Investigation on the Positive Chronotropic Action of 6- Nitrodopamine in the Rat Isolated Atria

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Abstract

6-Nitrodopamine (6-ND) is released from rat isolated atria being 100-times more potent than noradrenaline and adrenaline, and 10,000-times more potent than dopamine as a positive chronotropic agent. The present study aimed to investigate the interactions of 6-ND with the classical catecholamines, phosphodiesterase (PDE)-3 and PDE4 and the protein kinase A in rat isolated atria. Atrial incubation with 1 pM of dopamine, noradrenaline or adrenaline had no effect on atrial frequency. Similar results were observed when the atria were incubated with 0.01 pM of 6-ND. However, co-incubation of 6-ND (0.01 pM) with dopamine, noradrenaline or adrenaline (1 pM each) resulted in significant increases in atrial rate, which persisted over 30 min after washout of the agonists. The increased atrial frequency induced by co-incubation of 6-ND with the catecholamines was significantly reduced by the voltage-gated sodium channel blocker tetrodotoxin (1 mM, 30 min), indicating that the positive chronotropic effect of 6-ND is due in part to activation of nerve terminals. Pre-treatment of the animals with reserpine had no effect on the positive chronotropic effect induced by dopamine, noradrenaline or adrenaline; however, reserpine markedly reduced the 6-ND (1 pM)-induced positive chronotropic effect. Incubation of the rat isolated atria with the protein kinase A inhibitor H-89 (1 mM, 30 min) abolished the increased atrial frequency induced by dopamine, noradrenaline and adrenaline, but only attenuated the increases induced by 6-ND. 6-ND induces catecholamine release from adrenergic terminals and increases atrial frequency independently of PKA activation.

1. Introduction

The catecholamines noradrenaline and adrenaline are reported as the most powerful stimulators of cardiac function through activation of b-adrenoceptors (Vandecasteele & Bedioune, 2021). Activation of these receptors generates intracellular signaling events through G protein and effectors such as adenylyl cyclase, a family of enzymes that produce cyclic AMP (cAMP; Fu et al., 2013). Although the mammalian heart presents five phosphodiesterases (PDE1-5; Bender and Beavo, 2006), cardiac cAMP is hydrolysed mainly by PDE3 and/or PDE4 (Fischmeister et al., 2006). Inhibition of PDE3 or PDE4 potentiates the positive inotropic effects induced by noradrenaline in human (Christ et al., 2009) and rat ventricular and atrial myocardium (Katano & Endoh, 1992; Christ et al., 2009). However, the modulation of the β1-adrenoceptor-mediated positive inotropism by PDEs is considerably different from their role in modulating the chronotropism. For instance, inhibition of cAMP-dependent protein kinase A (PKA) elicits significant bradycardia in cardiac pacemaker cells (Vinogradova et al., 2006) whereas selective inhibition of either PDE3 or PDE4 in the rat and mouse causes sinoatrial tachycardia (Galindo-Tobar and Kaumann, 2008). In addition, neither PDE3 nor PDE4 inhibitions potentiated the tachycardia elicited by b_1-adrenoceptor activation in response to catecholamines (Kaumann et al., 2009). Whether this lack of potentiation reflects distinct compartments of PDE or else, that the increase in heart rate induced by b_1-adrenoceptor stimulation is independent of increases in cAMP, is not clear.

6-nitrodopamine (6-ND) is a novel catecholamine that is released from vascular tissues such as human umbilical arteries and vein (Britto-Júnior et al., 2021a) and aortic rings of the reptiles *Chelonoidis*

carbonarius and *Panterophis guttatus* (Campos et al., 2020; Lima et al., 2022). In the vascular tissues, 6-ND is a potent relaxant agent, acting as a truly selective dopamine D_2-like-receptor antagonist (Lima et al., 2022). 6-nitrodopamine is also released from rat isolated atria where it exerts potent positive chronotropism (Britto-Júnior et al., 2022). As a positive chronotropic agent, 6-ND is 100-times more potent than noradrenaline and adrenaline and 10,000-times more potent than dopamine (Britto-Júnior et al., 2022. Incubation of rat atrial preparations with b_1-adrenoceptor antagonists blocked 6-ND-induced positive chronotropism at concentrations that did not affect the positive chronotropic action of dopamine, noradrenaline, and adrenaline, indicating that the negative chronotropic effect of b-adrenoceptors antagonists is due to the selective blockade of 6-ND receptor in the atria (Britto-Júnior et al., 2022). Here it was investigated the mechanism(s) of actions of 6-ND as a positive chronotropic agent and its interactions with the classical catecholamines dopamine, noradrenaline and adrenaline in atrial frequency.

