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RESEARCH ARTICLE

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6-Nitrodopamine potentiates catecholamine-induced contractions of human isolated vas deferens

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Abstract

6-Nitrodopamine (6-ND) is the main catecholamine released from human isolated vas deferens and the adrenergic nervous system is known to play a major role in the contractions of the epididymal portion of the vas deferens. Here it was investigated the interactions of 6-ND on the contractions of the human isolated vas deferens induced by either classical catecholamines or electric-field stimulation (EFS). The vas deferens obtained from 106 patients who underwent vasectomy surgery were mounted in a 10-mL glass chamber filled with warmed (37°C) and oxygenated Krebs–Henseleit's solution. The strips were pretreated (30 min) with 6-ND (0.1–100 nM) and exposed to increasing concentrations of noradrenaline (0.01–300 M), dopamine (0.00001–10 mM), or adrenaline (0.01–300 M). The strips were also submitted to EFS in tissues pre-incubated or not with 6-ND (1–100 nM), noradrenaline (100 nM), adrenaline (100 nM), or dopamine (100 nM). Catecholamine basal release was evaluated by LC–MS/MS and expression of tyrosine hydroxylase by both immunohistochemistry (IC) and fluorescence in-situ hybridization (FISH). Pre-incubation of the vas deferens with 6-ND caused marked potentiation of the contractions induced by noradrenaline, adrenaline, and dopamine, as characterized by significant increases in E_{max}, without changes in pEC_{50} values. 6-nitrodopamine also caused significant increases in the EFS-induced contractions. The basal release of 6-ND was not affected by pre-treatment of the tissues with tetrodotoxin. Tyrosine hydroxylase was detected in epithelial cells of human vas deferens samples by both IC and FISH. The results clearly demonstrate that epithelium-derived 6-ND is a major modulator of human vas deferens contractility.

KEYWORDS

epithelium, liquid chromatography coupled to mass spectrometry, nitrocatecholamines, synergism

Antonio Tiago Lima and Sami Jabbour were contributed equally to this work.

1 | INTRODUCTION

The vas deferens receives its main excitatory input from a network (plexus) of sympathetic nerves. When stimulated, these nerves co-release noradrenaline and ATP as the primary chemical messengers.[1,2] The purinergic component is crucial for the function of the vas deferens in the prostate, while the adrenergic component is likely more important in the epididymis.[3] Additionally, other neurotransmitters, including dopamine, neuropeptide Y, and acetylcholine may also influence vas deferens motility.[4–6] The vas deferens has a thick layer of smooth muscle and functions as a channel, transporting sperm from the epididymis to the urethra, with the epithelium having the main absorptive, secretory functions, besides modulating ejaculation.[7]

6-nitrodopamine (6-ND) is the main catecholamine release from human,[8] rat[9,10] and mouse isolated vas deferens.[11] In the rat isolated epididymal vas deferens, 6-ND significantly enhances the contractions induced by the classical catecholamines noradrenaline, adrenaline, and dopamine.[12] Interestingly, the contractions of the human isolated epididymal vas deferens induced by 6-ND are antagonized by tricyclic antidepressants[8] and by α_1-adrenoceptor antagonists,[13] both of which are drug categories known to induce ejaculatory disorders.[14–16] In fact, both classes of drugs are therapeutically indicated for premature ejaculation relief.[17,18]

Since 6-ND is known as an endogenous mediator of the vas deferens contractility, this study evaluated the interactions of 6-ND with the contractions induced by classical catecholamines and electric-field stimulation (EFS) in human isolated vas deferens.

2 | PATIENTS AND METHODS

2.1 | Patients

Patients who have undergone vasectomy surgery from the Hospital Paulo Sacramento HapVida (Jundiaí, Sao Paulo) were asked to sign an approved informed consent by the local institutional review board (Protocol number 4.468.508). The tissue was collected from 106 participants (24–53 years of age) and the surgery performed under local anesthesia.[19] The resected component (1.5 cm length) was removed at approximately 9–10 cm from the cauda epididymis.

