

B1 - and B1 /B2 -adrenergic receptor antagonists block 6-nitrodopamine- induced contractions on the rat isolated epididymal vas deferens

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Abstract

6-nitrodopamine (6-ND) is an endogenous modulator of the contractility in the rat isolated epididymal vas deferens (RIEVD) and considered to be the main peripheral mediator of the emission process. Use of selective and unselective β -adrenergic receptor antagonists have been associated with ejaculatory failure. Here, the effects of selective β_1 - and β_1/β_2 - adrenergic receptor antagonists on RIEVD contractions induced by 6-ND, dopamine, noradrenaline, adrenaline and EFS were investigated. The selective β_1 adrenergic receptor antagonists atenolol (0.1 and 1mM), betaxolol (1mM) and metoprolol (1mM) and the unselective β_1/β_2 - adrenergic receptor antagonists propranolol (1 and 10mM) and pindolol (10mM) caused significant rightward shifts of the concentration-response curve to 6-ND (pA_2 6.41, 6.91, 6.75, 6.47 and 5.74; for atenolol, betaxolol, metoprolol, propranolol and pindolol), but had no effect on dopamine-, noradrenaline- and adrenaline-induced contractions. The effects of selective β_1 - and β_1/β_2 - adrenergic receptor antagonists at a higher concentration (atenolol 1mM, betaxolol 1mM, metoprolol 1mM, propranolol 10mM and pindolol 10mM) also reduced the EFS-induced RIEVD contractions in control but not in RIEVD obtained from L-NAME-treated animals. The selective β_1 - adrenoceptor agonist RO-363, the selective β_2 - adrenoceptor agonist salbutamol and the selective β_3 -adrenoceptor agonist mirabegron, up to 300 mM, had no effect on the RIEVD tone. The results demonstrate that β_1 - and β_1/β_2 - adrenoceptor receptor antagonists act as 6-ND receptor antagonists in RIEVD, further confirming the main role of 6-ND in the RIEVD contractility.

1. Introduction

6-nitrodopamine (6-ND) is a novel catecholamine released from vascular tissues such as human umbilical cord vessels (Britto-Jr et al., 2021a), *Chelonoidis carbonaria* aorta (Campos et al., 2020), and from rat vas deferens (Britto-Jr et al., 2021). The synthesis / release of 6-ND is coupled to nitric oxide (NO) synthesis, since it is reduced by the NO synthase inhibitor N^W-nitro-L-arginine methyl ester (L-NAME).

In the rat epididymal vas deferens, 6-ND has been characterized as a major endogenous modulator of the contractility of this tissue (Britto-Jr et al., 2021b; Britto-Jr et al., 2022). Tricyclic antidepressants such as clomipramine (Millan et al., 2001), desipramine (Cusack et al., 1994) and amitriptyline (Sánchez et al., 1999) and α_1 -adrenergic receptor antagonists such as 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 alfuzosin (Ramsay et al., 1985) act as 6-ND receptor antagonist in the rat vas deferens (Britto-Jr et al., 2021b; 2022). One known adverse reaction of these two classes of drugs is the impairment of the ejaculatory process (Beaumont, 1977; Cavallini, 1995; Hsieh et al., 1999; Debruyne, 2000). Indeed, both classes of drugs are used for the treatment of premature ejaculation (Hellstrom and Sikka, 2006; Basar et al., 2005), indicating a major role for 6-ND in the ejaculatory process. In pre-clinical studies, male rats treated for 16 weeks with the non-selective β -adrenoceptor antagonist propranolol (1.25 mg/day) exhibited an impairment in the ejaculation and copulatory pattern (Srilatha et al., 1999). Subcutaneous administration of the non-selective β -blocker pindolol (4 mg/kg, 30 min) to male rats was also associated

