# Alpha1-adrenergic antagonists act as 6-nitrodopamine receptor antagonists in the human vas deferens

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# Abstract

Background: 6-Nitrodopamine (6-ND) is released from human vas deferens and plays a modulatory role in the male ejaculation. Therapeutical use of $\alpha_1$-adrenoceptor antagonists is associated with ejaculatory abnormalities.

Objectives: To evaluate the effect of $\alpha_1$-adrenoceptor antagonists on the contractions induced by 6-ND, dopamine, noradrenaline, and adrenaline in the human epididymal vas deferens (HEVD).

Methods: HEVD strips were suspended in glass chambers containing heated and oxygenated Krebs-Henseleit's solution. Cumulative concentration-response curves to catecholamines (10 nM-300 mM) were constructed in HEVD strips pre-incubated (30 min) with doxazosin (0.1-1nM), tamsulosin (1-10 nM), prazosin (10-100 nM) and/or silodosin (0.1-10 nM). The effects of these $\alpha_1$-adrenoceptor antagonists were also evaluated in the electric-field stimulation (EFS, 2-32 Hz)-induced contractions.

Results: Doxazosin (0.1 nM) caused significant reductions in 6-ND-induced HEVD contractions without affecting the contractions induced by dopamine, noradrenaline, and adrenaline. Similar results were observed with tamsulosin (1 nM) and prazosin (10 nM). At these concentrations, these $\alpha_1$-adrenoceptor antagonists largely reduced the EFS-induced contractions. Silodosin (1 nM) caused concentration-dependent rightward shifts of the concentration-response curves to 6-ND but had no effect on the contractions induced by dopamine and adrenaline. Silodosin (0.1 nM) only inhibited the contractions induced by noradrenaline. Silodosin at 1 nM, but not at 0.1 nM, caused significant reductions in the EFS-induced contractions.

Discussion and conclusion: The results indicate that 6-ND plays a major role in the human vas deferens contractility and doxazosin, tamsulosin, prazosin and silodosin cause ejaculation disorders in man by blocking the 6-ND receptor rather than $\alpha_1$-adrenoceptors.


# INTRODUCTION

6-nitrodopamine (6-ND) is a novel catecholamine that is released from human umbilical cord vessels (Britto-Júnior et al., 2021a), human popliteal artery and vein (Oliveira et al., 2023) and human vas deferens (Britto-Júnior et al., 2022a), as detected by liquid chromatography coupled to tandem mass spectrometry. Although in human vascular tissues 6-ND acts mainly as a potent vasorelaxant, in human vas deferens 6-ND causes smooth muscle contraction, which is blocked by tricyclic antidepressants such as amitriptyline and desipramine, at concentrations that do not affect the spasmogenic actions of dopamine, noradrenaline, and adrenaline (Britto-Júnior et al., 2022a). Similar results have been observed in the rat epididymal vas deferens (Britto-Júnior et al., 2021b).

The $\alpha_1$-adrenoceptor family is composed of three receptor subtypes, namely $a_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$ (Andersson et al., 2000). All three receptor subtypes are expressed in the human prostate, although in different locations (Walden et al., 1999). Blockade of $\alpha_1$-adrenoceptors play an important role in the clinical

management of patients with lower urinary tract symptoms (LUTS) caused by prostate hyperplasia (Roehrborn and Schwinn, 2004), and the $\alpha_1$-adrenoceptor antagonists doxazosin, tamsulosin and silodosin are often used for LUTS relief (Debruyne, 2000). Although the treatment with $\alpha_1$-adrenoceptor antagonists is generally well tolerated, one of the most frequent adverse reactions is abnormal ejaculation, as characterized by delayed ejaculation or absence of ejaculation during orgasm (Cho and Yoo, 2014). These adverse reactions could be due to inhibition of the emission phase of the ejaculation, in which the contraction of prostate, vas deferens, seminal vesicles and cauda epididymis occurs (Alwaal et al., 2015). Interestingly, $\alpha_1$-adrenoceptor antagonists are now clinically used for the treatment of premature ejaculation (Gul et al., 2022).

In the rat isolated epididymal vas deferens, the contractions induced by 6-ND and by electric-field stimulation (EFS) are antagonized by pre-treatment of the vas deferens with doxazosin, tamsulosin, alfuzosin, terazosin and prazosin, at concentrations that do not block the contractions induced by dopamine, noradrenaline and adrenaline (Britto-Júnior et al., 2022c), indicating that these drugs could be acting as selective antagonists of 6-ND receptor rather than as $\alpha_1$-adrenoceptor antagonists. Fluorescence histochemistry and biochemical detection of catecholamines revealed significant differences between rat and human vas deferens (Kaleczyc, 1998), i.e., the former receives numerous adrenergic nerve fibres (Owman & Sjöstrand, 1965), whereas the latter presents less dense adrenergic innervation (Alm, 1982; Baumgarten et al., 1968). Thus, we have investigated whether $\alpha_1$-adrenoceptor antagonists would have inhibitory action on the contractions induced by 6-ND in human vas deferens.

# MATERIALS AND METHODS

## Study participants

Participants who underwent vasectomy surgery in Centro Clínico NotreDame Intermedicas (Jundiaí, Sao Paulo) signed an informed consent approved by the Institute of Biomedical Sciences (ICB-USP) Institutional Review Board (Protocol number 4.468.508). The human epididymal vas deferens (HEVD) were obtained from 125 participants aged 28–59 years. The surgical procedure (Rogers & Kolettis, 2013) was performed under local anaesthesia and the excised segment (1.5 cm lenght) was taken at approximately 9 cm from the cauda epididymis.