2.2 | Basal release of catecholamines from human epididymal vas deferens (HEVD)

Two HEVD strips were incubated for 30 min in 3 mL of Krebs–Henseleit's solution. The solution was continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37°C using a heated circulator (PolyScience, Illinois, USA). A 2 mL aliquot of the KHS supernatant was transferred to black Eppendorf tubes and stored at −20°C until analysis. The basal release of substances from the HEVD was then measured under two conditions: one in the absence of any drug, and another in the presence of tetrodotoxin (TTX), a selective inhibitor of voltage-gated sodium (Na^+) channels, at a concentration of 1 μM. The LC–MS/MS method was described elsewhere.[20]

2.3 | Functional studies

The strips (1.5 cm) of HEVD were mounted in a 10-mL glass chamber filled with a warm (37°C) and oxygenated (95% O_2: 5% CO_2) KHS. The isometric tension of the tissue was maintained at 10 mN and recorded by a PowerLab system (ADInstruments, Dunedin, New Zealand). After resting for 45 min, the HEVD strips were stimulated with potassium chloride (KCl, 80 mM) to ensure the tissue was healthy. KCl was then removed, and the tissue tension was allowed to return to baseline (around 15 min). After KCl removal and return to the baseline (approximately 15 min), HEVD strips were pretreated or not with 6-ND (6-ND; 0.1, 1, 10 or 100 nM for 30 min) and then exposed to increasing concentrations of noradrenaline (0.01–300 M) to build concentration-response curves. Similar protocols were carried out for dopamine (0.00001–10 mM), and adrenaline (0.01–300 M). Additionally, in separate set of experiments, cumulative concentration-response curves to noradrenaline (0.01–300 M), adrenaline (0.01–300 M), and dopamine (0.00001–10 mM) were conducted in TTX-pretreated HEVD strips (1 μM, 30 min), in the absence and the presence of 6-ND (100 nM, 30 min).

2.4 | EFS in HEVD preparations

EFS was applied using a Grass S88 stimulator (Astro-Medical, Industrial Park, RI, USA). The HEVD strips were stimulated with square-wave pulses at 60 V for 20 s, with a frequency range of 2–32 Hz, a pulse width of 0.3 ms, and a delay of 0.1 ms. This stimulation was performed on both control tissues and tissues that were pre-incubated with 6-ND (1, 10 or 100 nM), noradrenaline (100 nM), adrenaline (100 nM) or dopamine (100 nM).

2.5 | Immunohistochemical (IC) and fluorescence in-situ hybridization (FISH) assays for tyrosine hydroxylase (TH) and S100 protein

The methods employed for both IC and FISH have been previously described.[21]

2.6 | Chemical and reagents

The suppliers for the chemical and reagents employed are given as supplemental information.

2.7 | Data analysis

The pEC_{50} and E_{max} values represent the average along with the standard error of the mean (SEM) obtained from *n* independent experiments. E_{max} values are reported in millinewtons (mN). One vas deferens served as a control, while its contralateral counterpart received drug treatment. The specific methods used to determine both pEC_{50} and E_{max}, as well as the chosen statistical test (Student's two-tailed unpaired *t*-test) have been described elsewhere (8;13).

3 | RESULTS

3.1 | Basal release of catecholamines from HIVD

The HEVD strips released 6-ND (Figure 1A), dopamine (Figure 1B), adrenaline (Figure 1C), as quantified in the KHS by LC–MS/MS. The release of noradrenaline was below the limit of quantification (LOQ) of 0.1 ng/mL ($n = 7$). Treatment of the tissue with TTX (1 M for 30 min) had no significant effect the basal release of 6-ND (Figure 1A) and dopamine (Figure 1B), but abolished the adrenaline release (Figure 1C).

3.2 | Potentiation by 6-ND of the HEVD contractions induced by noradrenaline

Noradrenaline produced concentration-dependent HEVD contractions (Figure 2A–C). Pre-incubation of 6-ND (0.1 nM for 30 min) had no effect on the noradrenaline-induced concentration curve (Figure 1A); however, at 1 nM (Figure 2B) and 10 nM (Figure 2C), 6-ND significantly augmented the E_{max} values for noradrenaline. Pre-incubations of the tissues with 6-ND at 100 nM also increased the E_{max} values of the concentration-response curves to noradrenaline (Table 1). The values for pEC_{50}, E_{max}, the number of strips employed, and the *p*-values are presented in Table 1.