2. Materials And Methods

2.1. Animals

Adult male Wistar rats (280 to 320 g) were provided by both the Central Animal House of the University of Campinas (CEMIB-UNICAMP; São Paulo, Brazil) and Animais de Laboratório Criação e Com. LTDA (ANILAB; Paulinia, São Paulo, Brazil). All experimental protocols were approved by the Ethics Committee for Animal Use of UNICAMP (CEUA; Protocol No. 5746-1/2021; 5831-1/2021) following the Brazilian Guidelines for the Production, Maintenance and Use of Animals for Teaching or Research from the National Council of Control in Animal Experimentation (CONCEA; Andersen, 2016) as well as by the ARRIVE guidelines (Percie du Sert et al., 2020). Animals were housed in cages (three per cage) located in ventilated cage shelters with a constant humidity of 55 ± 5% and temperature of 24 ± 1°C under a 12-hour light-dark cycle. Animals received filtered water and standard rodent food *ad libitum*.

2.2. Isolated right atrium 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 euthanasia. After euthanasia, the heart was removed, and the right atrium was isolated. The right atrium was mounted between two metal hooks in 10-mL custom designed glass chambers containing Krebs-Henseleit's solution (KHS), continuously gassed with a mixture of 95%O_2: 5%CO_2 at 37°C using a heated circulator (PolyScience, Illinois, USA). Tissues were allowed to equilibrate under a resting tension of 10 mN for one hour, and the isometric tension was registered using a PowerLab system (ADInstruments, Sydney, Australia; Riado et al., 1999).

2.3. Interaction of 6-ND with catecholamines in the rat isolated atrial rate

A single concentration of dopamine (1 pM), noradrenaline (1 pM) or adrenaline (1 pM) was added to the organ bath and the changes in atrial rate were monitored for 30 min. To evaluate the interaction of 6-ND with the other catecholamines, 6-ND (0.01 pM) was co-incubated with either dopamine (1 pM), noradrenaline (1 pM) or adrenaline (1 pM) and the changes in atrial rate were monitored for 30 min. The atria were then washed with fresh KHS to remove any drug residue, and an additional 30 min period was recorded. One atrium was used for each drug and each concentration.

2.4. Interaction of dopamine with noradrenaline or adrenaline in the rat isolated atrial rate

To evaluate the interaction of dopamine with noradrenaline and adrenaline, dopamine (1 pM) was co-incubated with either noradrenaline (1 pM) or adrenaline (1 pM) and the changes in atrial rate were monitored for 30 min. The atria were then washed with fresh KHS to remove any drug residue, and an additional 30 min period was recorded. One atrium was used for each drug and each concentration.

2.5. Interaction of noradrenaline with adrenaline in the rat isolated atrial rate

To evaluate the interaction of noradrenaline with adrenaline, noradrenaline (1 pM) was co-incubated with adrenaline (1 pM) and the changes in atrial rate were monitored for 30 min. The atria were then washed with fresh KHS to remove any drug residue, and an additional 30 min period was recorded. One atrium was used for each drug and each concentration.

2.6. Effect of tetrodotoxin (TTX) on interaction of 6-ND with catecholamines in the rat isolated atrial rate

A single concentration of TTX (1 mM) was added to the organ bath and the changes in atrial rate was monitored for 30 min. After this period, 6-ND (0.01 pM) was co-incubated with dopamine (1 pM), noradrenaline (1 pM) or adrenaline (1 pM) and the changes in atrial rate were monitored for another 30 min. The atria were then washed with fresh KHS to remove any drug residue, and an additional 30 min period was recorded. In separate preparations, the effect of TTX (1 mM, 30 min) was investigated in atrial rate changes induced by 6-ND (1 pM), dopamine (100 pM-100 mM), noradrenaline (100 pM-10 mM) or adrenaline (100 pM-10 mM) individually were evaluated.

