Roles for $\alpha_1$-adrenoceptors during contractions by electrical field stimulation in mouse vas deferens

Hadeel A. Alsufyani$^1$ and James R. Docherty$^{2, *}$

$^1$Department of Physiology, King Abdulaziz University, Jeddah 21589, Saudi Arabia, $^2$Department of Physiology, Royal College of Surgeons in Ireland (RCSI), Dublin D02 YN77, Ireland

ABSTRACT We have investigated the relative roles of $\alpha_1$-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 $\alpha_{1A}$-adrenoceptor antagonist RS100329 (10$^{-9}$M–10$^{-7}$M) significantly reduced the response to the first pulse, the $\alpha_{1D}$-adrenoceptor antagonist BMY7378 (10$^{-7}$M–10$^{-6}$M) significantly reduced the response to the first two pulses, and the non-selective $\alpha_1$-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 $\alpha_{1A}$-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 $\alpha_{1D}$-adrenoceptor and a larger early $\alpha_{1A}$-adrenoceptor component to stimulation-evoked contractions of mouse vas deferens, but the major $\alpha_1$-adrenoceptor component is revealed by prazosin to be $\alpha_{1A}$-adrenoceptor mediated. $\alpha_{1A}$-Adrenoceptor activation probably facilitates contractions mediated by other $\alpha_1$-adrenoceptors and by purinoceptors. These results suggest that combined non-selective $\alpha_1$-adrenoceptor blockade, particularly $\alpha_{1B}$-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 $\alpha$-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 $\alpha_{1A}$-adrenoceptors and P2X1 receptors, with a lesser role for $\alpha_{1D}$-adrenoceptors [2-4]. $\alpha_{1A}$-Adrenoceptor knockout ($\alpha_{1A}$-KO) reduces the maintained contraction to high frequency stimulation in mouse vas deferens, but even after combined $\alpha_{1A}$-adrenoceptor and P2X1 KO, a small portion of the initial spike and later maintained response remains [5], and $\alpha_{1D}$-adrenoceptor KO ($\alpha_{1D}$-KO) reduced responses to low frequency stimulation and low concentrations of NA [2]. There was no evidence for involvement of $\alpha_{1B}$-adrenoceptors in contractions of vas deferens in these studies.

It has been proposed that P2X1-purinoceptor blockade...
combined with \(\alpha_{1A}\)-adrenoceptor blockade could prove useful as a male contraceptive [5]. In this study we have chosen the \(\alpha_{1A}\)-adrenoceptor antagonist RS100329, the \(\alpha_{1D}\)-adrenoceptor antagonist BMY7378, the non-selective \(\alpha_{1}\)-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 \(\alpha_{1A}\)-adrenoceptors have an important role in contractions, particularly in response to high frequency stimulation, of mouse vas deferens and to confirm whether \(\alpha_{1A}\)-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): NaCl 119; NaHCO\(_3\) 25; D-glucose 111.1; KCl 4.7; CaCl\(_2\) 2.5; KH\(_2\)PO\(_4\) 1.2; MgSO\(_4\) 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\(^{-9}\)M–10\(^{-3}\)M), BMY7378 (10\(^{-6}\)M–10\(^{-3}\)M), prazosin (10\(^{-4}\)M–10\(^{-3}\)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\(^{-5}\)M) and prazosin (10\(^{-5}\)M), etc. RS17053 was employed only in 10 Hz studies.