3.3 | Potentiation by 6-ND of the HEVD contractions induced by adrenaline

Adrenaline produced concentration-dependent HEVD contractions (Figure 2D–F). Pre-incubation of 6-ND (1 nM

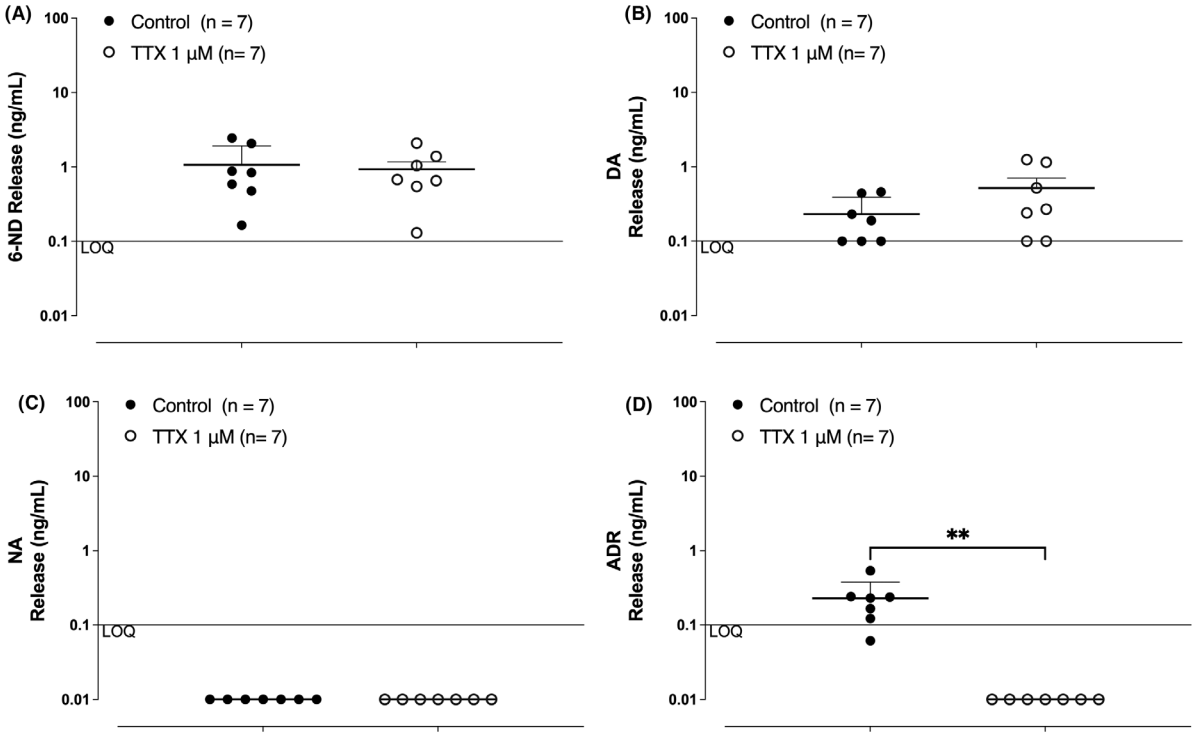

FIGURE 1 Basal release of 6-nitrodopamine (6-ND); (Panel A), dopamine (DA; Panel B), noradrenaline (NA); (Panel C), and adrenaline (ADR); (Panel D) were detected in the human epididymal vas deferens (HEVD). The basal release was measured by LC–MS/MS following a 30 min period incubation in Krebs–Henseleit's solution in the absence and the presence of TTX (1 µM). $^{**}p < .01$ control versus TTX. LOQ, limit of quantitation.