## HEVD functional assays

The HEVD strips (1.5 cm length) were suspended in a 10-mL glass chamber containing heated (37°C) and oxygenated (95%$O_2$: 5%$CO_2$) KHS. The isometric tension was kept at 10 mN and registered by a PowerLab system (ADInstruments, Dunedin, New Zealand). After a 45-min stabilization period, the HEVD strips were initially contracted with potassium chloride (KCl, 80 mM) to evaluate the tissue viability. After KCl removal and return to the baseline (15 min approximately), cumulative concentration-response curves to 6-ND (0.00001-1 mM) were constructed in control HEVD strips and in tissues pre-incubated with doxazosin (0.1, 0.3 and 1 nM, 30 min), tamsulosin (0.1, 1 and 10 nM, 30 min), prazosin (10, 30 and 100

nM, 30 min) and/or silodosin (0.1, 1, 3 and 10 nM, 30 min). Cumulative concentration-response curves to noradrenaline were constructed in HEVD pre-incubated or not with doxazosin (0.1 nM, 30 min), tamsulosin (1 nM, 30 min), prazosin (10 nM, 30 min) and/or silodosin (0.1 and 10 nM, 30 min). Cumulative concentration-response dopamine and adrenaline were constructed in HEVD pre-incubated or not with doxazosin (0.1 nM, 30 min), tamsulosin (1 nM, 30 min), prazosin (10 nM, 30 min) and/or silodosin (1 nM, 30 min).

# Electric-field stimulation (EFS) in HEVD preparations

The HEVD strips were submitted to EFS (60 V for 20 sec, at 2–32 Hz in square-wave pulses, 0.3 ms pulse width, and 0.1 ms delay), using a Grass S88 stimulator (Astro-Medical, Industrial Park, RI, USA). The EFS-induced HEVD contractions were conducted in control and tissues pre-incubated with doxazosin (0.1 nM, 30 min), tamsulosin (1 nM, 30 min), prazosin (10 nM, 30 min) and/or silodosin (0.1 and 1 nM, 30 min).

# Materials and drugs

Dopamine and prazosin were obtained from Sigma-Aldrich Chemicals Co. (St Louis, Missouri, USA). Adrenaline, noradrenaline and silodosin were purchased from Cayman Chemical Co (Michigan, USA). 6-Nitrodopamine was acquired from Toronto Research Chemicals (Ontario, CA). Tamsulosin was obtained from Swati Spentose Pvt Ltd (Vapi, Gujarat, India). Doxazosin was obtained from Nifty Labs Pvt Ltd (Hyderabad, Telangana, India). Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), sodium bicarbonate ($NaHCO_3$), potassium phosphate monobasic ($KH_2PO_4$) and glucose were acquired from Merck KGaA (Darmstadt, Germany).

# Data Analysis

The values of $pEC_{50}$ and Emax data represent the mean ± standard error of the mean (S.E.M.) of *n* experiments. Values of $E_{max}$ were expressed in mN. One was used as the control response and the other HEVD was incubated with an antagonist / inhibitor.

The $pA_2$ values of the antagonists were calculated from application of the equation; $pA_2$ = log (antagonist concentration) – log (CR-1) -log (antagonist concentration). The methods for determining both pEC50 and Emax, and the statistical tests employed, were described elsewhere (Britto-Júnior et al., 2022a).

## RESULTS

# Effect of doxazosin

Cumulative addition of 6-ND ($10^{-8}$ to $10^{-3}$ M; Fig. 1A), dopamine ($10^{-8}$ to $10^{-3}$ M; Fig. 1B), noradrenaline ($10^{-8}$ to $3\times10^{-4}$ M; Fig. 1C) and adrenaline ($10^{-8}$ to $3\times10^{-4}$ M; Fig. 1D) produced concentration-dependent contractions in the HEVD strips (Fig. 1A). The in vitro pre-treatment (30 min) of the HEVD with doxazosin (0.1-1 nM) significantly reduced the Emax values to 6-ND with a $pA_2$ value of *10.38* ± 0.20 (n = 6). However, doxazosin (0.1 nM) did not affect Emax and $pEC_{50}$ the HEVD contractions induced by

dopamine (Fig. 1B), noradrenaline (Fig. 1C) and adrenaline (Fig. 1D). The $pEC_{50}$ and Emax data for 6-ND, dopamine, noradrenaline and adrenaline in control and doxazosin-treated preparations are shown in Table 1.

Table 1
The effect of doxazosin on the potency ($pEC_{50}$) and maximal responses ($E_{max}$) of the contractions induced by 6-nitrodopamine (6-ND), noradrenaline (NA), adrenaline (ADR), and dopamine (DA) in the human isolated epididymal vas deferens.

| Catecholamine | $pEC_{50}$ (log[M]) | $E_{max}$ (mN) | n |
|---|---|---|---|
| **6-ND** | 4.10 ± 0.15 | 57.08 ± 8.49 | 6 |
| + doxazosin 0.1 nM | 3.71 ± 0.19* | 44.66 ± 10.98 | 6 |
| + doxazosin 0.3 nM | 3.54 ± 0.22* | 35.66 ± 7.87* | 6 |
| + doxazosin 1 nM | 4.20 ± 0.31 | 20.01 ± 8.10* | 4 |
| **DA** | 3.99 ± 0.30 | 33.53 ± 12.01 | 6 |
| + doxazosin 1 nM | 4.01 ± 0.30 | 41.77 ± 13.98 | 6 |
| **NA** | 4.19 ± 0.14 | 31.9 ± 11.8 | 4 |
| + doxazosin 1 nM | 4.04 ± 0.31 | 26.7 ± 5.1 | 4 |
| **ADR** | 4.85 ± 0.19 | 35.4 ± 17.1 | 4 |
| + doxazosin 1 nM | 5.06 ± 0.52 | 33.6 ± 25.8 | 4 |

*pEC50 is defined as the negative logarithm of the $EC_{50}$. Emax* is the maximal effect at the highest drug concentration. The *$pEC_{50}$and Emax were expressed as mean ± SEM (n means the number of vas deferens strips). *P < 0.05 compared with respective control values.*

Electrical-field stimulation (EFS; 2–32 Hz) evoked frequency-dependent contractile responses in the HEVD strips (Fig. 1E), which were significantly reduced by doxazosin (0.1 nM) in all frequencies tested (p = 0.0001; n = 6; Fig. 1E).