with inhibition of the sexual behaviour, as evidenced by an increase in mounts, intromissions and time to ejaculate (Ahlenius & Larsson, 1991). In patients with arterial hypertension, coronary artery disease or heart failure, meta-analytic data have shown that b-blockers are associated with a small, but significant, increase in risk of reported sexual dysfunction, which was not related to the lipid-soluble b-blockers (Ko et al., 2002). The use of the β_1 -, β_2 - and α_1 -adrenergic receptor antagonist labetalol was associated with ejaculatory failure soon after the initiation of therapy, that resolved with drug discontinuation (O'Meara and White, 1988). In a double-blind, placebo-controlled trial comprising eighty-six paroxetine-refractory patients, pindolol, at the dose of 7.5 mg/d, increased significantly the mean intravaginal ejaculatory latency time after six weeks of treatment (Safarinejad, 2008). Thus, both the experimental and clinical observations open the interesting possibility that b-blockers could act as 6-ND receptor antagonists in the vas deferens, as observed with tricyclic antidepressants and α_1 -adrenergic receptor antagonists.

2. Materials And Methods

2.1. Animals

Adult male Wistar rats (280–320 g) were obtained from the animal care of University of Campinas (UNICAMP; Campinas, São Paulo, Brazil) and Animais de Laboratorio Criação e Com. LTDA (ANILAB; Paulinia, São Paulo, Brazil). All experimental protocols were authorized by the Ethics Committee in Animal Use of UNICAMP (CEUA/UNICAMP, protocol numbers 5952-1/2022 and 5831-1/2021) and followed the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines (Percie du Sert et al., 2020). Animals were housed in cages (three per cage) located in ventilated cage shelters with constant humidity of $55\% \pm 5\%$ and temperature of $24 \pm 1^\circ\text{C}$ under a 12-hour light-dark cycle. Animals received filtered water and standard food *ad libitum*.

2.2. Chronic L-NAME treatment

Animals were treated with L-NAME dissolved in the drinking filtered water at a concentration of approximately 20 mg/rat/day for a minimum of 4 weeks (Ribeiro et al., 1992). Control animals received filtered water alone.

2.3. Rat isolated epididymal vas deferens (RIEVD) isolation and preparation

Euthanasia was performed by isoflurane overdose, in which animals were exposed to a concentration greater than 5% until 1 min after the breathing stops. Exsanguination was performed to confirm the euthanasia. The vas deferens was removed and immediately placed in Krebs-Henseleit's solution (KHS). Epididymal portions of vas deferens were surgically dissected (length, 1.5 cm each) for the functional studies. The RIEVD strips were suspended vertically between metal hooks in 10-mL organ baths containing KHS, continuously gassed with a mixture of 95% O_2 : 5% CO_2 at 37°C . Tissues were allowed to equilibrate under a resting tension of 10 mN, and the isometric tension was registered using a PowerLab

system (ADInstruments, Sydney, Australia). Following a 45-min stabilization period, the RIEVD strips were initially contracted with a single concentration of noradrenaline (NA, 10 μ M) to verify the tissue viability.

2.4. *In vitro* functional assays in RIEVD preparations

Cumulative concentration-response curves to 6-ND were performed in RIEVD strips in the absence and the presence of atenolol (0.1 and 1 mM, 30 min), betaxolol (0.1 and 1 mM, 30 min), metoprolol (0.1 and 1 mM, 30 min), propranolol (1 and 10 mM, 30 min) or pindolol (1, 3 and 10 mM, 30 min). In separate RIEVD preparations, cumulative concentration-response curves to dopamine, noradrenaline and adrenaline were performed in the absence and presence of atenolol (1 mM, 30 min) or betaxolol (1 mM, 30 min), metoprolol (1 mM, 30 min), propranolol (10 mM, 30 min) or pindolol (10 mM, 30 min).

Cumulative concentration-response curves to selective β 1-adrenoceptor agonist RO-3630 (0.001-300 mM), selective β 2-adrenoceptor agonist salbutamol (0.001-300 mM) and selective β 3-adrenoceptor agonist mirabegron (0.001-300 mM) were performed in RIEVD strips of animal control.