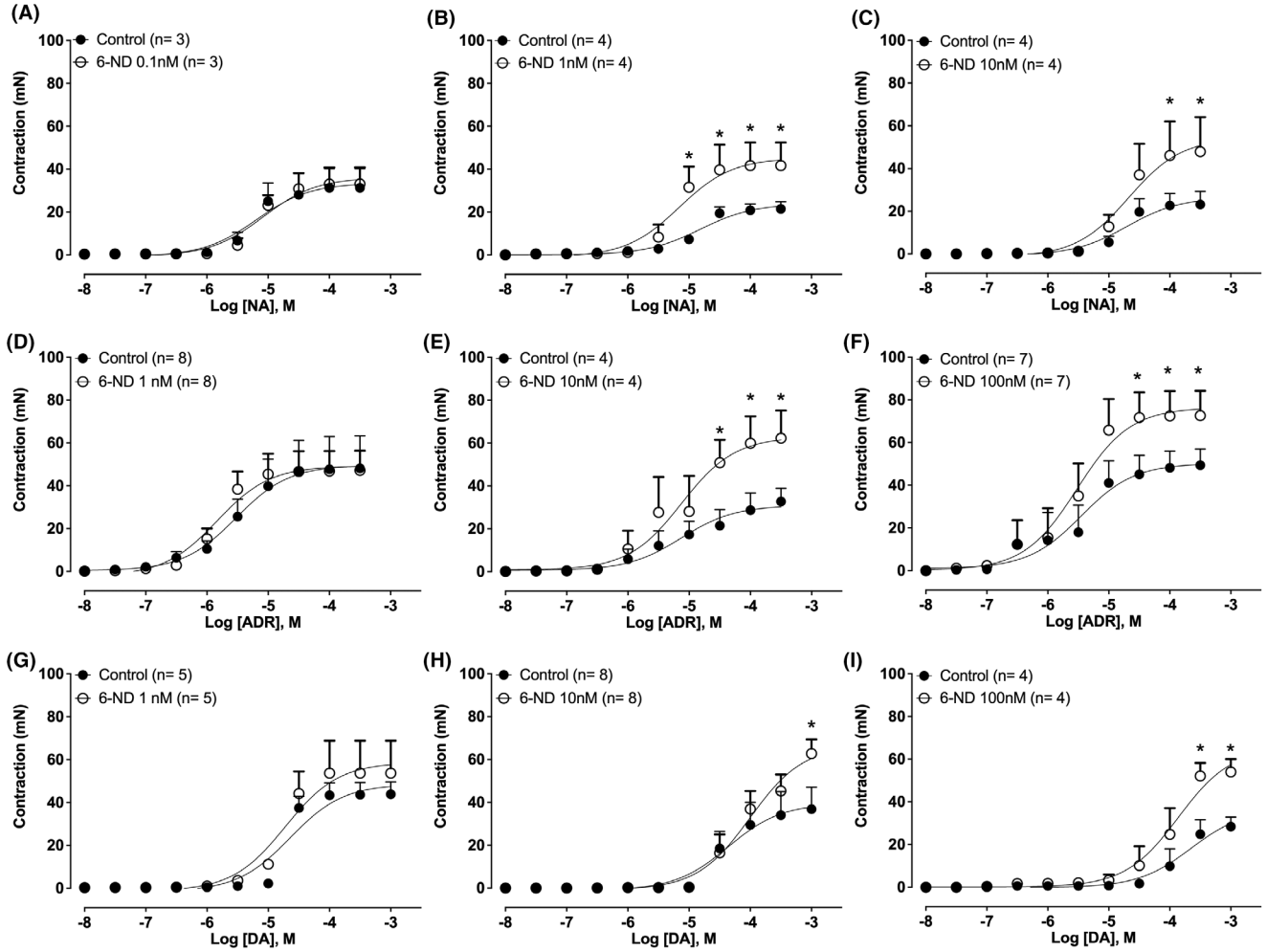

FIGURE 2 Effect of 6-nitrodopamine on the concentration-dependent contractions of human epididymal vas deferens (HEVD) strips Induced by noradrenaline, adrenaline, and dopamine. Noradrenaline (NA; 10 nM–300 μM) induced concentration-dependent contractions of the HEVD (A–C). Pre-incubation with 6-ND (30 min, 0.1 nM) had no effect (A), but at 1 nM (B) and 10 nM (C), 6-ND significantly increased the E_{max}. Adrenaline (ADR; 10 nM–300 μM) induced concentration-dependent contractions (D–F). Pre-incubation with 6-ND (30 min, 1 nM) had no effect (D), but at 10 nM (E) and 100 nM (F), it significantly increased the E_{max}. Dopamine (DA; 10 nM–1 mM) induced concentration-dependent contractions of the HEVD (G–I). Pre-incubation with 6-ND (30 min, 1 nM) had no effect (G), but at 10 nM (H) and 100 nM (I), 6-ND significantly increased the E_{max}. * denotes $p < 0.05$, as calculated by two-tail unpaired *t*-test.

for 30 min) had no effect on the concentration-response curve to adrenaline (Figure 2D); however, at 10 nM (Figure 2E) and 100 nM (Figure 2F), 6-ND significantly augmented the E_{max} values for adrenaline (Table 1). The pEC_{50} values did not significantly change between control and 6-ND groups (Table 1).

3.4 | Potentiation by 6-ND of the HEVD contractions induced by dopamine

Dopamine produced concentration-dependent HEVD contractions (Figure 2G–I). Pre-incubation of 6-ND (1 nM for 30 min) had no effect on the concentration-response curve to dopamine (Figure 2G); however, at 10 nM (Figure 2H) and 100 nM (Figure 2I), 6-ND significantly increased the E_{max} values for dopamine (Table 1). The pEC_{50} values did not significantly change between control and 6-ND groups (Table 1).