2.7. Effect of the reserpine treatment in the rat isolated atrial rate

Rats were treated with reserpine (5 mg/kg, i.p.) or saline (2 mL/kg, i.p) once daily, starting two days before the experiment (Murnaghan, 1968). After treatment, the right atrium was isolated and cumulative concentration-response curves to tyramine (1 nM-100 mM), isoprenaline (100 pM – 10 mM), dopamine (1 nM – 30 mM), noradrenaline (1 nM-30 mM) or adrenaline (1 nM – 30 mM) were performed. In a separate set of reserpine-treated rats, the right atrium was incubated (30 min) with 6-ND (1 pM), and the atrial rate monitored during the incubation and for a further 30 min after KHS was changed to remove this agonist.

2.8. Interaction of dopamine, noradrenaline and adrenaline with PDE3 and PDE4 inhibitors in the rat isolated atrial rate

Cumulative concentration-response curves to dipyridamole (10 nM − 1 mM), cilostazol (10 nM − 100 mM), milrinone (10 nM − 1 mM) and rolipram (10 pM − 1 mM) were performed in isolated atria preparations in the absence and the presence (30 min) of 6-ND (0.01 pM), dopamine (100 pM), noradrenaline (1 pM) or adrenaline (1 pM).

2.9. Effect of the protein kinase A inhibitor H-89 in the rat isolated atrial rate

A single concentration of the protein kinase A inhibitor H-89 (1 mM) was added to the organ bath and the changes in atrial rate monitored for 60 min. After this period, 6-ND (1 pM), dopamine (100 nM), noradrenaline (100 pM) or adrenaline (100 pM) were incubated and the changes in atrial rate monitored for 30 min. One atrium was used for each drug and each concentration.

2.10. Drugs and solutions

6-nitrodopamine was bought from Toronto Research Chemicals Inc (Toronto, Ontario, Canada). Adrenaline, H-89, milrinone, noradrenaline, tetrodotoxin, rolipram were purchased from Cayman Chemical Co (Michigan, USA). Cilostazol, dipyridamole, dopamine and reserpine were obtained from Sigma-Aldrich Chemicals Co. (St Louis, Missouri, USA). 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). The composition of the KHS was in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and dextrose 5.6.

2.11. Data analysis

Nonlinear regression analysis to determine the pEC_{50} was carried out using GraphPad Prism (GraphPad Software, version 9.4, 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 mean ± standard error of the mean (SEM) of *n* experiments. Data of atrial rate are presented as beats per minute (bpm) before and after the respective stimulation or as the delta increase of atrial rate. Student's two-tail unpaired t-test was employed and the differences between groups. A p value of less than 0.05 was considered statistically significant. Since the study has an exploratory character, the p values should be considered descriptive (Motulsky,2014; Michel et al., 2020).

3. Results

3.1. Interactions of 6-ND with dopamine, noradrenaline, and adrenaline

Incubation (30 min) of the rat isolated atrium with dopamine (1 pM, Fig. 1A), noradrenaline (1 pM, Fig. 1B), or adrenaline (1 pM Fig. 1C) did not increase the atrial frequency. Atrial incubation with 6-ND (0.01 pM) also had no effect on the atrial frequency (data not shown); however, co-incubation of 6-ND (0.01 pM) with dopamine (1 pM, Fig. 1A), noradrenaline (1 pM, Fig. 1B) or adrenaline (1 pM Fig. 1C) resulted in significant increases in atrial rate, which persisted for at least 30 min after washout of the agonists. Co-incubation of 6-ND (0.01 pM) with lower concentrations of dopamine (0.1 pM), noradrenaline (0.1 pM) or adrenaline (0.1 pM) did not result in significant increases in atrial rate (data not shown).

Co-incubation of dopamine (1 pM) with 1 pM of either noradrenaline (Fig. S1A) or adrenaline (Fig. S1B) did not result in significant increases in atrial frequency. Co-incubation of noradrenaline (1 pM) with 1 pM of adrenaline (Fig. S1C) also failed to significantly increase atrial rate.