2.5. Electric-field stimulation (EFS) in RIEVD preparations

The EFS-induced contractions from RIEVD were evaluated in control and L-NAME-treated rats. Briefly, RIEVD strips were submitted to EFS (60 V for 20 sec, at 2–16 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). In control animals, EFS-induced contractions were evaluated in the absence and the presence of atenolol (0.1 and 1 mM, 30 min), betaxolol (0.1 and 1 mM, 30 min), metoprolol (0.1 and 1 mM, 30 min), propranolol (1 and 10 mM, 30 min) or pindolol (1 and 10 mM, 30 min). In L-NAME-treated rats, EFS-induced contractions were evaluated in the absence and in the presence of atenolol (1 mM, 30 min), betaxolol (1 mM, 30 min), metoprolol (1 mM, 30 min), propranolol (10 mM, 30 min) or pindolol (10 mM, 30 min).

2.6. Drugs and Solutions

Atenolol, dopamine, metoprolol, mirabegron, N^ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), salbutamol and propranolol were obtained from Sigma-Aldrich Chemicals Co. (St Louis, Missouri, USA). Adrenaline, betaxolol, noradrenaline and pindolol, were purchased from Cayman Chemical Co (Michigan, USA). 6-nitrodopamine was bought from Toronto Research Chemicals Inc (Toronto, Ontario, Canada). RO-363 was provided by MedChem Express (New Jersey, USA). Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride (CaCl₂), magnesium sulfate (MgSO₄), sodium bicarbonate (NaHCO₃), potassium phosphate monobasic (KH₂PO₄) and glucose were acquired from Merck KGaA (Darmstadt, Germany). The composition of the KHS was in mM: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and dextrose 5.6.

2.7. Data Analysis

Nonlinear regression analysis to determine the pEC₅₀ was carried out using GraphPad Prism (GraphPad Software, version 9.0, San Diego, California, USA) with the constraint that F = 0. All concentration–response data were evaluated for a fit to a logistics function in the form: $E = E_{\max} / [1 + (10c / 10x)^n] + F$,

where E represents the increase in response contractile induced by the agonist, E_{max} is the effect agonist maximum, c is the logarithm of concentration of the agonist that produces 50% of E_{max} , x is the logarithm of the concentration of the drug; the exponential term, n, is a curve fitting parameter that defines the slope of the concentration–response line, and F is the response observed in the absence of added drug. The values of pEC_{50} data represent the mean \pm standard error of the mean (SEM) of n experiments. Values of E_{max} were expressed in mN. One strip was used as the control response and the other strip was incubated with an antagonist/inhibitor. Student's two-tail unpaired t-test was employed and the differences between groups. In addition, standard ANOVA, followed by the Newman–Keuls post-test, were used when more than two groups were involved. A p value of less than 0.05 was considered statistically significant. For 6-ND, the pA_2 values of the antagonists were calculated from the intercept on the concentration axis and by application of the equation; $pA_2 = \log(\text{antagonist concentration}) - \log(CR-1) - \log(\text{antagonist concentration})$ (Arunlakshana and Schild, 1959).

3. Results

3.1. *Effect of atenolol on RIEVD contractions induced by catecholamines and EFS*

Atenolol (0.1 and 1 mM) produced concentration-dependent rightward shifts on the concentration-response curves to 6-ND (Fig. 1A; $p = 0.0284$ and $p = 0.0068$, respectively) with a pA_2 value of 6.51 ± 0.54 ($n = 4$). Atenolol (1 mM) had no effect on the RIEVD contractions induced by dopamine (Fig. 1B; $p = 0.4540$), noradrenaline (Fig. 1C; $p = 0.4234$) and adrenaline (Fig. 1D; $p = 0.4570$).

Atenolol (0.1 mM) had no effect on the EFS-induced contractions of RIEVD (Fig. 1F), but at a higher concentration (1 mM), atenolol caused significant reductions on the EFS-induced contractions of the RIEVD in all frequencies tested (Figs. 1F), which was not observed in RIEVD obtained from animals chronically treated with L-NAME (Fig. 1G).