3.5 | Potentiation by 6-ND of the EFS-induced HEVD contractions

EFS (2–32 Hz) caused frequency-dependent HEVD contractions (Figure 3A–F). Pre-treatment (30 min) of the tissue with 6-ND (1 nM) had no effect on the EFS-induced contractions (Figure 3A). However, 6-ND at 10 nM (Figure 3B) and 100 nM (Figure 2C) augmented significantly the contractions induced by EFS at the frequencies of 4–32 Hz. Pre-incubation (30 min) of the tissue with either noradrenaline (100 nM; Figure 3D), adrenaline (100 nM; Figure 3E), or dopamine (100 nM; Figure 3F) had no effect on the EFS-induced contractions in any frequency.

TABLE 1 The potency (pEC_{50}) and the maximal responses (E_{max}) of noradrenaline, adrenaline and dopamine in the human isolated epididymal vas deferens in the absence (control) and presence of 6-nitrodopamine (6-ND).

	pEC_{50} (log[M])	E_{max} (mN)	*n*
Noradrenaline			
Control	5.19 ± 0.22	31.32 ± 9.07	3
6-ND (0.1 nM)	5.08 ± 0.17	33.09 ± 7.75	3
Control	4.81 ± 0.12	21.46 ± 3.32	4
6-ND (1 nM)	5.16 ± 0.20	41.68 ± 10.76*	4
Control	4.70 ± 0.22	23.20. ± 6.06	4
6-ND (10 nM)	4.67 ± 0.27	47.90 ± 16.19*	4
Control	4.59 ± 0.17	26.73 ± 5.82	5
6-ND (100 nM)	4.86 ± 0.10	49.07 ± 5.95*	5
Adrenaline			
Control	5.53 ± 0.30	48.10 ± 15.17	8
6-ND (1 nM)	5.83 ± 0.21	47.18 ± 9.18	8
Control	5.13 ± 0.27	32.73 ± 6.18	4
6-ND (10 nM)	5.12 ± 0.26	62.26 ± 12.88*	4
Control	5.45 ± 0.24	49.42 ± 7.35	6
6-ND (100 nM)	5.55 ± 0.19	72.63 ± 11.61*	6
Dopamine			
Control	4.64 ± 0.13	43.83 ± 5.70	5
6-ND (1 nM)	4.72 ± 0.22	53.69 ± 15.09	5
Control	4.36 ± 0.24	36.88 ± 10.25	6
6-ND (10 nM)	4.01 ± 0.13	62.75 ± 6.59*	6
Control	3.65 ± 0.19	28.40 ± 4.43	4
6-ND (100 nM)	3.86 ± 0.14	54.00 ± 6.10*	4

Note: pEC_{50} is defined as the negative logarithm of the EC_{50}. E_{max} is the maximal effect at the highest drug concentration. The pEC_{50} and E_{max} were expressed as mean ± SEM (n means the number of vas deferens strips). Two-tail unpaired *t*-test was performed on control vs treated values. *Represents $p < .05$.

3.6 | Effect of TTX on the potentiation by 6-ND of noradrenaline-, adrenaline-, and dopamine-induced HEVD contractions

In HEVD strips pre-treated with TTX (1 μM, 30 min), 6-ND at 100 nM had no potentiating effect in the HEVD contractions induced by noradrenaline (Figure 4A), adrenaline (Figure 4B), or dopamine (Figure 4C).

3.7 | IC and FISH assays for TH

TH was detected in epithelial cells of all human vas deferens samples, with the intensity of positivity being moderate to strong, as illustrated in Figure 3A,B. In most specimens, sparse nerve fibers were morphologically observed within tunica media (Figure 5B) and adventitia (Figure 5C,D), and they were positive for TH. As expected, TH was also detected in the endothelium of some vessels of tunica adventitia, as illustrated in Figure 1D. TH expression in vas deferens was confirmed by the FISH assay, as demonstrated in Figure 3E–G. S100 protein was negative in epithelial cells (Figure S1A), being positive only in neural structures within tunica media and adventitia (Figure S1B–D).