# Effect of tamsulosin

Pre-treatment (30 min) of the HEVD with tamsulosin (1 and 10 nM) caused significant rightward shifts on the concentration-response curves to 6-ND (Fig. 2A) with a $pA_2$ value of 9.63 ± 0.06 (n = 5). At 10 nM, tamsulosin nearly abolished the 6-ND-induced contractions (Fig. 2A). Tamsulosin (1 nM) did not affect the HEVD contractions induced by dopamine (Fig. 2B), noradrenaline (Fig. 2C) and adrenaline (Fig. 2D). The $pEC_{50}$ and Emax data for 6-ND, dopamine, noradrenaline and adrenaline in control and tamsulosin-treated preparations are shown in Table 2.

Table 2
The effect of tamsulosin on the potency ($pEC_{50}$) and maximal responses ($E_{max}$) of the contractions induced by 6-nitrodopamine (6-ND), noradrenaline (NA), adrenaline (ADR), and dopamine (DA) in the human isolated epididymal vas deferens.

| Catecholamine | $pEC_{50}$ (log[M]) | $E_{max}$ (mN) | n |
|---|---|---|---|
| **6-ND** | 4.01 ± 0.12 | 41.93 ± 4.52 | 8 |
| + tamsulosin 0.1 nM | 4.00 ± 0.10 | 45.75 ± 6.63 | 4 |
| + tamsulosin 1 nM | 3.45 ± 0.07* | 37.36 ± 11.47 | 5 |
| + tamsulosin 10 nM | | 11.58 ± 7.01* | 4 |
| **DA** | 4.59 ± 0.22 | 30.23 ± 0.91 | 3 |
| + tamsulosin 1 nM | 4.03 ± 0.10 | 37.54 ± 5.06 | 3 |
| **NA** | 4.37 ± 0.11 | 40.32 ± 9.45 | 6 |
| + tamsulosin 1 nM | 4.38 ± 0.08 | 42.01 ± 5.21 | 6 |
| **ADR** | 5.03 ± 0.18 | 37.15 ± 19.33 | 3 |
| + tamsulosin 1 nM | 4.86 ± 0.24 | 35.93 ± 16.99 | 3 |

*pEC50 is defined as the negative logarithm of the $EC_{50}$. Emax* is the maximal effect at the highest drug concentration. The *pEC50* and *Emax were expressed as mean ± SEM (n means the number of vas deferens strips). *P < 0.05 compared with respective control values.*

Tamsulosin (1 nM) caused marked reductions (p = 0.0002) in the HEVD contractions induced by EFS (2–32 Hz; Fig. 2E).

# Effect of prazosin

Pre-treatment (30 min) of the HEVD with prazosin (10–100 nM) caused significant rightward shifts on the concentration-response curves to 6-ND (Fig. 3A) with a $pA_2$ value of *8.23* ± 0.52 (n = 6). At 100 nM, prazosin markedly reduced the maximal responses to 6-ND (Fig. 3A). Prazosin (10 nM) did not affect the HEVD contractions induced by dopamine (Fig. 3B), noradrenaline (Fig. 3C) and adrenaline (Fig. 3D). The $pEC_{50}$ and Emax data for 6-ND, dopamine, noradrenaline and adrenaline in control and prazosin-treated preparations are shown in Table 3.

Table 3
The effect of prazosin on the potency ($pEC_{50}$) and maximal responses ($E_{max}$) of the contractions induced by of 6-nitrodopamine (6-ND), noradrenaline (NA), adrenaline (ADR), and dopamine (DA) in the human isolated epididymal vas deferens.

| Catecholamine | $pEC_{50}$ (log[M]) | $E_{max}$ (mN) | n |
|---|---|---|---|
| **6-ND** | 4.62 ± 0.11 | 62.55 ± 8.31 | 6 |
| + prazosin 10 nM | 4.08 ± 0.25* | 49.64 ± 6.67 | 6 |
| + prazosin 30 nM | 3.47 ± 0.30* | 58.42 ± 13.26 | 4 |
| + prazosin 100 nM | 3.12 ± 1.24* | 27.89 ± 11.66* | 4 |
| **DA** | 4.62 ± 0.05 | 33.42 ± 9.26 | 4 |
| + prazosin 10 nM | 4.55 ± 0.10 | 31.39 ± 9.44 | 4 |
| **NA** | 4.73 ± 0.34 | 32.25 ± 4.9 | 4 |
| + prazosin 10 nM | 4.68 ± 0.22 | 34.79 ± 7.55 | 4 |
| **ADR** | 5.14 ± 0.29 | 57.76 ± 11.25 | 3 |
| + prazosin 10 nM | 5.19 ± 0.33 | 54.14±14.53 | 3 |

*pEC50 is defined as the negative logarithm of the $EC_{50}$. Emax* is the maximal effect at the highest drug concentration. The *$pEC_{50}$and Emax were expressed as mean ± SEM. n means the number of vas deferens strips. *P < 0.05 compared with respective control values.*

Prazosin (10 nM) caused significant reductions (p = 0.0042) in the HEVD contractions induced by EFS (2–32 Hz; Fig. 3E).

# Effect of silodosin

Pre-treatment of the HVED with silodosin (1–10 nM) caused significant rightward shifts on the concentration-response curves to 6-ND (Fig. 4A) with a $pA_2$ value of 9.84 ± 0.06 (n = 3). At 10 nM, silodosin almost abolished the contractions induced by 6-ND (Fig. 4A). Silodosin (1 nM) did not affect the HEVD contractions induced by dopamine (Fig. 4B) and adrenaline (Fig. 4D). Silodosin (0.1 and 1 nM) caused significant rightward shifts on the concentration-response curves to noradrenaline (Fig. 4C) with a $pA_2$ value of 10.71 ± 0.12 (n = 6). The $pEC_{50}$ and Emax data for 6-ND, dopamine, noradrenaline and adrenaline in control and silodosin-treated preparations are shown in Table 4.