3.2. *Effect of betaxolol on RIEVD contractions induced by catecholamines and EFS*

Betaxolol at 0.1 mM had no effect on 6-ND-induced RIEVD contractions, but at 1 mM it caused a significant rightward shift on the concentration-response curve to 6-ND (Fig. 2A; $p = 0.0046$) with a pA_2 value of 6.91 ± 0.03 ($n = 4$). Betaxolol (1 mM) had no effect on the RIEVD contractions induced by dopamine (Fig. 2B; $p = 0.4608$), noradrenaline (Fig. 2C; $p = 0.2830$) and adrenaline (Fig. 2D; $p = 0.1571$).

Betaxolol (0.1 mM) had no significant effect on EFS-induced RIEVD contractions (Fig. 2F); however, at 1 mM betaxolol caused significant reductions of the EFS-induced contractions in all frequencies tested (Figs. 2F). In RIEVD obtained from animals chronically treated with L-NAME, betaxolol (1 mM) had no effect on the EFS-induced contractions (Fig. G).

2.3. Effect of metoprolol on RIEVD contractions induced by catecholamines and EFS

Metoprolol at 0.1 mM had no effect on 6-ND-induced RIEVD contractions, but at 1 mM it caused a significant rightward shift on the concentration-response curve to 6-ND (Fig. 3A; $p = 0.0159$) with a pA_2 value of 6.75 ± 0.08 ($n = 4$). Metoprolol (1 mM) had no effect on the RIEVD contractions induced by dopamine (Fig. 3B; $p = 0.4540$), noradrenaline (Fig. 3C; $p = 0.1887$) and adrenaline (Fig. 3D; $p = 0.3795$).

Metoprolol at 0.1 mM had no significant effect on EFS-induced RIEVD contractions (Fig. 3E); however, at 1 mM metoprolol caused significant reductions of the EFS-induced contractions in all frequencies tested (Figs. 3F). In RIEVD obtained from animals chronically treated with L-NAME, metoprolol (1 mM) had no effect on the EFS-induced contractions (Fig. 3G).

2.4. Effect of propranolol on RIEVD contractions induced by catecholamines and EFS

Propranolol (1 and 10 mM) produced concentration-dependent rightward shifts on the concentration-response curves to 6-ND (Fig. 4A; $p = 0.029$ and $p = 0.0345$ for 1 and 10 mM, respectively) with a pA_2 value of 6.47 ± 0.35 ($n = 4$). Propranolol (10 mM) had no effect on the RIEVD contractions induced by dopamine (Fig. 4B; $p = 0.1073$), noradrenaline (Fig. 4C; $p = 0.4481$) and adrenaline (Fig. 4D; $p = 0.3986$).

Propranolol at 1 mM had no significant effect on EFS-induced RIEVD contractions (Fig. 4E); however, at 10 mM propranolol caused significant reductions of the EFS-induced contractions at the frequencies of 4 to 16 Hz (Figs. 4F), which was not observed in RIEVD obtained from animals chronically treated with L-NAME (Fig. 4G).

2.5. Effect of pindolol on RIEVD contractions induced by catecholamines and EFS

Pindolol (10 mM) caused a rightward shift on the concentration-response curve to 6-ND (Fig. 5A; $p = 0.0184$) with a pA_2 value of 5.74 ± 0.15 ($n = 4$). Lower concentrations of pindolol (1 and 3 mM) had no significant effect on the contractions induced by 6-ND (Fig. 5A). Pindolol (10 mM) had no effect on the RIEVD contractions induced by dopamine (Fig. 5B; $p = 0.2102$), noradrenaline (Fig. 5C; $p = 0.3951$) and adrenaline (Fig. 5D; $p = 0.2394$).

Pindolol at 1 mM had no significant effect on EFS-induced RIEVD contractions (Fig. 5E); however, at 10 mM, pindolol caused significant reductions of the EFS-induced contractions at the frequencies of 4 to 16 Hz (Figs. 5F), which was not observed in RIEVD obtained from animals chronically treated with L-NAME (Fig. 5G).

2.6. Effect of RO-363, salbutamol and mirabregron on RIEVD tone

The selective β_1 -adrenoceptor agonist RO-363 (Fig. 6A), the selective β_2 -adrenoceptor agonist salbutamol (Fig. 6B) and the selective β_3 -adrenoceptor agonist mirabegron (Fig. 6C), up to 300 μ M, had no effect on the RIEVD tone.

