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Original Article

Purinergic contraction of the rat vas deferens in *L*-NAME-induced hypertension: effect of sildenafil

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Abstract

Hypertension (HTN) is a risk factor for erectile dysfunction, but its effect on vas deferens (VD) contractility and the ejaculatory response has not been delineated. NG-nitro-*L*-arginine methyl ester (*L*-NAME), a nitric oxide synthase inhibitor, was used for induction of nitric oxide (NO)-deficient HTN. Our aim was to evaluate the effects of *L*-NAME-induced HTN on rat VD contractility and to determine whether sildenafil affects VD contractility. A total of 36 male rats were divided into (1) control, (2) *L*-NAME–HTN, (3) sildenafil treated *L*-NAME–HTN groups. Group 2 was treated with *L*-NAME (40 mg kg^{-1} per day) in drinking water for 4 weeks. Group 3 received sildenafil (1.5 mg kg^{-1} per day, by oral gavage) concomitantly with *L*-NAME. The prostatic portion of the VD was subjected to electrical field stimulation (EFS, 1–20 Hz), and the $P2X_1$ agonist α,β-methylene ATP (α,β-meATP, 100 μmol L^{-1}–1 μmol L^{-1}) and the α1-adrenoceptor agonist phenylephrine (Phe, 100 μmol L^{-1}–1 mmol L^{-1}) were used to construct concentration-response curves. These experiments were repeated in the presence of P2X receptor antagonist, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, 30 μmol L^{-1}). VD contractions in response to EFS, α,β-meATP and Phe were significantly enhanced by *L*-NAME. Sildenafil treatment in the *L*-NAME group improved the contractile response of VD to EFS (20 Hz). In the presence of PPADS, the enhanced contractile response of VD to EFS and α,β-meATP in hypertensive rats was reversed. In the rat model of chronic NO depletion, the purinergic and adrenergic components and EFS affect VD contractility. The VD contractile response may be mediated more by the purinergic system than the adrenergic system, and sildenafil may alter the ejaculatory response in men with PE.

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1 Introduction

Spermatozoal transport involves contraction of the muscle layers of the vas deferens (VD). Studies employing rat VD suggested that ejaculatory disorders may be caused by alterations in the contractile mechanism [1, 2]. Recently, it has been shown that ATP and noradrenaline (NA) are co-transmitters in the contraction of the human VD [3–5]. Medina *et al*. [4] reported that sildenafil inhibits adrenergic neurotransmission in the human VD. ATP, released from sympathetic nerves, acts through ion-gated P2X receptors and stimulate the initial fast phase of contraction, whereas NA is

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responsible for more sustained and slower contractions through the G-protein-coupled α-adrenoceptor action [6, 7]. Adenosine, derived from enzymatic dephosphorylation, contributes to the relaxant effect of ATP, apparently by activation of a smooth muscle adenosine receptor linked to the accumulation of cAMP and activation of protein kinase A [8].

Hypertensive patients (~30%) have erectile dysfunction (ED) and severe hypertension (HTN) is directly proportional of the severity of ED [9]. In addition, the majority of ED in hypertensive patients seems to be vasculogenic [10]. Various hypertensive models display ED that is characterized by decreased erectile response or intracavernosal pressure/mean arterial pressure (ICP/MAP) ratio after electrical stimulation of the major pelvic ganglion [11–13].

In previous studies carried out in spontaneously hypertensive rats (SHR), VD displayed an altered post-junctional functionality of α_1-adrenoceptors [14, 15]. Similarly, an increased purinergic contribution to the co-transmission process has been described in nerve-mediated contractions by electrical field stimulation (EFS) recorded in isolated caudal arteries of SHR [10, 16, 17]. Commonly, SHR smooth muscle shows an increased post-junctional responsiveness to sympathetic stimulation [17] and greater post-synaptic contribution of electrically evoked ATP [18].

Nitric oxide (NO) deficiency induced by administration of NG-nitro-*L*-arginine methyl ester (*L*-NAME) produces a persistent increase in arterial blood pressure [19]. In *L*-NAME-treated hypertensive rats, adrenergic tone due to NO deficiency has an important role in the maintenance of blood pressure [20].