Table 4
The effect of silodosin on the potency ($pEC_{50}$) and the maximum response ($E_{max}$) of the contractions induced by of 6-nitrodopamine (6-ND), noradrenaline (NA), adrenaline (ADR), and dopamine (DA), in the human isolated epididymal vas deferens.

| Catecholamine | $pEC_{50}$ (log[M]) | $E_{max}$ (mN) | n |
|---|---|---|---|
| **6-ND** | 3.93 ± 0.16 | 45.43 ± 7.38 | 7 |
| + silodosin 0.1 nM | 4.15 ± 0.12 | 40.81 ± 17.69 | 3 |
| + silodosin 1 nM | 3.19 ± 0.13* | 21.57 ± 5.94* | 3 |
| + silodosin 3 nM | - | 27.53 ± 7.26* | 4 |
| + silodosin 10 nM | - | 6.02 ± 1.18* | 4 |
| **DA** | 4.35 ± 0.23 | 28.71 ± 8.33 | 4 |
| + silodosin 1 nM | 4.09 ± 0.32 | 25.50 ± 6.44 | 4 |
| **NA** | 5.04 ± 0.17 | 49.37 ± 8.6 | 10 |
| + silodosin 0.1 nM | 4.51 ± 0.22* | 38.82 ± 12.96 | 4 |
| + silodosin 1 nM | 3.56 ± 0.35* | 36.56 ± 6.33 | 6 |
| **ADR** | 4.92 ± 0.15 | 38.68 ± 8.83 | 6 |
| + silodosin 1 nM | 4.63 ± 0.25 | 46.98 ± 14.90 | 6 |

*pEC50 is defined as the negative logarithm of the $EC_{50}$. Emax* is the maximal effect at the highest drug concentration. The *pEC50, Emax* and *the mean shifts were expressed as mean ± SEM (n means the number of vas deferens strips). *P < 0.05 compared with respective control values.*

Silodosin (0.1 nM) did not affect the EFS-induced HEVD contractions (Fig. 4E). However, at 1 nM, silodosin caused significant reductions in the HEVD contractions induced by EFS (Fig. 4F).

# DISCUSSION

Incidence of abnormal ejaculation is a typical adverse reaction of $\alpha_1$-adrenoceptor antagonists (Michel, 2007). This class of drugs induces relaxations of prostate, urethra and bladder neck smooth muscle (Andersson, 2000), reducing the resistance of the prostatic urethra and bladder neck to the ejaculate coming from the vas deferens, thus allowing retrograde ejaculation to occur (van Dijk et al., 2006). However, in healthy volunteers treated for 5 days with either tamsulosin or alfuzosin, few individuals (95%) had increased post-ejaculate in the urine, indicating that $\alpha_1$-adrenoceptor antagonists may cause anejaculation rather than retrograde ejaculation (Hellstrom et al., 2005); Thus, evaluation of the effect of these compounds in human vas deferens and seminal vesicles may provide useful information on the mechanism(s) involved.

Silodosin (KMD-3213; Shibata et al., 1995) has an increased affinity to adrenoceptor $\alpha_{1A}$ (0.039 nM) compared to $\alpha_{1B}$ (6.5 nM) and $\alpha_{1D}$ (2.2 nM; Tatemichi et al., 2006) in the lower urinary tract. In comparison to the $\alpha_1$-adrenoceptor antagonists alfuzosin (Ramsay et al., 1988), doxazosin (Elliott et al., 1982; Wilt and MacDonald, 2006), tamsulosin (Lepor et al., 1988; Dunn et al., 2002), terazosin (Frishman et al., 1988; O'Leary 2001), and prazosin (U'Prichard et al., 1978), silodosin was the most potent antagonist on the contractions induced by noradrenaline in the rat epididymal vas deferens (Britto-Júnior et al., 2022c). As shown here, silodosin was also more potent than tamsulosin, doxazosin and prazosin to inhibit noradrenaline-induced HEVD contractions. Interestingly, silodosin was the only $\alpha_1$-adrenoceptor antagonist that was more potent to inhibit HEVD contractions induced by noradrenaline, as compared to those induced by 6-ND. Since tamsulosin and silodosin are the $\alpha_1$-adrenoceptor antagonists more associated with abnormal ejaculation (Cui et al., 2012), selectivity for the $\alpha_{1A}$-adrenoceptor subtype could be the mechanism involved in this adverse reaction. Indeed, incidence of abnormal ejaculation in patients treated with prazosin is rare, and in the rat isolated vas deferens, prazosin presents low potency on $\alpha_{1A}$- and high potency at $\alpha_{1D}$-adrenoceptor (Docherty, 2013).

6-Nitrodopamine concentration-dependently contract both rat (Britto-Júnior et al., 2021b) and human (Britto-Júnior et al., 2022a) vas deferens, but more importantly, potentiates the contractile action of the dopamine, noradrenaline, and adrenaline in rat epididymal vas deferens at a very low concentration such as 10 pM (Britto-Júnior et al., 2023a). Although this mechanism is not clear, the potentiation is abolished when the vas deferens is pre-treated with the voltage-gated sodium channel blocker tetrodotoxin, suggesting that may be due to activation of adrenergic terminals (Britto-Júnior et al., 2023a). A similar potentiation of 6-ND on the increased atrial frequency induced by dopamine, noradrenaline and adrenaline was observed in the rat isolated atria (Britto-Júnior et al., 2023b). The blockade of $\alpha_{1A}$ adrenoceptors in the rat vas deferens could lead to infertility (Michel et al., 2007); however, in $\alpha_1$-adrenoceptor subtype-selective knockout mice ($\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptor knockout), the contractions of the vas deferens induced by electric-field stimulation was reduced by 60–70%, but not abolished (Sanbe et al., 2007), indicating the presence of another receptor responsible for the observed contractions. This receptor could be the 6-ND receptor. Our finding that silodosin, at a concentration (100 pM) that caused marked reductions in noradrenaline-induced contractions, did not affect the contractions induced by EFS, reinforces the concept that 6-ND is a major modulator of human vas deferens contractility (Britto-Júnior et al., 2022a). Indeed, inhibition of the EFS-induced HEVD contractions was only observed when the $\alpha_1$-adrenoceptor antagonists were used at concentrations that inhibited those induced by 6-ND.

