition and indeed increased the early part of the contraction, presumably due to prejunctional α_2-adrenoceptor antagonism. RS17053 (10^{-5}M) significantly reduced responses at all time points, both at peak and plateau, but it was the only antagonist to have markedly more inhibitory actions against plateau than against peak (Fig. 4E).

Nifedipine (10^{-5}M) markedly reduced both the peak and plateau of response to 10 Hz stimulation so that the shape of response was similar to that in the absence of nifedipine, but markedly smaller and with a more marked peak response and very small residual plateau component (Fig. 3G). Suramin (10^{-4}M) markedly reduced responses to 10 Hz stimulation, and behaved largely like nifedipine, leaving a small peak response and a very small residual plateau (Fig. 3G).

Antagonist inhibitory potency

Antagonist potency at producing 50% inhibition (pIC_{50}, –log M) of contractions was calculated where antagonists produced at least 50% inhibition. RS100329 showed highest potency against a single stimulus (pIC_{50} of 8.46), with prazosin slightly less potent (8.05) and with BMY7378 much less potent (6.58) (Table 1). Similarly, the peak response to 10 Hz was inhibited most potently by RS100329 (8.19), then by prazosin (7.94), with RS17053 much less potent (5.24) (Table 1). Only prazosin and RS17053 produced more than 50% inhibition of the plateau response to 10 Hz, with values of 7.71 and 5.74, respectively (Table 1).

DISCUSSION

In this study, we have examined α_1-adrenoceptor subtypes involved in contractions of mouse vas deferens, employing the α_{1A}-adrenoceptor antagonist RS100329 [6], the α_{1D}-adrenoceptor antagonist BMY7378 [7], the non-selective antagonist prazosin [3,8] and the antagonist RS17053 [9].

This study relies on the selectivity of antagonists. BMY7378 has high potency at α_{1D}-adrenoceptors (average of 8.60, –log