4 | DISCUSSION

The results clearly demonstrated that 6-ND can significantly increase the contractions of human isolated vas deferens induced by the classical catecholamines noradrenaline, adrenaline, and dopamine. Although similar results have been reported for rat isolated epididymal vas deferens, there are some striking differences between the release and actions of 6-ND in human and rodent vas deferens. For instance, the basal release of 6-ND from human vas deferens is not affected by TTX pre-incubation, whereas in rat[10] and mouse[11] vas deferens, the release of 6-ND was virtually abolished. This indicates a possible epithelial-source for 6-ND in human vas deferens, like that observed in human isolated seminal vesicles.[22] Indeed, expression of TH in the epithelium was confirmed here by both immunohistochemistry and fluorescence in vitro hybridization. Human epithelial cells are known to synthetise both catecholamines[23] and nitric oxide[24]; therefore, it is possible that human vas deferens epithelium could produce dopamine, nitric oxide, and 6-ND. The finding that the release of 6-ND is abolished in rodents by pre-incubation with TTX does not indicate that 6-ND is necessarily coming from nerve terminals; one alternative explanation could be that dopamine comes from the epithelium and is "nitrated" by nitric oxide released from nitrergic terminals. Like human vas deferens, the epithelium of rat vas deferens also expresses TH.[10]

Identifying that the epithelium releases 6-ND may have relevant implications in the maturation of spermatozoa. Sperm maturation is a complex phenomenon, and in mammals the sperm acquire fertilization and motility when it passes through the epididymis[25–27] In the vervet monkey (*Cercopithecus aethiops*), motility improves as the spermatozoa move through the cauda epididymis and vas deferens.[28] It is interesting that human semen presents high concentrations of noradrenaline and DOPA higher than concentrations present in plasma, including the dopamine metabolite DOPAC.[29] Investigation on whether human isolated epididymis presents expression of TH and basal release of 6-ND may provide useful information on the role of this novel catecholamine in both maturation of spermatozoa and motility.