Inconsistencies in the interactions of $\alpha_1$-adrenoceptor antagonists and ejaculatory function have been observed in mouse, rat and human data (Michel, 2007), and as summarized in Table 5, they are also present in the pharmacology of 6-ND in rat and human vas deferens. The remarkable synergism between 6-ND and the classical catecholamines (dopamine, noradrenaline, and adrenaline) has been described so far only on the rat epididymal vas deferens (Britto-Júnior et al., 2023a) and rat isolated right atrium

(Britto-Júnior et al., 2023b). Thus, it would be important to confirm the synergism in HEVD. Furthermore, purification and sequence of the 6-ND receptor from human vas deferens should help to deep our understanding on the physiological processes involved in the ejaculation.

Table 5
6-ND pharmacology in rat and human vas deferens

| | Rat | Ref | Human | Ref |
|---|---|---|---|---|
| 6-ND release (ng/mL) | 1.4±0.4 | Britto-Júnior et al., 2021b | 1.9±0.8 | Britto-Júnior et al., 2022a |
| Noradrenaline (ng/mL) | 0.4±0.2 | Britto-Júnior et al., 2021b | 0.5±0.2 | Britto-Júnior et al., 2022a |
| Dopamine release (ng/mL) | - | Britto-Júnior et al., 2021b | 0.3±0.1 | Britto-Júnior et al., 2022a |
| Adrenaline release (ng/mL) | - | Britto-Júnior et al., 2021b | 0.2±0.1 | Britto-Júnior et al., 2022a |
| 6-ND contractile ($pEC_{50}$) | 5.2±0.2 | Britto-Júnior et al., 2023a | **4.6±0.1** | this manuscript |
| Abolished by TTX | + | Lima et al., 2022 | ? | |
| L-NAME on 6-ND release | ↓ | Britto-Júnior et al., 2021b | ↓ | Britto-Júnior et al., 2022a |
| L-NAME on EFS contraction | - | Britto-Júnior et al., 2021b | ↓ | Britto-Júnior et al., 2022a |
| $b_1$ and $b_1/b_2$ antagonists | + | Lima et al., 2022 | ? | |
| Potentiates DA, NA and ADR | + | Britto-Júnior et al., 2023a | ? | |
| Tricyclic compounds ($pA_2$): | | | | |
| Amitriptyline | 7.7±0.5 | Britto-Júnior et al., 2021b | 8.9±0.1 | Britto-Júnior et al., 2022a |
| Desipramine | 8.2±0.1 | Britto-Júnior et al., 2021b | 9.5±0.1 | Britto-Júnior et al., 2022a |
| Carbamazepine | 7.7±0.4 | Britto-Júnior et al., 2021b | 8.8±0.1 | Britto-Júnior et al., 2022a |
| $\alpha_1$ antagonists ($pA_2$): | | | | |
| Doxazosin | 9.1±0.1 | Britto-Júnior et al., 2021c | **10.3±0.2** | this manuscript |
| Tamsulosin | 9.7±0.1 | Britto-Júnior et al., 2021c | **9.3±0.1** | this manuscript |
| Silodosin | 8.8±0.1 | Britto-Júnior et al., 2021c | **9.8±0.1** | this manuscript |

| | Rat | Ref | Human | Ref |
|---|---|---|---|---|
| Prazosin | 7.7±0.2 | Britto-Júnior et al., 2021c | **8.2±0.5** | this manuscript |

# CONCLUSION

The $\alpha_1$-adrenoceptor antagonists doxazosin, tamsulosin, prazosin and silodosin only block the contractions of human isolated vas deferens at concentrations that inhibit 6-ND induced contractions reinforcing the concept that this novel catecholamine acts as a major modulator of human vas deferens contractility.

# Declarations

### Ethical Approval

The investigation followed the principles outlined in the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo – ICB/USP (protocol number 4.468.508), and the patients were asked to sign an informed consent.

### Consent to Participate

Not applicable.

### Consent to Publish

The authors authorize the submission and publication of this article in *Naunyn-Schmiedeberg's Archives of Pharmacology*

### Author Contributions Statement

Conceptualization: JBJ, GDN.

Data curation: JBJ, GDN.

Formal analysis: GDN

Funding acquisition: EA, GDN.

Investigation: RABG, JBJ, ATL, DLO, GQJ, GAOS.

Methodology: RABG, ATL.

Project administration: GDN.

Supervision:  EA and GDN.

Visualization: AF, EA, GDN.

Writing – original draft: JBJ, GDN

The authors declare that all data were generated in-house and that no paper mill was used.

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**Availability of data and materials**

The authors authorize the availability of any data used in this study.