4. Discussion

The results clearly indicate that both selective and non-selective β -blockers can antagonize the contractions of the rat epididymal vas deferens induced by 6-ND, as observed with α_1 -adrenergic receptor antagonists and tricyclic depressants. These findings also reinforce the role of 6-ND as the major modulator of rat epididymal vas deferens contractility, since the contractions induced by electric field stimulation were inhibited by the β -blockers only at the concentrations that caused right-shifts of the 6-ND concentration-response curves. The inhibition of RIEVD contractions by the β -blockers was not observed in the vas deferens obtained from animals chronically treated with L-NAME, further supporting the concept that the inhibition of EFS-induced by β -receptor antagonists is due to blockade of 6-ND action.

β_1 -adrenergic receptors are not considered relevant for contractile activity in the rat vas deferens, since this tissue contains a homogenous population of β_2 -adrenoceptors that inhibit field-stimulated contractions (Vohra, 1979). Radio-ligand binding using [125 I]-pindolol in the rat vas deferens labelled a single class of high affinity binding sites with properties consistent with a population of β_2 -adrenoceptors (May et al., 1985). In the rat vas deferens, β_2 -adrenergic antagonists such as carazolol is more potent to displace [3 H]-dihydroalprenolol binding compared to β_1 -adrenergic antagonists such as atenolol and practolol (Chang and Lotti, 1983). Indeed, the finding that the selective β_1 -adrenergic receptor agonist RO-363 (Iakovidis et al., 1980) had no contractile activity per se confirms the relative unimportance of modulatory role for this subclass of receptors in the vas deferens. The lack of contractile activity of RO-363 clearly demonstrates that the contractions induced by 6-ND are not due to activation of β_1 -adrenergic receptors. Similar results were obtained with the selective β_2 -adrenergic agonist salbutamol and the selective β_3 -adrenergic agonist mirabegron, indicating that the contractile activity induced by 6-ND is independent of β -adrenergic receptor activation. 6-ND has been considered the endogenous mediator of EFS-induced contractions in the rat vas deferens (Britto-Júnior et al., 2021b), and these results further support the concept that 6-ND is acting on a specific 6-ND receptor.

Although ejaculatory disorders have been reported with the use of selective and unselective β -blockers, the incidence is rather low (reported cases) when compared to α_1 -adrenergic receptor antagonists (4–11%; Höfner et al., 1999). This major difference in incidence (Djavan et al., 2004) could be easily attributed to the observed major potency (over 100 times) of α_1 -blockers (the pA_2 values are 9.66, 9.15, 8.86, 7.70, 7.20 and 8.82 for tamsulosin, doxazosin, alfuzosin, silodosin, terazosin and prazosin; Britto-Júnior et al 2022) in blocking 6-ND contractile activity compared to the β -blockers (6.41, 6.91, 6.75, 6.47 and 5.74; for atenolol, betaxolol, metoprolol, propranolol and pindolol, respectively; this manuscript), further supporting a major role of 6-ND in the ejaculatory process. Although β_1 -, β_2 - and β_3 -adrenergic

receptor agonists per se did not induce contractions in the rat vas deferens, these findings do not exclude a potential modulatory role on vas deferens contractility.

Conclusion

The inhibitory effect of β_1 - and β_1/β_2 -adrenergic receptor antagonists on the RIEVD contractions induced by both the EFS and 6-ND is due to blockade of the 6-ND receptor.

Declarations

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Ethical Approval

All experimental protocols were authorized by the Ethics Committee in Animal Use of UNICAMP (CEUA/UNICAMP, protocol numbers 5952-1/2022 and 5831-1/2021).

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: ATL, ACA, JBJ, RRC, GDN.

Methodology: ATL, ACA, JBJ, RRC, AF, EA, FZM, GDN.

Project administration: GDN.

Supervision: FZM, EA.

Visualization: AF, EA, GDN.

Writing – original draft: JBJ, AF, EA, GDN.