Contraction 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 \(\alpha_{1}\)-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-\(\alpha_{1}\)-dimethyl-1H-indole-3-ethanamine hydrochloride) (Tocris); RS100329 (5-methyl-3-[3-[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 nonlinear 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 α₁D-adrenoceptor antagonist BMY7378, the α₁A-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 (10⁻⁷ 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. 3B). The $\alpha_{1D}$-adrenoceptor antagonist BMY7378 ($10^{-6}\text{M}$) had only small effects on the peak response (Fig. 3C). The $\alpha_{1A}$-adrenoceptor antagonist RS100329 ($10^{-5}\text{M}$–$10^{-3}\text{M}$) reduced the peak response but had less effect on the plateau response (Fig. 3D), whereas prazosin ($10^{-4}\text{M}$–$10^{-2}\text{M}$) antagonized both peak and plateau components markedly (Fig. 3E). RS17053 ($10^{-3}\text{M}$) 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 ($10^{-4}\text{M}$) did not significantly affect the peak or plateau response (Fig. 4B). RS100329 ($10^{-3}\text{M}$) significantly reduced the peak response, but RS100329 ($10^{-2}\text{M}$) had no further effect (Fig. 4C). Prazosin ($10^{-3}\text{M}$) significantly reduced both the peak and plateau response, and prazosin ($10^{-2}\text{M}$) produced further inhibition (Fig. 4D). Concentrations of prazosin at $10^{-4}\text{M}$ and above produced no further inhibition and indeed increased the early part of the contraction, presumably due to prejunctional $\alpha_{2}$-adrenoceptor antagonism. RS17053 ($10^{-3}\text{M}$) 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 ($10^{-3}\text{M}$) 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 ($10^{-4}\text{M}$) 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 $\alpha_{1}$-adrenoceptor subtypes involved in contractions of mouse vas deferens, employing the $\alpha_{1A}$-adrenoceptor antagonist RS100329 [6], the $\alpha_{1D}$-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 $\alpha_{1D}$-adrenoceptors (average of 8.60, –log...
but low potency at $\alpha_1A$- and $\alpha_1B$-adrenoceptors (average of 6.55 and 7.07, respectively) [8]. Conversely, RS100329 has high potency (average of 9.40) at $\alpha_1A$-adrenoceptors, but low potency (average of around 8.15) at other $\alpha_1$-adrenoceptors ($\alpha_1B$- or $\alpha_1D$-adrenoceptors) [8]. Allowing for sub-optimal conditions, BMY7378 ($10^{-8}$–$10^{-7}$ M) and RS100329 ($10^{-9}$–$10^{-8}$ M) might be expected to produce selective actions at $\alpha_1D$- and $\alpha_1A$-adrenoceptors, respectively. The major problem in terms of $\alpha_1$-adrenoceptor antagonist selectivity is the absence of a reliable selective $\alpha_1B$-adrenoceptor antagonist. Currently, there is no reliable selective antagonist for $\alpha_1B$-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 $\alpha_1B$-adrenoceptor antagonists, actions of low concentrations of prazosin not shared by low and subtype selective concentrations of BMY7378 and RS100329, suggesting the involvement of $\alpha_1B$-adrenoceptors in contractions [11]. In rat portal vein, no $\alpha_1D$-adrenoceptor antagonists could be demonstrated, but prazosin had differing actions from RS100329, again suggesting involvement of $\alpha_1B$-adrenoceptors, particularly in tonic contractions [10]. Hence, this strategy is able to identify $\alpha_1B$-adrenoceptor mediated responses. RS17053 has relatively high affinity at $\alpha_1A$-adrenoceptor ligand binding sites of 7.5–8.5 (–log M) [9,12], and lower affinity at $\alpha_1B$- and $\alpha_1A$-adrenoceptor (7.3 & 7.1, respectively) [9]. The functional selectivity of RS17053 is less clear. RS17053 has high potency at $\alpha_1A$-adrenoceptors in CHO cells (8.24) [13], intermediate potency at $\alpha_1B$-adrenoceptors in CHO cells (7.63) [13] and rat spleen (7.2, 7.28) [13,14], and similar or lower potency at $\alpha_1D$-adrenoceptors (noncompetitive in rat aorta) [14]. In rat portal vein, where contractions involve both $\alpha_1A$- and $\alpha_1B$-adrenoceptors [10], RS17053 markedly reduced contractions over the concentration range $10^{-8}$–$3 \times 10^{-6}$ M [14].