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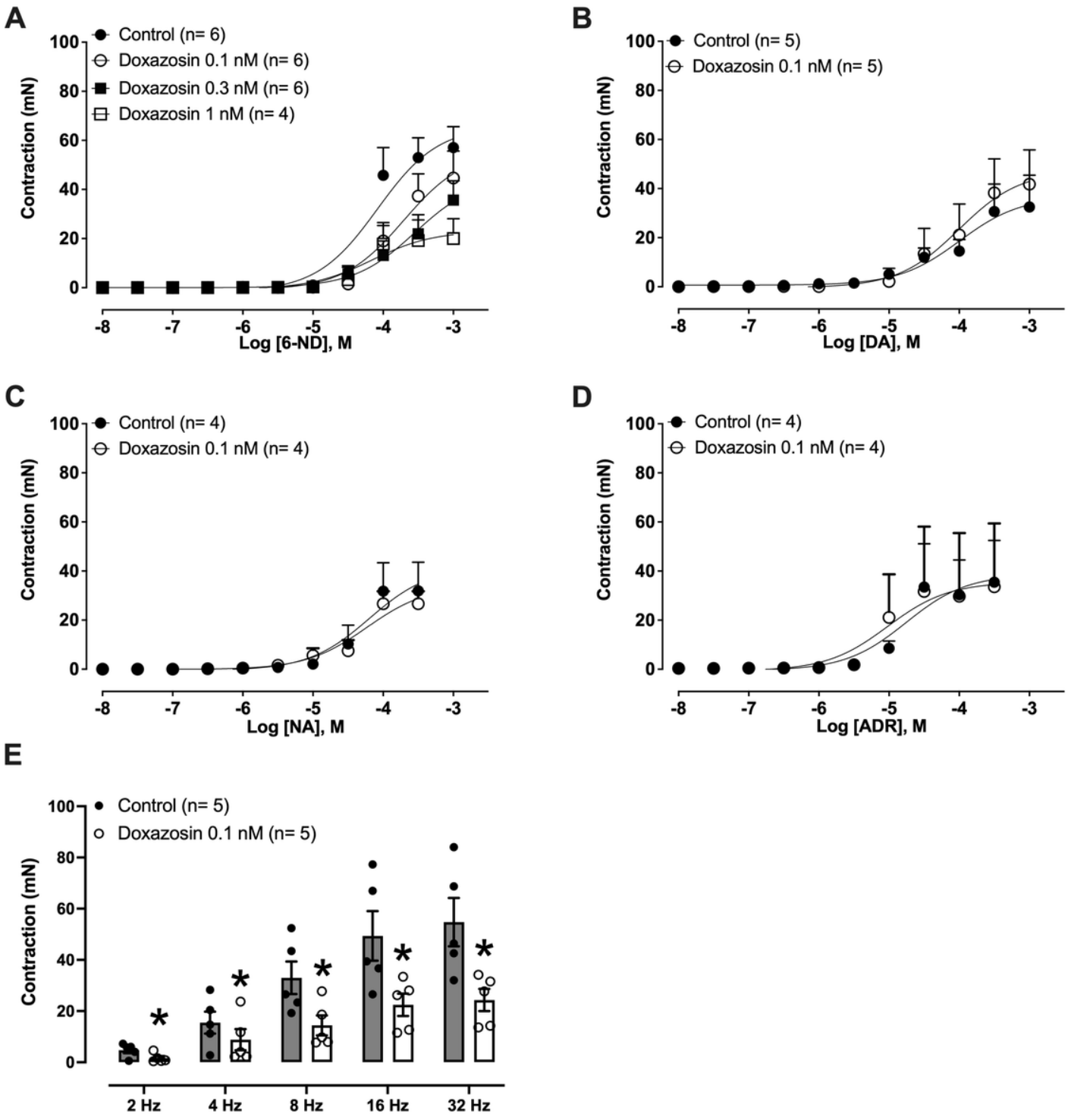
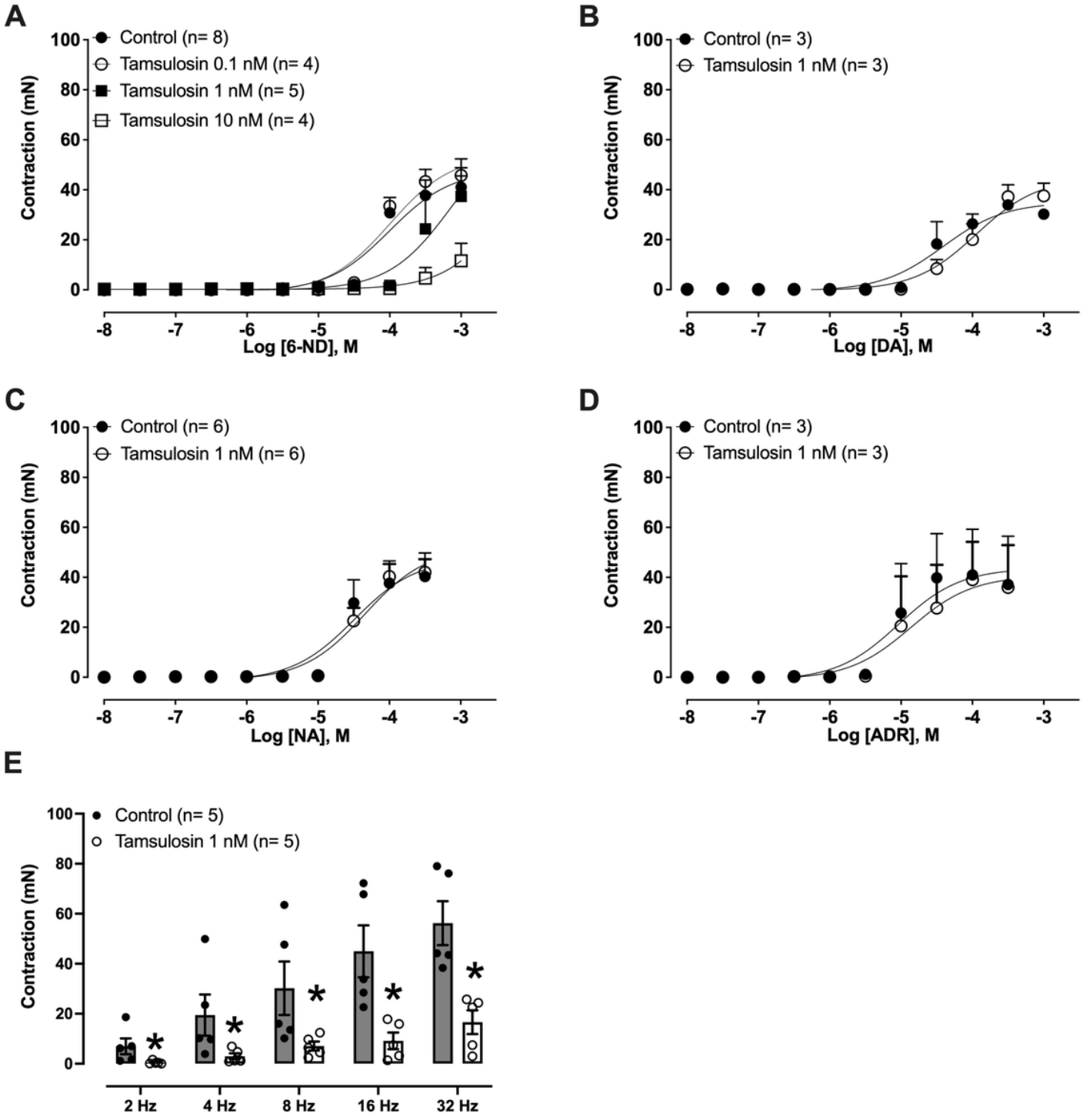
# Figures