As of now, data are not available on the contractile features of VD from *L*-NAME-induced hypertensive (HTN) rats. The aims of the present study were: 1) to characterise the EFS-induced and agonist-induced contractile properties of the prostatic end of VD in *L*-NAME HTN rats; 2) to assess the contribution of the purinergic component to the increased sympathetic response in HTN VD; 3) to evaluate the possible restorative effects of sildenafil (phosphodiesterase 5 [PDE5] inhibitor) treatment on VD contractility in this model.

2 Materials and methods

2.1 Animals and treatment

A total of 36 adult male Sprague-Dawley rats were divided equally into three groups: Group 1 received normal drinking water. Group 2 rats were made hypertensive by adding *L*-NAME (40 mg kg^{-1} per day) in the drinking water for 4 weeks. Group 3 rats received *L*-NAME-treated drinking water and sildenafil at a daily dose of 1.5 mg kg^{-1}. Sildenafil (Viagra 50 mg tablets, Pfizer Pharmaceuticals, New York, USA) was administered orally by gavage once a day for 4 weeks. Viagra tablets were crushed and suspended in 0.5% carboxymethylcellulose (vehicle; 2 mL kg^{-1}). The 1.5 mg kg^{-1} per dose of sildenafil used is approximately equivalent to the clinical dose of 100 mg for a 70-kg patient [21].

2.2 Measurement of mean arterial pressure (MAP)

Rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), the trachea cannulated (PE-240 tubing) to maintain the airway and the carotid artery was cannulated (PE-50 tubing) to measure MAP (mm Hg) using a transducer (Statham, Oxnard, CA, USA) attached to a data acquisition system (Biopac MP 100 System; Biopac, Santa Barbara, CA, USA).

2.3 Measurement of isometric tension in VD strips

The VD of the rat was dissected and the prostatic side marked before being placed in a petri dish containing Krebs bicarbonate solution (containing [concentrations in mmol L^{-1}] NaCl: 118.1, KCl: 4.7, KH_2PO_4: 1.0, $MgSO_4$: 1.0, $NaHCO_3$: 25.0, $CaCl_2$: 2.5 and glucose: 11.1) and oxygenated with a mixture of 95% O_2 and 5% CO_2. The prostatic portion of VD was cut into strips (1 × 1 × 6 mm dimension) and mounted under 1 g of resting tension in a 20-mL organ bath chamber.

In the first series of experiments, EFS was delivered as a train of square-wave pulses (pulse width 0.5 ms, intensity 20 V) at frequencies of 1–20 Hz across paired platinum electrodes placed on either side of the tissue strips. EFS-induced contractile responses were repeated in the presence of the $P2X_1$ antagonist, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS; 30 μmol L^{-1}). In the second series of experiments, the contractile responses to the α_1-adrenoceptor agonist phenylephrine (Phe) and α,β-methylene ATP (α,β-meATP, $P2X_1$ receptor agonist) were evaluated in the absence and presence of PPADS (30 μmol L^{-1}).

2.4 Statistical analysis

All data are expressed as mean ± SEM. Contractions are reported as absolute values (in grams) or as percentages of control responses. pD_2 values (receptor

affinity and negative logarithm of the molar concentration at which one half of the maximal contraction occurs) were determined from individual concentration-response curves by nonlinear regression analysis. Differences between the means were identified by paired *t*-test. Statistical differences were determined by ANOVA (analysis of variance) followed by Bonferroni's complementary analysis, using GraphPad 4 Prism (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was accepted at $P < 0.05$.

2.5 Drugs

Relevant drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3 Results

3.1 Body and VD weights and MAP

The body weights and the VD weights were not significantly different among the groups (Table 1). MAP was increased in the *L*-NAME group, which did not return to the control levels after sildenafil treatment (Table 1).

3.2 EFS-induced contractile responses of VD

The amplitude of contraction produced by EFS at 20 Hz (intensity 20 V, pulse width 0.5 ms) in the tissue from the *L*-NAME group was greater than in the control group ($P < 0.05$). Sildenafil treatment in HTN rats normalized this *L*-NAME-induced response (Table 2, Figure 1). At low frequencies (1–15 Hz), there was no difference in contraction to EFS in the different groups, although sildenafil treatment showed a lower contractile response compared with other groups (Figure 1). PPADS treatment also reduced the EFS-induced enhancement of responses to high-frequency stimulation (20 Hz) in HTN rats (0.930 ± 0.075 *vs.* 0.280 ± 0.036, $P < 0.01$).