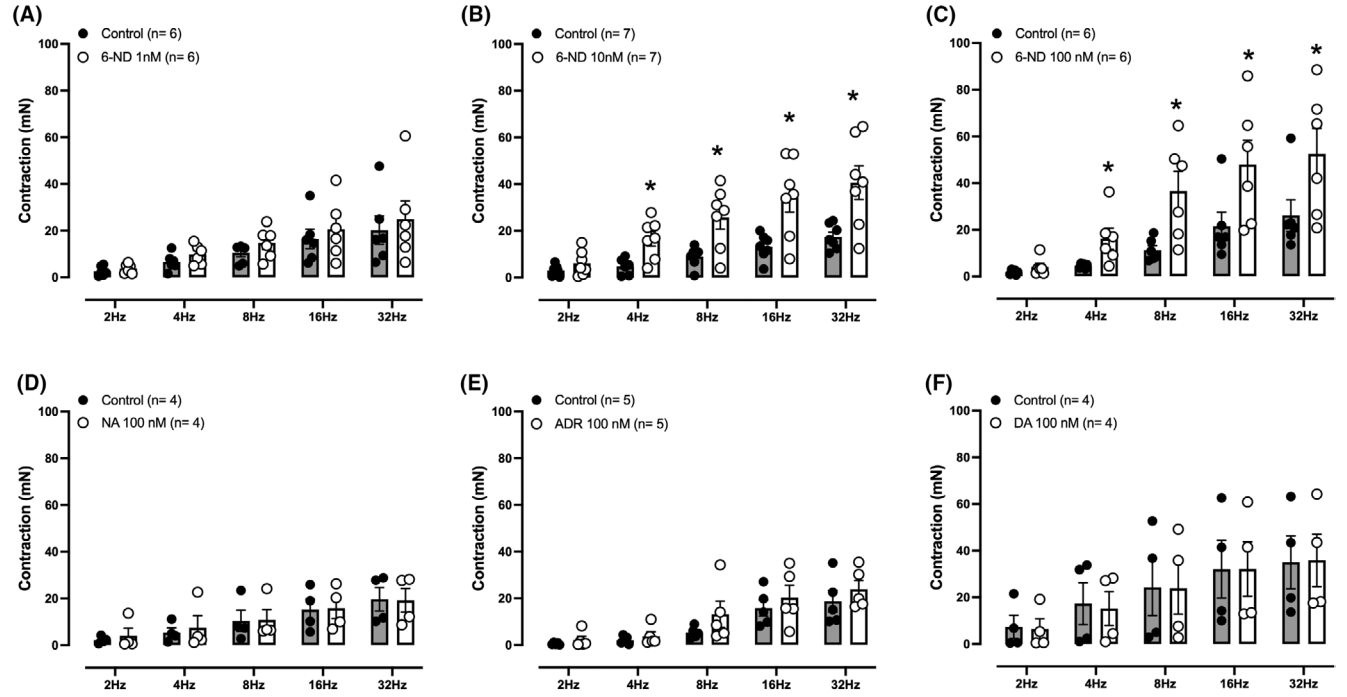

FIGURE 3 Effect of catecholamine pre-incubation on the electric-field stimulation (EFS)-induced contraction in the human epididymal vas deferens (HEVD) strips. Pre-incubation (30 min) of the HEVD with 6-nitrodopamine (6-ND, 1 nM) had no significant effect on the EFS-induced contractions (A). Pre-incubation (30 min) of the HEVD with 6-ND 10 nM (B) and 100 nM (C) potentiated the EFS-induced contractions in the frequencies from 4 to 32 Hz. Noradrenaline (100 nM; D), adrenaline (100 nM; E), and dopamine (100 nM; F) did not affect the EFS-induced contractions. *indicates $p < 0.05$.

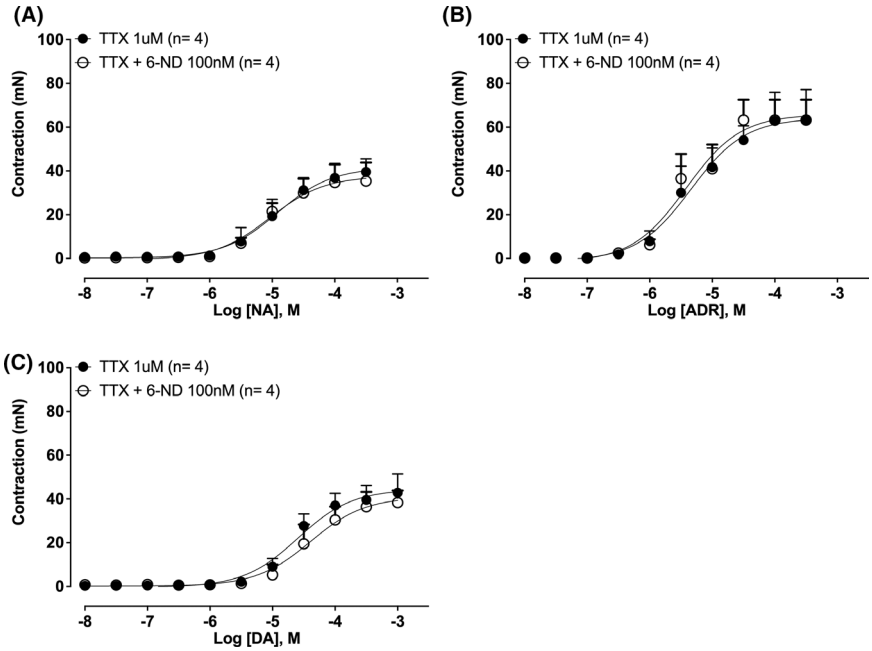

FIGURE 4 Effect of tetrodotoxin on the potentiation induced by 6-nitrodopamine (6-ND) of the concentration-dependent contractions of human epididymal vas deferens (HEVD) strips induced by noradrenaline (NA), adrenaline (ADR), and dopamine (DA). In TTX (30 min, 1 μM) pre-treated strips, the incubation of the tissue with 6-ND (100 nM) had no effect on the concentration-dependent contractions induced by noradrenaline (A), adrenaline (B), and dopamine (C).

Another major difference between human and rodent vas deferens relies on the interaction with the classical catecholamines. Whereas in the rat isolated vas deferens, the potentiation of the noradrenaline-, adrenaline-, and dopamine-induced contractions by 6-ND is characterized by significant leftward shifts of the concentration-response curves,[12] in the human vas deferens the distinguishing feature is a significant increase in Emax. This cannot be attributed to species differences (human vs. rat), since in the human isolated seminal vesicles, the potentiation induced by 6-ND in the concentration-response curves to noradrenaline was also characterized by a significant