## Figure 1

Effect of doxazosin in the human epididymal vas deferens (HEVD) contractions induced by 6-nitrodopamine (6-ND), dopamine, noradrenaline, adrenaline and electric-field stimulation (EFS). Doxazosin (0.1-1 nM) induced concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Doxazosin (0.1 nM) had no effect on the contractions induced by dopamine (DA, Panel B), noradrenaline (NA, Panel C) and adrenaline (ADR, Panel D). Doxazosin (0.1 nM; Panel E)

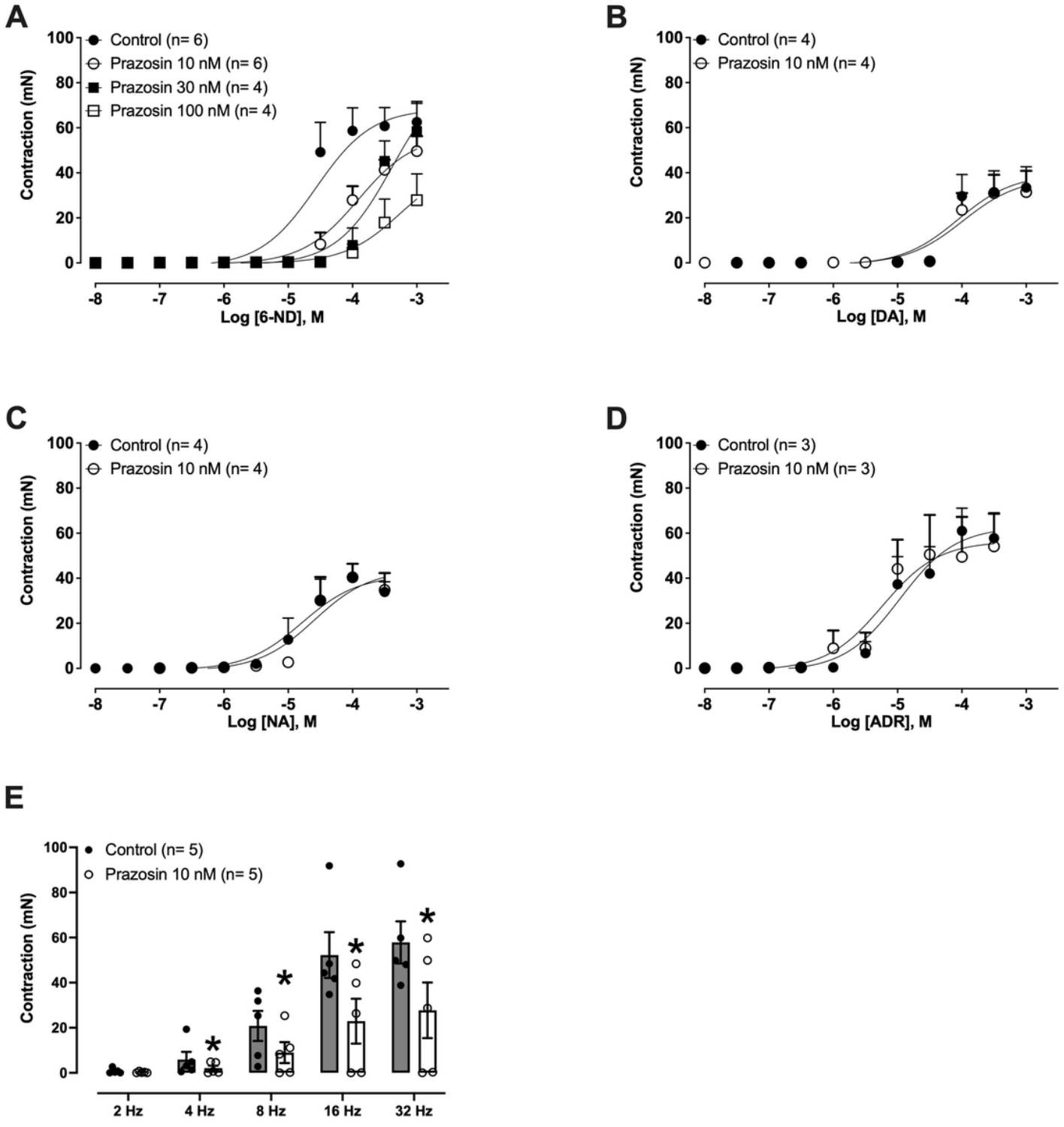
caused significant inhibition of EFS-induced HEVD contractions. Data are expressed as mean ± SEM. *P<0.05 compared with respective control values (*n* means the number of vas deferens strips).