In the present study, it can be clearly seem from the effects of BMY7378 ($10^{-8}$ M) against a single pulse (first pulse at 1 Hz) and against low frequency (1 Hz) stimulation that an $\alpha_1D$-
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<sub>1D</sub>-adrenoceptor response involving T-type Ca<sup>2+</sup> 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<sup>−9</sup>M), so that the initial response at 1 Hz involves both α<sub>1A</sub> and α<sub>1D</sub> adrenoceptors. Prazosin has more effect than either BMY7378 or RS100329 alone, presumably by combined actions at both α<sub>1A</sub> and α<sub>1D</sub>-adrenoceptors, or possibly by additional actions at α<sub>1B</sub>-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<sub>1D</sub>-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<sup>−8</sup>M) inhibited the peak response to 10 Hz stimulation, demonstrating an α<sub>1A</sub>-adrenoceptor mediated component. However, RS100329 even in higher concentrations did not significantly affect the plateau response to 10 Hz stimulation. This suggests that α<sub>1A</sub>-adrenoceptor activation may have only a small effect on the plateau response. However, prazosin (10<sup>−8</sup>M) markedly reduced both the peak

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### Table 1. Antagonist potency at producing 50% inhibition (pIC<sub>50</sub> – 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.

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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<sub>1D</sub>-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<sup>−8</sup>M) inhibited the peak response to 10 Hz stimulation, demonstrating an α<sub>1A</sub>-adrenoceptor mediated component. However, RS100329 even in higher concentrations did not significantly affect the plateau response to 10 Hz stimulation. This suggests that α<sub>1A</sub>-adrenoceptor activation may have only a small effect on the plateau response. However, prazosin (10<sup>−8</sup>M) markedly reduced both the peak
and plateau response to 10 Hz stimulation, demonstrating that $\alpha_{1A}$-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 $\alpha_{1A}$- over $\alpha_{1B}$-adrenoceptors, but lowest potency at $\alpha_{1D}$-adrenoceptors (see above). Hence, these actions of RS17053 are consistent with actions mainly at $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors, and so differ from the effects of prazosin in that, lacking $\alpha_{1A}$-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 $\alpha_{1}$-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 $\alpha_{1A}$-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 pIC50 of around 6.6, showing that $\alpha_{1D}$-adrenoceptors have a minor role in contractions.

However, $\alpha_{1}$-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 $\alpha_{1D}$-adrenoceptors is also P2X1-purinoceptor mediated. We have previously shown that tonic contractions of rat portal vein involve predominantly $\alpha_{1D}$-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 $\alpha_{1A}$-adrenoceptors in mouse vas deferens is to facilitate P2X1-purinoceptor mediated contractions.

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

Prazosin in the high concentration of 10$^{-4}$M acted to reverse the inhibition of 10 Hz stimulation-evoked contractions achieved by 10$^{-3}$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 $\alpha_{1D}$-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 $\alpha_{1D}$-adrenoceptor antagonists yohimbine and BRL44408 have been shown to potentiate the early component of contractions to 10 Hz stimulation in mouse vas deferens [21].

$\alpha_{1A}$-Adrenoceptor knockout caused a 50% loss of fertility, triple $\alpha_{1}$-adrenoceptor knock-out a 92% loss, P2X1 purinoceptor knockout an 86% loss, and combined P2X1/$\alpha_{1A}$-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 $\alpha_{1}$-adrenoceptor antagonists. In rats, the non-selective $\alpha_{1}$-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 $\alpha_{1A}$-adrenoceptor blockade could prove useful as a male contraceptive [5]. In mice, $\alpha_{1A}$-KO or $\alpha_{1D}$-KO, but not $\alpha_{1B}$-KO, reduces resting blood pressure [8]; hence, $\alpha_{1B}$-adrenoceptor blockade may have lesser effects on blood pressure.

These results suggest that there is a relatively minor early $\alpha_{1D}$-adrenoceptor and a larger $\alpha_{1A}$-adrenoceptor component to stimulation-evoked contractions of mouse vas deferens, but the major $\alpha_{1D}$-adrenoceptor component is revealed to be $\alpha_{1D}$-adrenoceptor mediated by the non-selective antagonist prazosin. $\alpha_{1D}$-Adrenoceptors mediate contractions and/or facilitate contractions to other $\alpha_{1}$-adrenoceptor and purinoceptor activation. These results suggest that combined non-selective $\alpha_{1}$-adrenoceptor blockade, particularly $\alpha_{1B}$-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 $\alpha_{1A}$-adrenoceptor antagonism.

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.
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