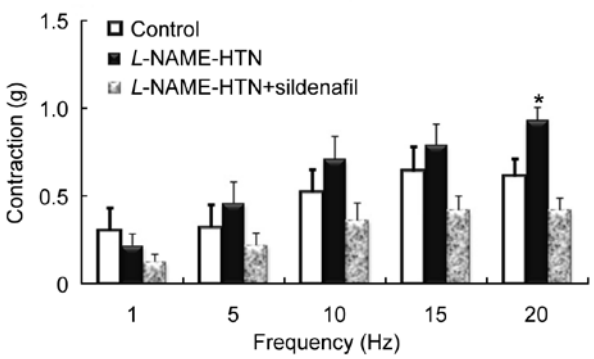

Figure 1. Bar graphs showing electrical field stimulation (EFS; 1–20 Hz frequency, pulse width 0.5 ms, intensity 20 V)-induced contractions of the prostatic end of the VD in organ bath preparations. Data are represented as mean ± SEM ($n = 8$–10). $^{*}P < 0.05$, compared with the control group.

Table 1. Several characteristics of animals groups.

	Control	*L*-NAME-induced HTN	*L*-NAME-induced HTN + sildenafil
Body weight (g)	369.0 ± 7.1	373.8 ± 5.6	374.0 ± 8.5
VD strip weight (g)	0.035 ± 0.002	0.034 ± 0.002	0.038 ± 0.001
Mean arterial pressure (mm Hg)	114.13 ± 3.28	152.89 ± 4.39***	167.21 ± 5.99***

Abbreviations: *L*-NAME, NG-nitro-*L*-arginine methyl ester; HTN, hypertensive; VD, vas deferens. $^{***}P < 0.001$, compared with controls.

Table 2. Maximum contractile responses to EFS and agonists.

	Control	*L*-NAME-HTN	*L*-NAME-HTN + sildenafil
EFS	0.610 ± 0.100	0.930 ± 0.075*	0.420 ± 0.068
Phenylephrine (contraction g)	0.370 ± 0.058	0.810 ± 0.043**	0.850 ± 0.102**
pD_2 value	5.40 ± 0.36	4.91 ± 0.23	4.48 ± 0.17
α,β-Methylene ATP (contraction g)	0.394 ± 0.049	1.060 ± 0.013***	1.036 ± 0.014***
pD_2 value	7.76 ± 0.29	7.29 ± 0.09	7.09 ± 0.06

Abbreviations: EFS, electrical field stimulation; *L*-NAME, NG-nitro-*L*-arginine methyl ester; HTN, hypertensive. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with corresponding controls. Data also include pD_2 values for phenylephrine and α,β Methylene ATP.

3.3 α_1-adrenoceptor agonist-induced contractile response of VD

Maximum contractile response to Phe (1 mmol L^{-1}) was significantly increased ($P < 0.01$) in the *L*-NAME hypertensive groups (Table 2, Figure 2). This response was not normalized by sildenafil. At low concentrations ($< 10^{-4}$ mol L^{-1}), there was no difference in contractions to Phe in the different groups (Figure 2). pD_2 values reflecting α-receptor affinity for Phe were not changed in the different groups (Table 2).

3.4 α,β-meATP-induced contractile responses of VD

The contractile response to the $P2X_1$ receptor agonist, α,β-meATP, at 1 μmol L^{-1} was augmented ($P < 0.001$) in VD from the *L*-NAME group, when compared with control (Table 2, Figure 3). At low concentrations ($< 10^{-7}$mol L^{-1}), there was no difference in contractions to α,β-meATP in the different groups (Figure 3). pD_2 values reflecting $P2X_1$-receptor affinity for α,β-meATP were not changed in the different groups (Table 2). Sildenafil treatment did not restore the enhancement of the $P2X_1$ receptor agonist-induced contraction at 1 μmol L^{-1} concentration in the VD of HTN rats (Table 2). This augmented contractile response was significantly inhibited by PPADS in the HTN groups (1.060 ± 0.013 *vs.* 0.400 ± 0.085, $P < 0.001$). Although inhibition percentage by PPADS was 72% in control, there was no significant difference in HTN and sildenafil treated HTN groups when compared with controls in the absence of PPADS.