## Figure 2

Effect of tamsulosin in the human epididymal vas deferens (HEVD) contractions induced by 6-nitrodopamine (6-ND), dopamine, noradrenaline, adrenaline and electric-field stimulation (EFS). Tamsulosin (1 and 10 nM) provoked concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Tamsulosin (1 nM) had no effect on the contractions induced by

dopamine (DA, Panel B), noradrenaline (NA, Panel C) and adrenaline (ADR, Panel D). Tamsulosin (1 nM; Panel E) caused significant inhibition of EFS-induced HEVD contractions. Data are expressed as mean ± SEM. *P<0.05 compared with respective control values (*n* means the number of vas deferens strips).




## Figure 3

Effect of prazosin in the human epididymal vas deferens (HEVD) contractions induced by 6-nitrodopamine (6-ND), dopamine, noradrenaline, adrenaline and electric-field stimulation (EFS). Prazosin

(10-30 nM) caused concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Prazosin (10 nM) had no effect on the contractions induced by dopamine (DA, Panel B), noradrenaline (NA, Panel C) and adrenaline (ADR, Panel D). Prazosin (10 nM; Panel E) caused significant inhibition of EFS-induced contractions of the HEVD. Data are expressed as mean ± SEM. *P<0.05 compared with respective control values (*n* means the number of vas deferens strips).

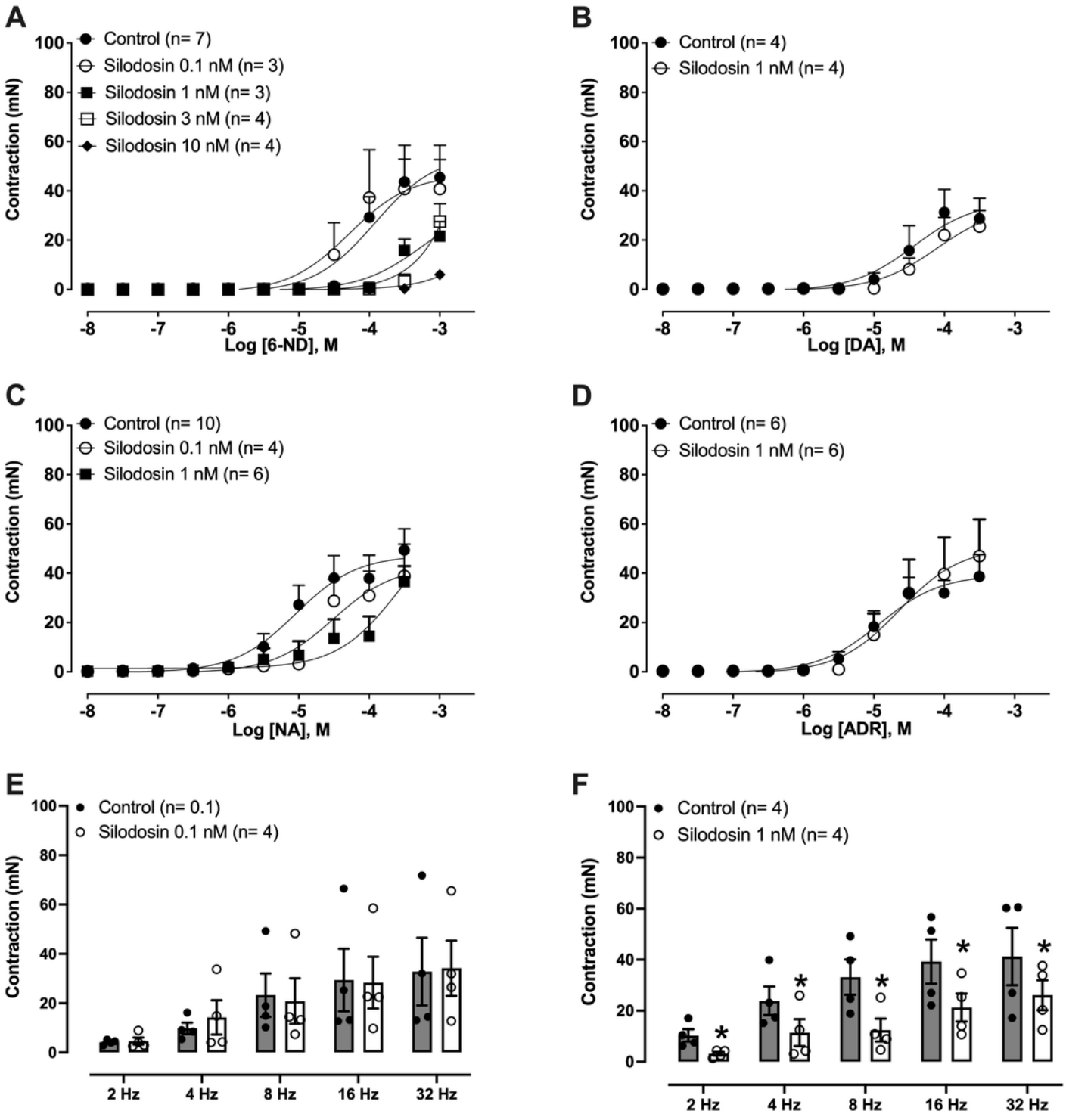



Figure 4

Effect of silodosin in the human epididymal vas deferens (HEVD) contractions induced by 6-nitrodopamine (6-ND), dopamine, noradrenaline, adrenaline and electric-field stimulation (EFS). Silodosin (1-10 nM) caused concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Silodosin (1 nM) had no effect on the contractions induced by dopamine (DA, Panel B) and adrenaline (ADR, Panel D), but caused concentration-dependent rightward shifts of the concentration-response curves to noradrenaline (NA, Panel C). Silodosin (0.1 nM) significantly reduced the EFS-induced contractions at 1 nM (Panel F), but not at 0.1 nM (1 nM; Panel E). Data are expressed as mean ± SEM. *P<0.05 compared with respective control values (*n* means the number of vas deferens strips).