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

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Competing interests

The authors declare no competing or financial interests

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 atenolol in the rat isolated epididymal vas deferens (RIEVD). Atenolol (0.1 and 1 mM) caused significant concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Atenolol (1 mM) causes had no effect on the RIEVD contractions induced by dopamine (Panel B), noradrenaline (Panel C) and adrenaline (Panel D) concentration-response curves. Atenolol (0.1 mM; Panel E) had no effect on the EFS-induced contractions but atenolol (1 mM; Panel F) caused no reduction in the contractions induced by EFS but at higher concentrations but had no effect on the EFS-induced contractions of the RIEVD obtained from animals chronically treated with L-NAME (Panel G). Data are expressed as mean \pm SEM. *P < 0.05 compared with respective control values. n means the number of vas deferens strips.

Figure 2

Effect of betaxolol in the rat isolated epididymal vas deferens (RIEVD). Betaxolol (1 mM) caused significant concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Betaxolol (1 mM) causes had no effect on the RIEVD contractions induced by dopamine (Panel B), noradrenaline (Panel C) and adrenaline (Panel D) concentration-response curves. Betaxolol (0.1 mM; Panel E) had no effect on the EFS-induced contractions but betaxolol (1 mM; Panel F) caused no reduction in the contractions induced by EFS but at higher concentrations but had no effect on the EFS-induced contractions of the RIEVD obtained from animals chronically treated with L-NAME (Panel G). Data are expressed as mean \pm SEM. *P < 0.05 compared with respective control values. n means the number of vas deferens strips.

Figure 3

Effect of metoprolol in the rat isolated epididymal vas deferens (RIEVD). Metoprolol (1 mM) caused significant concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Metoprolol (1 mM) causes had no effect on the RIEVD contractions induced by dopamine (Panel B), noradrenaline (Panel C) and adrenaline (Panel D) concentration-response curves. Metoprolol (0.1 mM; Panel E) had no effect on the EFS-induced contractions but metoprolol (1 mM; Panel F) caused no reduction in the contractions induced by EFS but at higher concentrations but had no effect on the EFS-induced contractions of the RIEVD obtained from animals chronically treated with L-NAME (Panel G). Data are expressed as mean \pm SEM. *P < 0.05 compared with respective control values. n means the number of vas deferens strips.

Figure 4

Effect of propranolol in the rat isolated epididymal vas deferens (RIEVD). Propranolol (1 and 10 mM) caused significant concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Propranolol (10 mM) causes had no effect on the RIEVD contractions induced by dopamine (Panel B), noradrenaline (Panel C) and adrenaline (Panel D) concentration-response curves. Propranolol (1 mM; Panel E) had no effect on the EFS-induced contractions but propranolol (10 mM; Panel F) caused significant reductions on the EFS-induced contractions of the RIEVD but caused no reduction in the contractions induced by EFS but at higher concentrations but had no effect on the EFS-induced contractions of the RIEVD obtained from animals chronically treated with L-NAME (Panel G). Data are expressed as mean \pm SEM. *P < 0.05 compared with respective control values. n means the number of vas deferens strips.

Figure 5

Effect of pindolol in the rat isolated epididymal vas deferens (RIEVD). Pindolol (10 mM) caused significant concentration-dependent rightward shifts of the concentration-response curves to 6-ND (Panel A). Pindolol (10 mM) causes had no effect on the RIEVD contractions induced by dopamine (Panel B), noradrenaline (Panel C) and adrenaline (Panel D) concentration-response curves. Pindolol (1 mM; Panel E) had no effect on the EFS-induced contractions but pindolol (10 mM; Panel F) caused significant reductions on the EFS-induced contractions of the RIEVD but caused no reduction in the contractions induced by EFS but at higher concentrations but had no effect on the EFS-induced contractions of the RIEVD obtained from animals chronically treated with L-NAME (Panel G). Data are expressed as mean \pm SEM. *P < 0.05 compared with respective control values. n means the number of vas deferens strips.