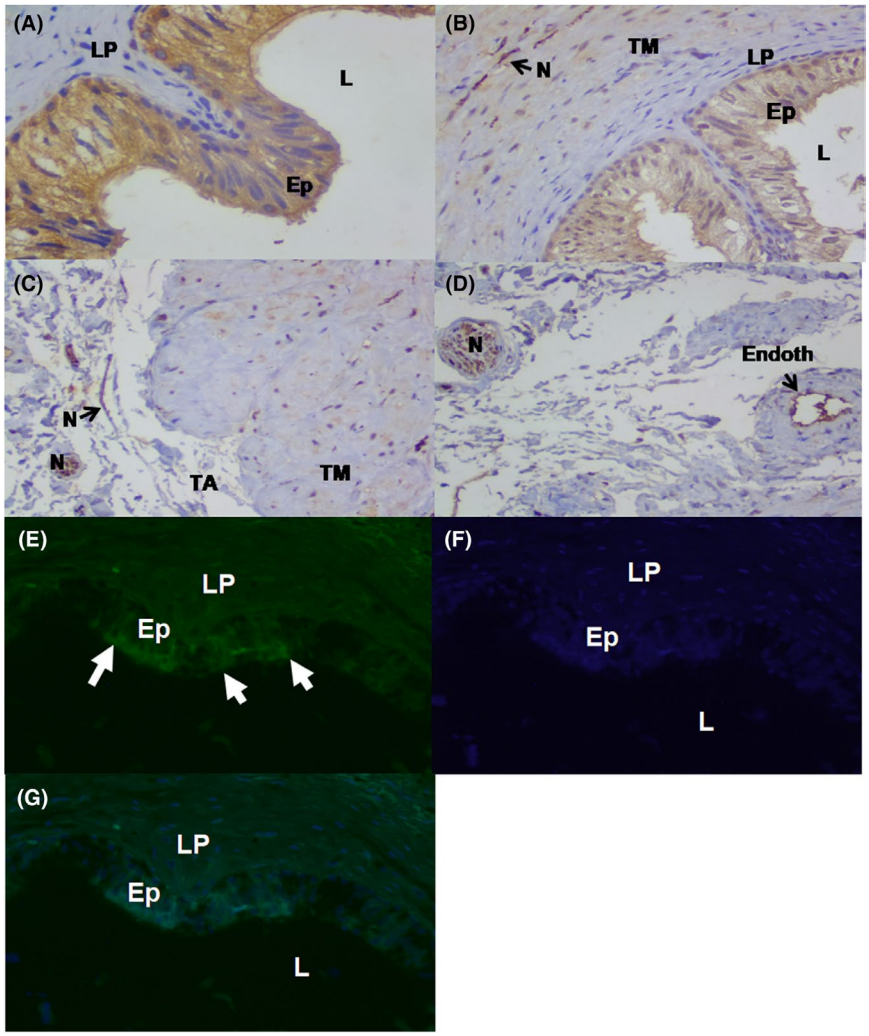

FIGURE 5 Representative images of tyrosine hydroxylase (TH) immunoexpression in human vas deferens. TH immunostaining was detected in epithelial cells of the human vas deferens (A, B); notice the positivity for TH also in nerve fibers (N) of the tunica media; panel C shows TH positivity in neural structures (N) of the adventitia (TA); a thich nerve and endothelial cells were also positive for TH as illustrated in panel D. Immunoperoxidase, 400× (A), 200× (B–D). (E–G) Detection of tyrosine hydroxylase mRNA by fluorescence in situ hybridization (FISH) in human vas deferens. (E) TH mRNA staining in the cytoplasm of epithelial cells (arrows); (F) DAPI stainings in nuclei; (G) overlay (DAPI + TH mRNA stainings); DAPI/6-FAM (400×, original magnification). Ep, epithelium; Endoth, endothelium; LP, lamina propria; L, lumen; N, nerve; TM, tunica media; TA, tunica adventitia.

leftward shift, without changes in Emax.[22] The increase in E_{max} induced by 6-ND in the human vas deferens cannot be attributed to an additive effect, since it was observed at a concentration that 6-ND does not provoke contraction in the human vas deferens. Whether 6-ND has two distinct mechanisms by which 6-ND can interact with classical catecholamines in the vas deferens is under current investigation.

AUTHOR CONTRIBUTIONS

Conceptualization: JBJ, GDN. Data curation: JBJ, GDN. Formal analysis: GDN, *Funding acquisition*: EA, GDN., *Investigation*: ATL, SJ, VBS, *Methodology*: JBJ, GDN, FVM, AAS, *Project administration*: JBJ, GDN, *Supervision*: EA and GDN., *Visualization*: JBJ, GDN, AF, *Writing—original draft*: JBJ, EA, GDN. The authors declare that all data were generated in-house and that no paper mill was used.

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DISCLOSURES

Authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The authors declare that all data will be disclosed upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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