4 Discussion

The contractile characteristics of the VD from *L*-NAME group differed significantly from those of the normotensive group—specifically 1) the contractile responses of the VD in response to EFS, a P2X purinoceptor agonist, or an α_1-adrenoceptor agonist were more pronounced; 2) although sildenafil had a protective effect on the neural responses of the VD in the HTN rat group, an overall significant functional benefit was not observed; 3) these results show that the neural release of ATP by electrical stimulation of VD may regulate PDE5 enzyme expression in HTN.

This study shows that the electrically evoked contractile response of VD in *L*-NAME-induced HTN rats was enhanced by 52.4%. Previous studies have also reported increased muscular contraction to EFS in VD of SHR rats [5, 17, 19]. Katsuragi *et al*. [17] have reported that twitch contractions evoked by EFS at frequencies ranging from 4 to 16 Hz were much greater in SHR than in Wistar Kyoto (WKY). In addition, the maximal nerve-mediated contractions in the presence of PPADS were inhibited by 69.9% in VD from HTN rats. This suggests that the enhanced contraction of the VD in *L*-NAME hypertensive rats are produced by increased ATP release, as the amplitude can be taken as an index of the amount of neurotransmitter released by each stimulus. Furthermore, it is possible that ATP is the major contributor to neural stimulation in the VD from HTN.

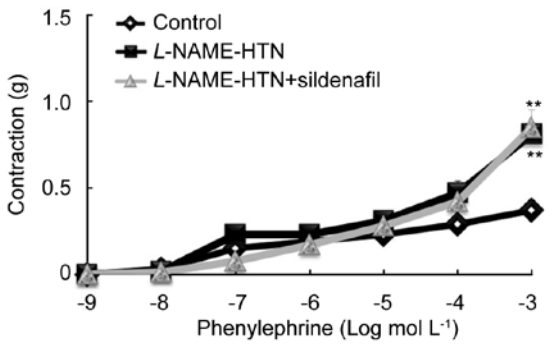

Figure 2. Dose-response graphs showing phenylephrine (10^{-8} to 10^{-3} mol L^{-1})-induced contractions of the prostatic end of the VD in organ bath preparations. Data are represented as mean ± SEM (n = 8–10). $^{**}P < 0.01$, compared with control group.

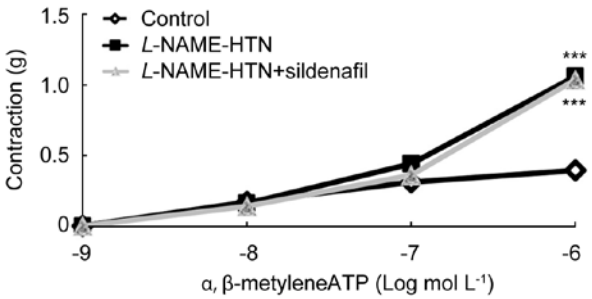

Figure 3. Dose-response graphs showing: α,β-meATP (10^{-9} to 10^{-6} mol L^{-1})-induced contractions of the prostatic end of the VD in organ bath preparations. Data are represented as mean ± SEM (n = 8–10). $^{***}P < 0.001$, compared with control group.

Prostatic VD segments were used in all experiments. In this segment, contraction is mostly due to release of ATP from sympathetic nerve terminals caused by EFS [22, 23]. We have thus confirmed that the increased responsiveness to EFS observed in the prostatic VD is due to an enhanced purinergic component in the co-transmission process in HTN animals. Interestingly, sildenafil treatment returned the augmented ATP release caused by EFS in a similar manner to PPADS. Sildenafil has an antagonistic effect on the ATP response, which may act by presynaptic purinergic P2X receptor blockade and/or against NO. A previous study reported that sildenafil antagonizes the ATP response noncompetitively in VD, similar to PPADS [24]. On the other hand, an increased release of NO has been shown experimentally by exogenously administering ATP [25].