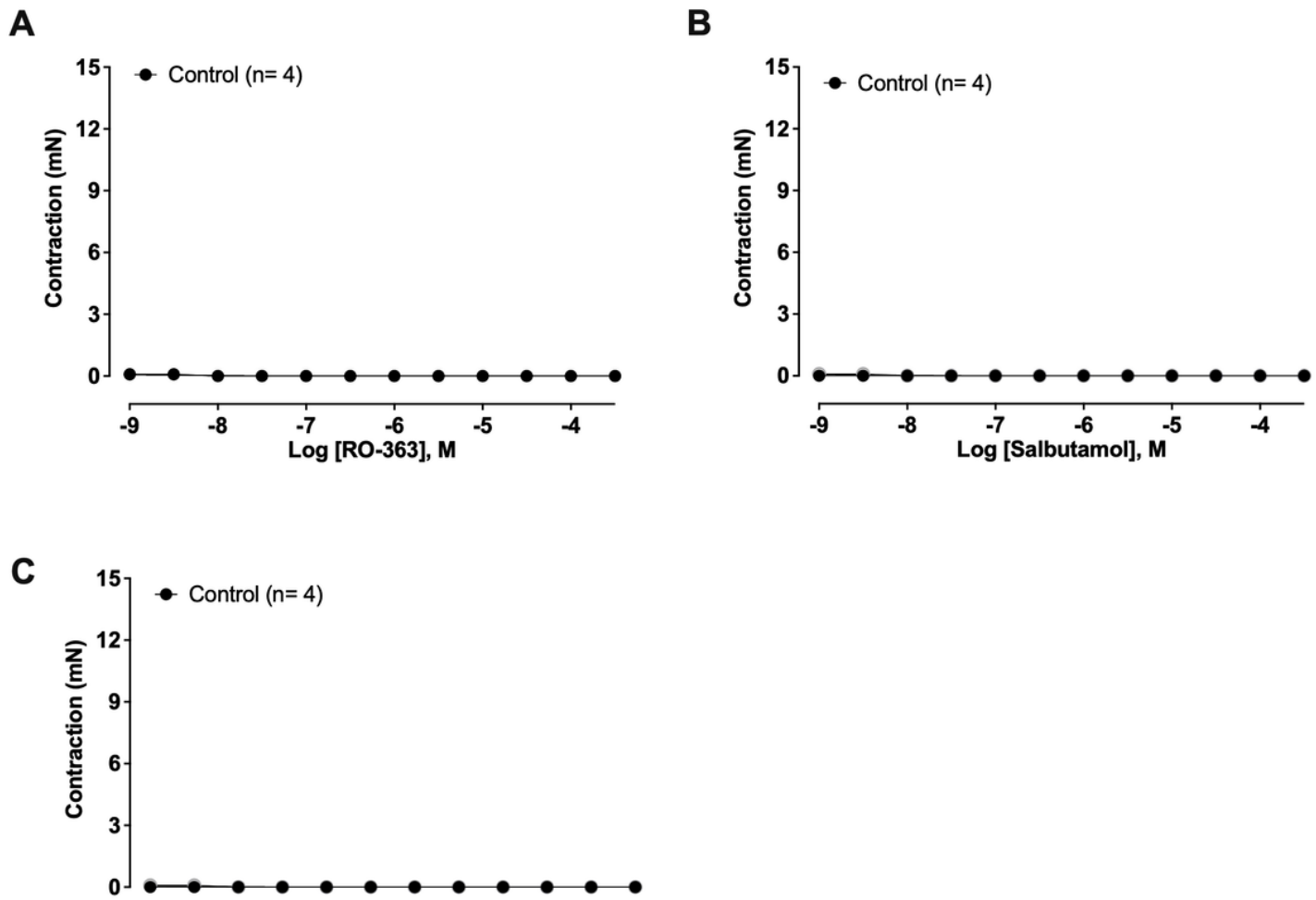


Figure 6

Effect of the β_1 -selective receptor agonist RO-363 (panel A), the β_2 -selective receptor agonist salbutamol (panel B) and of the β_3 -selective receptor agonist mirabregon (panel C) In the rat isolated epididymal vas deferens tone. n means the number of vas deferens strips.