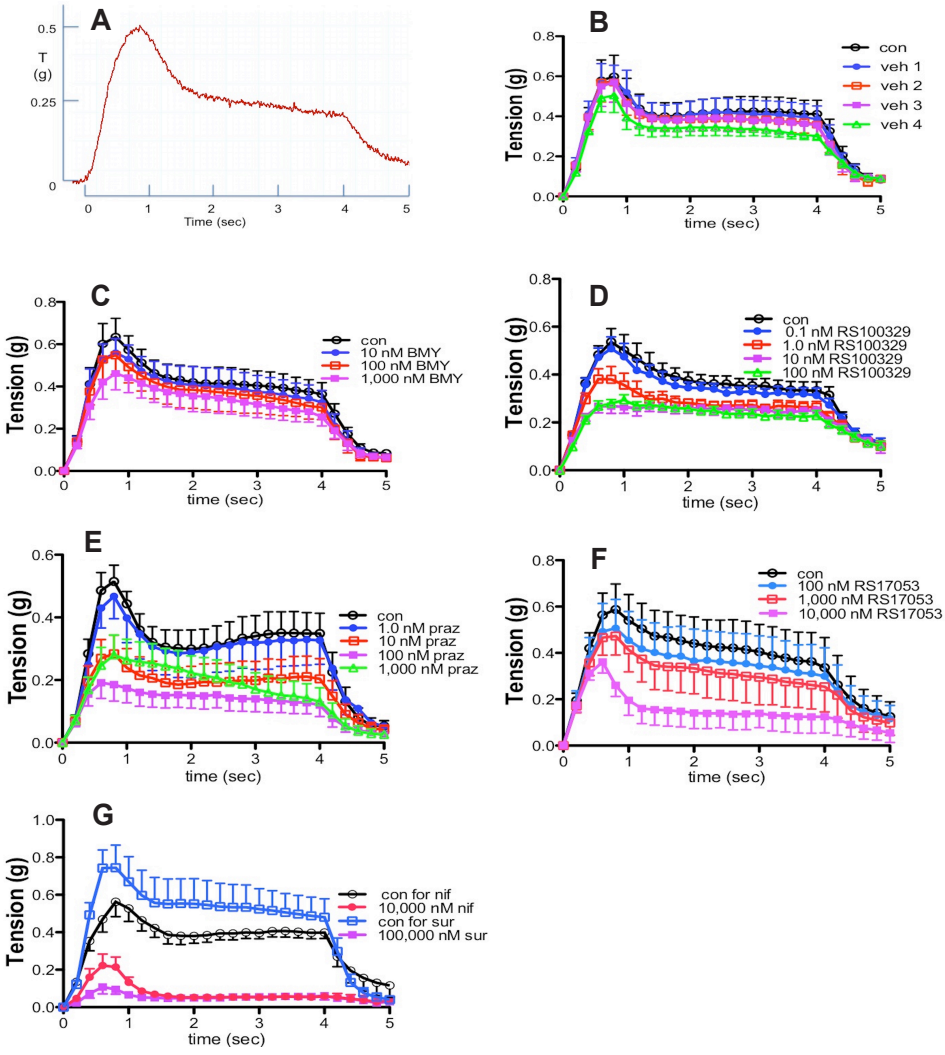

Fig. 3. Effects of vehicle and antagonists on isometric contractions of mouse vas deferens produced by 40 pulses at 10 Hz, expressed as tension in grams. (A) Original recordings of isometric contraction obtained to field stimulation in mouse vas deferens with 40 pulses at 10 Hz. The vertical axis shows tension in grams. The horizontal axis shows time in sec, with stimulation at 10 Hz beginning at time zero and ending at 4 sec. (B–G) The effects of (B) vehicle (veh) (4 additions), (C) BMY7378 (BMY) (10 nM–1 μM), (D) RS100329 (0.1 nM–100 nM), (E) prazosin (praz) (1 nM–1 μM) and (F) RS17053 (100 nM–10 μM) on isometric contractions to field stimulation with 40 pulses at 10 Hz in mouse vas deferens, expressed as absolute tension in grams. In (G) responses in the presence suramin (sur) 100 μM and nifedipine (nif) 100 μM are shown. Values are presented as mean ± SEM from 6 experiments, except for suramin and nifedipine, where n = 5, and RS17053, where n = 4. Statistical tests were carried out on data expressed as a percentage of control (see Fig. 4).

M), but low potency at α_{1A}- and α_{1B}-adrenoceptors (average of 6.55 and 7.07, respectively) [8]. Conversely, RS100329 has high potency (average of 9.40) at α_{1A}-adrenoceptors, but low potency (average of around 8.15) at other α_1-adrenoceptors (α_{1B}- or α_{1D}-adrenoceptors) [8]. Allowing for sub-optimal conditions, BMY7378 (10^{-8}M–10^{-7}M) and RS100329 (10^{-9}M–10^{-8}M) might be expected to produce selective actions at α_{1D}- and α_{1A}-adrenoceptors, respectively. The major problem in terms of α_1-adrenoceptor antagonist selectivity is the absence of a reliable selective α_{1B}-adrenoceptor antagonist. Currently, there is no reliable selective antagonist for α_{1B}-adrenoceptors [8]. However, studies with the antagonist RS17053 suggest that this antagonist has a different profile of action to the other antagonists, and this will be discussed below. Given the uncertainty about selectivities of α_{1B}-adrenoceptor antagonists, actions of low concentrations of prazosin not shared by low and subtype selective concentrations of BMY7378 or RS100329 (at the above concentrations) can be presumed to involve α_{1B}-adrenoceptors [10,11]. We have previously demonstrated in rat spleen that actions of prazosin were greater than the combined effects of selective concentrations of BMY7378 and RS100329, suggesting the involvement of α_{1B}-adrenoceptors in contractions [11]. In rat portal vein, no α_{1D}-adrenoceptors could be demonstrated, but prazosin had differing actions from RS100329, again suggesting involvement of α_{1B}-adrenoceptors, particularly in tonic contractions [10]. Hence, this strategy is able to identify α_{1B}-adrenoceptor mediated responses.

RS17053 has relatively high affinity at α_{1A}-adrenoceptor ligand binding sites of 7.5–8.5 (–log M) [9,12], and lower affinity at α_{1B}- and α_{1A}-adrenoceptor (7.3 & 7.1, respectively) [9]. The functional selectivity of RS17053 is less clear. RS17053 has high potency at α_{1A}-adrenoceptors in CHO cells (8.24) [13], intermediate potency at α_{1B}-adrenoceptors in CHO cells (7.63) [13] and rat spleen (7.2, 7.28) [13,14], and similar or lower potency at α_{1D}-adrenoceptors (noncompetitive in rat aorta) [14]. In rat portal vein, where contractions involve both α_{1A}- and α_{1B}-adrenoceptors [10], RS17053 markedly reduced contractions over the concentration range 10^{-7}M–3×10^{-6}M [14].