3.2. Effect of tetrodotoxin (TTX) on the positive chronotropic responses of catecholamines

Incubation with TTX (1 mM, 30 min) significantly decreased the basal atrial frequency (291±6 and 230±12 bpm, for control and TTX-treated atria, respectively; $p = 0.004$, $n = 4$) and abolished the increased atrial frequency induced by co-incubations of 6-ND (0.01 pM) with dopamine (1 pM, Fig. 1D), noradrenaline (1 pM, Fig. 1E) or adrenaline (1 pM, Fig. 1F).

Incubation with TTX (1 mM, 30 min) significantly attenuated the increase in atrial rate induced by 6-ND (1 pM; Fig. 2A) and abolished the increased atrial frequency observed after washout of the agonist (Fig. 2A), indicating that the positive chronotropic effect of 6-ND is due in part to activation of nerve terminals. Incubation with TTX (1 mM, 30 min) had no effect on the positive chronotropic effect induced by dopamine (Fig. 2B), noradrenaline (Fig. 2C) or adrenaline (Fig. 2D).

3.3. Effect of reserpine treatment on the ex vivo positive chronotropic effects induced by catecholamines

Tyramine produced concentration-dependent elevations of the atrial rate, which was nearly abolished by reserpine treatment, as expected (Fig. 3A). Reserpine treatment had no effect on the positive chronotropic effect induced by dopamine (Fig. 3B), noradrenaline (Fig. 3C) or adrenaline (Fig. 3D). Reserpine treatment significantly reduced the positive chronotropic effect induced by 6-ND (1 pM; Fig. 3E) and nearly abolished the increased atrial frequency observed after washout of the agonist (Fig. 3E), demonstrating that the positive chronotropic effect of 6-ND is partially due to release of catecholamines from nerve terminals.

3.4. Interactions of catecholamines with the PDE3 inhibitor cilostazol

Pre-incubation of the isolated atria with the PD3 inhibitor cilostazol resulted in concentration-dependent increases in the atrial frequency (Figs. 4A-D). Pre-incubation of the isolated atria (30 min) with 6-ND (0.01 pM) markedly reduced the increases in atrial rate induced by cilostazol (Fig. 4A). In contrast to 6-ND, pre-incubation of the atria (30 min) with dopamine (100 pM; Fig. 4B), noradrenaline (1 pM; Fig. 4C) or adrenaline (1 pM; Fig. 4D) had no effect on the increases in atrial rate induced by cilostazol.

3.5. Interactions of catecholamines with the PDE3 inhibitor dipyridamole

Pre-incubation of the isolated atria with the PD3 inhibitor dipyridamole resulted in concentration-dependent increases in the atrial frequency (Figs. 5A-D). Pre-incubation of the atria (30 min) with 6-ND (0.01 pM) abolished the increases in atrial rate induced by dipyridamole (Fig. 5A). In contrast to 6-ND, pre-incubation of the atria (30 min) with dopamine (100 pM; Fig. 5B), noradrenaline (1 pM; Fig. 5C) or adrenaline (1 pM; Fig. 5D) had no effect on the increases in atrial rate induced by dipyridamole.

3.6. Interactions of catecholamines with the PDE3 inhibitor milrinone

Pre-incubation of the isolated atria with the PD3 inhibitor milrinone resulted in concentration-dependent increases in the atrial frequency (Figs. 6A-D). Pre-incubation of the atria (30 min) with 6-ND (0.01 pM) significantly reduced the increases in atrial rate induced by milrinone (Fig. 6A). In contrast to 6-ND, pre-incubation of the atria (30 min) with dopamine (100 pM; Fig. 6B), noradrenaline (1 pM; Fig. 6C) or adrenaline (1 pM; Fig. 6D) had no effect on the increases in atrial rate induced by milrinone.

3.7. Interactions of catecholamines with the PDE4 inhibitor rolipram

Pre-incubation of the atria with the PD4 inhibitor rolipram resulted in concentration-dependent increases in the atrial frequency (Figs. 7A-D). Pre-incubation of the atria (30 min) with 6-ND (0.01 pM; Fig. 7A), dopamine (100 pM; Fig. 7B), noradrenaline (1 pM; Fig. 7C) or adrenaline (1 pM; Fig. 7D) had no effect on the increases in atrial rate induced by rolipram.