In rodent VD, EFS of sympathetic nerves releases ATP that stimulates post-synaptic purinergic $P2X_1$ receptor (purinergic contractions) [26]. Burnstock *et al*. [27] reported that α,β-meATP was ~100 times more potent than ATP in producing phasic contraction of VD smooth muscle. In our study, α,β-meATP, a selective $P2X_1$ and $P2X_3$ receptor agonist, induced contractions in the VD at a dose of 1 μmol L^{-1}. A repeated dose of agonist α,β-meATP produced desensitization to subsequent additions. PPADS blocked α,β-meATP-induced contraction, and sildenafil had no effect on the P2X receptor in VD from HTN rats. Guitart *et al*. [18] suggested a greater post-synaptic contribution of electrically evoked ATP in the increased responsiveness to EFS. The difference in sildenafil between presynaptic ATP release and post-synaptic ATP contractile effects may be due to different subtypes of the P2X receptor. We did not measure the release of ATP and related subtype of P2X receptor in HTN rats. During neural stimulation, it is noted that ATP activates $P2X_3$ receptors regarding nociceptive signaling as a pain mediator [28, 29]. Perhaps, ejaculation in the HTN rats of the present study may be painful. In previous data, painful ejaculation in the chronic prostatitis syndrome was significantly associated to the sonographic demonstration of enlargement, asymmetry or inflammatory changes of the seminal vesicles [30]. Furthermore, $P2X_3$-null mice have reduced pain-related behavior in response to injection of ATP [31]. In this study, the restorative effect of sildenafil on presynaptic neurogenic effect of ATP may be related to the reduction of pain-related mechanisms in *L*-NAME hypertensive rats.

The post-synaptic effects are induced by $P2X_1$ and other subtypes [32]. A study by Burnstock and co-workers revealed that $P2X_1$ and $P2X_2$ exist in the membranes of the smooth muscle layer, suggesting that these receptors are involved in the process of sperm transport and ejaculation [33]. Furthermore, there is no available data on interactions between post-synaptic P2X receptors and the NO/cGMP pathway. The relaxant effects of ATP may be mediated by P2Y receptor subtypes in addition to P2X receptor subtypes. Buvinic *et al*. [34] reported that $P2Y_1$ and $P2Y_2$ receptors are coupled to the NO/cGMP pathway. However, in our study, the contractile effects of P2X receptor subtypes are dominant over P2Y receptor effects on ATP, suggesting that the majority of relaxant effects of ATP in VD are mediated by P2X receptors.

Enhancement of Phe-induced cumulative concentration-dependent contractions occurred in the HTN rat prostatic VD portion. It is known that NA-induced contractions are associated with an increase in cytosolic calcium concentration through α_1-adrenoceptors [35]. In earlier studies, maximal effects induced by NA were significantly greater in SHR, without changes in affinity [14]. Katsuragi *et al*. [17] reported that the endogenous NA content in VD and electrically evoked release of NA from tissues was identical in SHR and WKY rats. As there was an increase in the maximum response, the possible mechanism underlying the hyperactivity of α_1-adrenoceptors in SHR was hypothesized to be an enhanced activity of protein kinase C by the activation of phospholipase C, regulating the intracellular Ca^{2+} messenger system because of post-signaling or receptor mechanisms [36]. PPADS, a P2 receptor antagonist, did not affect NA-induced contractions (data not shown). This finding suggests that *L*-NAME-induced HTN sensitizes the VD to depolarizing stimuli and has a facilitatory effect on α-receptors. Perhaps, the NA concentration in the synaptic cleft may activate presynaptic autoreceptors, which in turn suppresses further transmitter release, thereby maintaining a steady level of NA within the neurovascular junction.

On the basis of this novel data, we suggest that sildenafil may have beneficial effects in several chronic conditions and improve quality of life for treated men with premature ejaculation. PDE5 inhibitors have previously been suggested to help men with premature ejaculation, possibly by a peripheral inhibition of the contractile response of the VD, seminal vesicles, prostate

and urethra, as well as a reduced central sympathetic output through a prolonged NO effect [37]. Perhaps the combination of sildenafil with purinergic P2X receptor inhibitors and/or paroxetine might provide an even better response. Further well-designed clinical studies are needed to determine whether there is any role for sildenafil in the treatment of premature ejaculation in hypertensive patients. In summary, we postulate that ATP-induced purinergic contractions of VD seem to be very forceful at pre- and post-junctional levels. These contractions are responsible for sperm passage through the VD [5]. Enhanced contractile activity of the VD by purinergic contractions rather than by adrenergic contractions may be responsible for propulsion of sperm in patients with premature ejaculation. We further propose that in the HTN model, by NO deprivation, this is a new pathophysiological concept for study of premature ejaculation, and may be used for developing new therapeutic agents.

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