In the present study, it can be clearly seem from the effects of BMY7378 (10^{-7}M) against a single pulse (first pulse at 1 Hz) and against low frequency (1 Hz) stimulation that an α_{1D}-

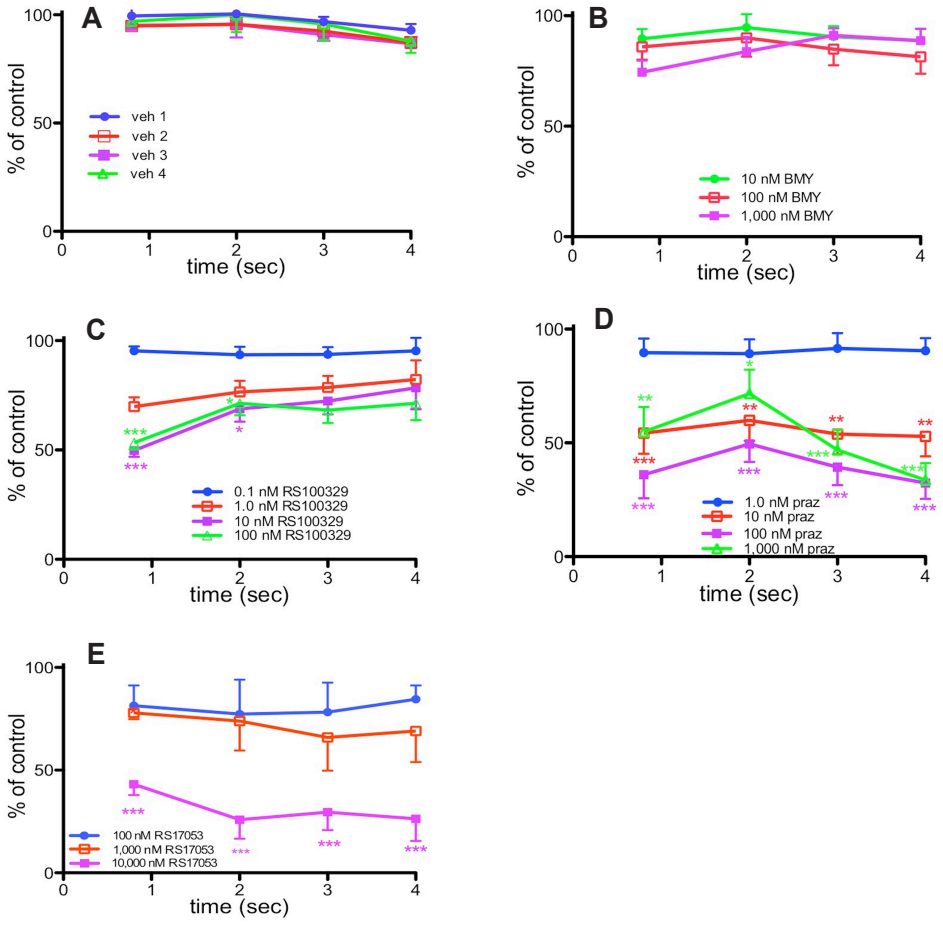

Fig. 4. Effects of vehicle and antagonists on isometric contractions of mouse vas deferens produced by 40 pulses at 10 Hz, expressed as % of control. The effects of (A) vehicle (veh) (4 additions), (B) BMY7378 (BMY) (10 nM–1 μM), (C) RS100329 (0.1 nM–100 nM), (D) prazosin (praz) (1 nM–1 μM) and (E) RS17053 (100 nM–10 μM) on isometric contractions to field stimulation with 40 pulses at 10 Hz in mouse vas deferens, expressed as a percentage of the respective control response (see Fig. 3 for responses expressed as absolute tension). Values are presented as mean ± SEM from 6 experiments, except for (E), where n = 4. Responses in the presence of antagonist were compared with the effects of respective vehicle by 2 way analysis of variance and Bonferroni *post-hoc* test. Asterisks denote effects of antagonist significantly different from the effects of vehicle at a given time point, and are colour coded to match the antagonist concentration symbol colour: *p < 0.05; **p < 0.01; ***p < 0.001.