3.8. Effect of the protein kinase A inhibitor H-89 on the positive chronotropic effect of catecholamines

Incubation of the rat isolated atria with protein kinase A inhibitor H-89 (1 mM, 60 min) resulted in a significant decrease of atrial frequency (296±9 and 273±8 bpm, for control and H89 respectively; $p = 0.044$, $n = 6$). Incubation of the isolated atria with H-89 abolished the increase in atrial frequency induced by dopamine (100 nM; Fig. 8A), noradrenaline (100 pM; Fig. 8B) or adrenaline (100 pM; Fig. 8C).

Incubation of the atria with H-89 (1 mM, 60 min) attenuated the positive chronotropic effect induced by 6-ND (1 pM; Fig. 8D) and reduced the increase in atrial frequency observed after washout of the agonist (Fig. 8D).

4. Discussion

The results clearly demonstrate that 6-ND, besides being the most potent endogenous catecholamine as a positive chronotropic agent in the rat isolated atrium, causes remarkable potentiation of the positive chronotropic effect induced by dopamine, noradrenaline and adrenaline. Although the mechanism(s) responsible for the 6-ND chronotropic action is not yet known, the results here presented give some interesting clues.

The attenuation of the chronotropic effect by TTX indicates that part of the chronotropic effect induced by 6-ND is due to activation of nerve terminals, most likely adrenergic nerve terminals. Indeed, the continuous activation of adrenergic terminals must be responsible for the prolonged chronotropic effect observed both *in vitro* and *in vivo*, following a single bolus administration of 6-ND (Britto-Júnior et al., 2022). This concept is further supported by the experiments with the indole alkaloid reserpine (Bein, 1953), which blocks the vesicular monoamine transporters VMAT1 and VMAT2 (Erickson et al., 1992), and reduces the stores of the monoamine neurotransmitters dopamine, noradrenaline and adrenaline (Liu et al., 1982). Pre-treatment of the animals with reserpine abolished the increase in atrial rate induced by tyramine, which acts as a catecholamine releasing agent (Murnaghan, 1968), and attenuated both the increase and duration in atrial rate induced by 6-ND, without affecting the responses to dopamine, noradrenaline and adrenaline. The concept that a nitro-catecholamine can release catecholamines is not new, since incubation of rat synaptosomes with 6-nitro-noradrenaline resulted in concentration-dependent release of noradrenaline (Li et al., 2000). Since 6-ND does not have a neurogenic origin in the heart (Britto-Júnior et al., 2022), what could be the mechanism(s) involved in the activation of the adrenergic terminals?

One possibility would be that 6-ND would be blocking the catecholamine uptake by the adrenergic terminals. Transporter-mediated uptake plays a major role in determining both the magnitude and duration of the catecholamine effect (Gasser, 2021). There are two types of monoamine transporter, namely, a high-affinity with low capacity to transport monoamines, such as NET (Gründemann et al., 2005; Engelhart et al 2020), a low-affinity with high capacity like organic cation transporters (OCT 1–3, Amphoux et al., 2006; Bacq et al., 2012) and the plasma membrane monoamine transporter (PMAT; Torres et al. 2003, Engel et al. 2004). For instance, 6-nitronoradrenaline inhibits noradrenaline uptake in rat brain tissue with an IC_{50} of 31 mM (Shintani et al., 1996). However, it is unlikely that 6-ND is blocking these transporters, since the potentiation induced by 6-ND is inhibited by TTX and these transporters are not dependent of sodium channel activation. Indeed, TTX does not affect dopamine-induced chronotropic effect and noradrenaline overflow from guinea-pig isolated heart (Habuchi et al., 1997). In addition, our results demonstrated that the chronotropic effect induced by noradrenaline and adrenaline is not affected by TTX. Another piece of evidence is that the inhibitors of OCT1, OCT2 and OCT3 such as d-

amphetamine, methamphetamine and cocaine act with IC_{50} in the mM range (Maier et al., 2021), whereas the potentiation of the chronotropic effect induced by 6-ND was observed with 10 fM.