Table 1. Antagonist potency at producing 50% inhibition (pIC_{50}, –log M) of contractions of mouse vas deferens produced by a single stimulus, and by 10 Hz stimulation, both peak response (0.8 sec) and plateau response (3 sec)

Antagonist	Single pulse	1 Hz (3rd pulse)	10 Hz peak (0.8 sec)	10 Hz plateau (3 sec)
BMY7378	6.58 ± 0.14	ND	ND	ND
RS100329	8.46 ± 0.28	ND	8.19 ± 0.10	ND
Prazosin	8.05 ± 0.28	ND	7.94 ± 0.22	7.71 ± 0.27
RS17053	-	-	5.24 ± 0.14	5.74 ± 0.49

Values are presented as mean ± SEM from 6 experiments, except for RS17053, where n = 4. ND, not determined, 50% inhibition not reached.

adrenoceptor mediated response is present only to the first pulse. This component can be clearly seen in the biphasic response of mouse or rat vas deferens to a single electrical stimulus [2,15]. Hence, the first stimulus in a train provokes the α_{1D}-adrenoceptor response involving T-type Ca^{2+} channels and stores of calcium and these stores are then either depleted or the system inactivates [15]. However, the response to the first pulse at 1 Hz is also significantly inhibited by low concentrations of RS100329 (10^{-9}M), so that the initial response at 1 Hz involves both α_{1A}- and α_{1D}-adrenoceptors. Prazosin has more effect that either BMY7378 or RS100329 alone, presumably by combined actions at both α_{1A}- and α_{1D}-adrenoceptors, or possibly by additional actions at α_{1B}-adrenoceptors.

In terms of responses to 10 Hz stimulation, BMY7378 had no significant effect at selective concentrations. Admittedly, BMY7378 reduced the peak response but this did not reach significance. The α_{1D}-adrenoceptor component to the response seen with 1 Hz stimulation is not well maintained by a high frequency stimulation protocol, presumably by exhaustion of the calcium stores. RS100329 (10^{-8}M) inhibited the peak response to 10 Hz stimulation, demonstrating an α_{1A}-adrenoceptor mediated component. However, RS100329 even in higher concentrations did not significantly affect the plateau response to 10 Hz stimulation. This suggests that α_{1A}-adrenoceptor activation may have only a small effect on the plateau response. However, prazosin (10^{-8}M) markedly reduced both the peak

and plateau response to 10 Hz stimulation, demonstrating that α_{1B}-adrenoceptors are involved in the plateau response. Most interestingly, RS17053 markedly reduced the plateau response revealing a greatly increased peak (in terms of the ratio of peak to plateau). None of the other antagonists had this action. RS17053 shows selectivity for α_{1A}- over α_{1B}-adrenoceptors, but lowest potency at α_{1D} -adrenoceptors (see above). Hence, these actions of RS17053 are consistent with actions mainly at α_{1A}- and α_{1B}-adrenoceptors, and so differ from the effects of prazosin in that, lacking α_{1D}-adrenoceptor antagonist potency, RS17053 leaves a clear peak response. Components of the response to 10 Hz stimulation are summarized in the graphical abstract.

In terms of potency at producing 50% inhibition of contractions, prazosin had similar potencies (7.7–8.0) at inhibiting the contraction to a single pulse and the peak and plateau contractions to 10 Hz stimulation, and this demonstrates that prazosin acts as a non-selective antagonist at all 3 subtypes of α_1-adrenoceptor. Like prazosin, RS17053 inhibited both the peak and plateau contractions to 10 Hz stimulation, although with much lower potency (5.2–5.7) than prazosin. In contrast, RS100329 showed slightly higher inhibitory potency than prazosin (8.2–8.5) against both the contraction to a single pulse and the peak contraction to 10 Hz stimulation, suggesting that these responses are mainly α_{1A}-adrenoceptor mediated. However, RS100329 did not produce significant inhibition of the plateau response to 10 Hz stimulation. BMY7378 significantly inhibited only the response to a single stimulus, with a pIC_{50} of around 6.6, showing that α_{1D}-adrenoceptors have a minor role in contractions.

However, α_1-adrenoceptors interact with P2X1-purinoceptors in producing contractions of mouse vas deferens. The P2X1-purinoceptor mediated response involving an intrinsic ion channel, depolarization and Ca^{2+} entry through nifedipine-sensitive calcium channels [16] and contractions to ATP are absent in vas deferens from P2X1-KO mice [17]. Suramin, a P2X1-purinoceptor antagonist, virtually abolishes the plateau response to 10 Hz stimulation, leaving mainly a small peak response. We cannot rule out the possibility that suramin in the high concentration of 10^{-4}M may have other actions at this high concentrations, but, assuming that actions are mainly at P2X1-purinoceptors, this demonstrates that the plateau response which partly involves α_{1B}-adrenoceptors is also P2X1-purinoceptor mediated. We have previously shown that tonic contractions of rat portal vein involve predominantly α_{1B}-adrenoceptors, and are at least partly mediated by mechanisms involving RhoA/Rho kinase [10]. RhoA activation causes inhibition of myosin light chain phosphatase to increase vasoconstriction by Ca^{2+} sensitization [18]. Hence, it is likely that the main action of activation of α_{1B}-adrenoceptors in mouse vas deferens is to facilitate P2X1-purinoceptor mediated contractions.

Although earlier studies failed to find evidence for α_{1B}-adrenoceptors in rat vas deferens by Northern blot [19], more recent studies using real time reverse transcription polymerase chain reaction have shown expression of α_{1B}-adrenoceptors in rat vas deferens and particularly in the epididymal portion, where expression was greater than for α_{1D}-adrenoceptors [20].

Prazosin in the high concentration of 10^{-6}M acted to reverse the inhibition of 10 Hz stimulation-evoked contractions achieved by 10^{-7}M (see Figs. 3E and 4D), and increased particularly the early component of the response. This action of high concentrations of prazosin is probably due to α_2-adrenoceptor antagonism to block negative feedback inhibition of neurotransmission by the neurotransmitter NA: NA acting at presynaptic receptors on the nerve terminals reduces transmitter release, so that blockade of these receptors increases release. The α_2-adrenoceptor antagonists yohimbine and BRL44408 have been shown to potentiate the early component of contractions to 10 Hz stimulation in mouse vas deferens [21].

α_{1A}-Adrenoceptor knockout caused a 50% loss of fertility, triple α_1-adrenoceptor knock-out a 92% loss, P2X1 purinoceptor knockout an 86% loss, and combined P2X1/α_{1A}-receptor knockout produced 100% loss of fertility [5,17,22]. These changes were caused by diminished sperm in the ejaculate [17,22]. Previous studies of male fertility have examined α_1-adrenoceptor antagonists. In rats, the non-selective α_1-adrenoceptor antagonist phenoxybenzamine (0.7 mg/kg for 5 weeks) caused infertility with increased spermatozoa number in the epididymis and vas deferens [23], and phenoxybenzamine (20 mg/day) produced similar effects in man and was also useful against premature ejaculation [24]. In rats, prazosin (1.4 mg/kg) significantly reduced sperm count [25], but in man, prazosin in the lower dose of 5 mg (approx 0.1 mg/kg) was ineffective as a male contraceptive [26]. It has been suggested that P2X1-purinoceptor blockade alone [27] or combined with α_{1A}-adrenoceptor blockade could prove useful as a male contraceptive [5]. In mice, α_{1A}-KO or α_{1D}-KO, but not α_{1B}-KO, reduces resting blood pressure [8]; hence, α_{1B}-adrenoceptor blockade may have lesser effects on blood pressure.

These results suggest that there is a relatively minor early α_{1D}-adrenoceptor and a larger α_{1A}-adrenoceptor component to stimulation-evoked contractions of mouse vas deferens, but the major α_1-adrenoceptor component is revealed to be α_{1B}-adrenoceptor mediated by the non-selective antagonist prazosin. α_{1B}-Adrenoceptors mediate contractions and/or facilitate contractions to other α_1-adrenoceptor and purinoceptor activation. These results suggest that combined non-selective α_1-adrenoceptor blockade, particularly α_{1B}-adrenoceptor blockade, in addition to P2X1 purinoceptor blockade may be useful in reducing contractility of vas deferens, and may have lesser effects on blood pressure than selective α_{1A}-adrenoceptor antagonism.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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