Another possible mechanism could involve the inhibition of monoamine oxidases (MAO), enzymes that are responsible for inactivation of catecholamines due to oxidative deamination (Edmondson and Binda, 2018). There are two isoforms, MAO-A and MAO-B, the former has a higher specificity for endogenous amines like noradrenaline and adrenaline, whereas both isoforms metabolize dopamine (Manzoor & Hoda, 2020). 6-ND inhibited MAO-B activity in rat brain homogenates only at a high concentration (1 mM, 60% inhibition) whereas the other nitro-catecholamines caused no inhibition up to 100 mM concentration (Huotari et al., 2001). Although there is no data on the effect of nitro-catecholamines in MAO-A activity, the finding that the potentiation induced by 6-ND is blocked by TTX makes unlikely that MAO inhibition could be the mechanism responsible for such potentiation. Catechol-O-methyltransferase (COMT) metabolizes noradrenaline and adrenaline to the inactive metabolites normetadrenaline and metadrenaline, respectively (Flohé, 1974). Rat isolated atria and ventricles present COMT activity (Magaribuchi et al., 1988) and both 6-nitronoradrenaline and 6-nitrodopamine inhibit COMT activity with an IC_{50} of 7.5 (Shintani et al., 1996) and 10 mM (Huotari et al., 2001), respectively. The finding that these nitro-catecholamines act as weak inhibitors of COMT and the remarkable low concentration of 6-ND (10 fM) necessary for the potentiation of the chronotropic effect may exclude COMT inhibition as the main mechanism. The exocytic noradrenaline release from the sympathetic nerves is triggered by activation of the TTX-sensitive sodium and w-conotoxin-sensitive N-type calcium channels (Hirning et al., 1988; Vega et al., 1995), so it is likely that activation of 6-ND receptor in the adrenergic terminals should cause exocytosis, but the exact mechanism is unclear at the moment.

The finding that 6-ND can partly (but significantly) increase the atrial rate even in the presence of TTX and/or after the pre-treatment of the animals with reserpine indicates that there must be a different receptor from the receptor on the adrenergic terminals mentioned above, most likely located on the sinoatrial mode. Inhibition of PDE3 and/or PDE4 increases the atrial rate (Dolce et al., 2021), and as mentioned in the introduction section, there is interaction between these inhibitors and the chronotropic effect induced by dopamine, noradrenaline and adrenaline (Staveren et al., 2001). It has been proposed that PDE3 and PDE4 may be in different compartments in the cell (Kerfant et al., 2007), and selective inhibition of 6-ND-induced positive chronotropic effect by the PDE3 inhibitors cilostazol, dipyridamole and milrinone contrasting with the lack of effect of the PDE4 inhibitor rolipram supports this hypothesis.

Although inhibition of these PDEs increases the atrial frequency and that 6-ND-induced increased atrial rate is abolished by PDE3 inhibitors, it is unlikely that 6-ND could be a direct and potent PDE3 inhibitor. Human platelets do express PDE3 activity (Katsel et al., 2003) but 6-ND does not induce cAMP increases in human washed platelets (Nash et al., 2022). Whether this is due to action of 6-ND in its adrenergic receptor or in the receptor located in the sinoatrial node, remains to further investigated. What is very clear is although 6-ND, like the classical catecholamines, causes increases in atrial frequency, the 6-ND mechanism(s) is(are) different from that employed by the classical catecholamines. This concept is further supported by the results obtained with the protein kinase A inhibitor H-89 (Chijiwa et al., 1990).

Intracellular cAMP activates protein kinase A which phosphorylates the sarcolemmal calcium channel and phospholamban, a protein closely related to the sarcoplasmic reticular calcium pump (Honerjäger, 1989). As expected, H-89 abolished the increase in atrial frequency induced by dopamine, noradrenaline and adrenaline, but 6-ND was still able to increase the atrial rate in the presence of this inhibitor, pointing again for a different mechanism.

Conclusion

6-nitrodopamine is the most potent modulator of atrial chronotropism and in contrast to the classical catecholamines dopamine, noradrenaline and adrenaline has a double mechanism of action: it causes catecholamine release from adrenergic terminals and causes increase in atrial frequency independently of PKA activation.

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 5746-1/2021; 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: JBJ, ATL, VF, GDN.

Methodology: JBJ, ATL, VF, EA, FZM, GDN.

Project administration: GDN.

Supervision: FZM, EA.

Visualization: EA, GDN.

Writing – original draft: JBJ, FZM, 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

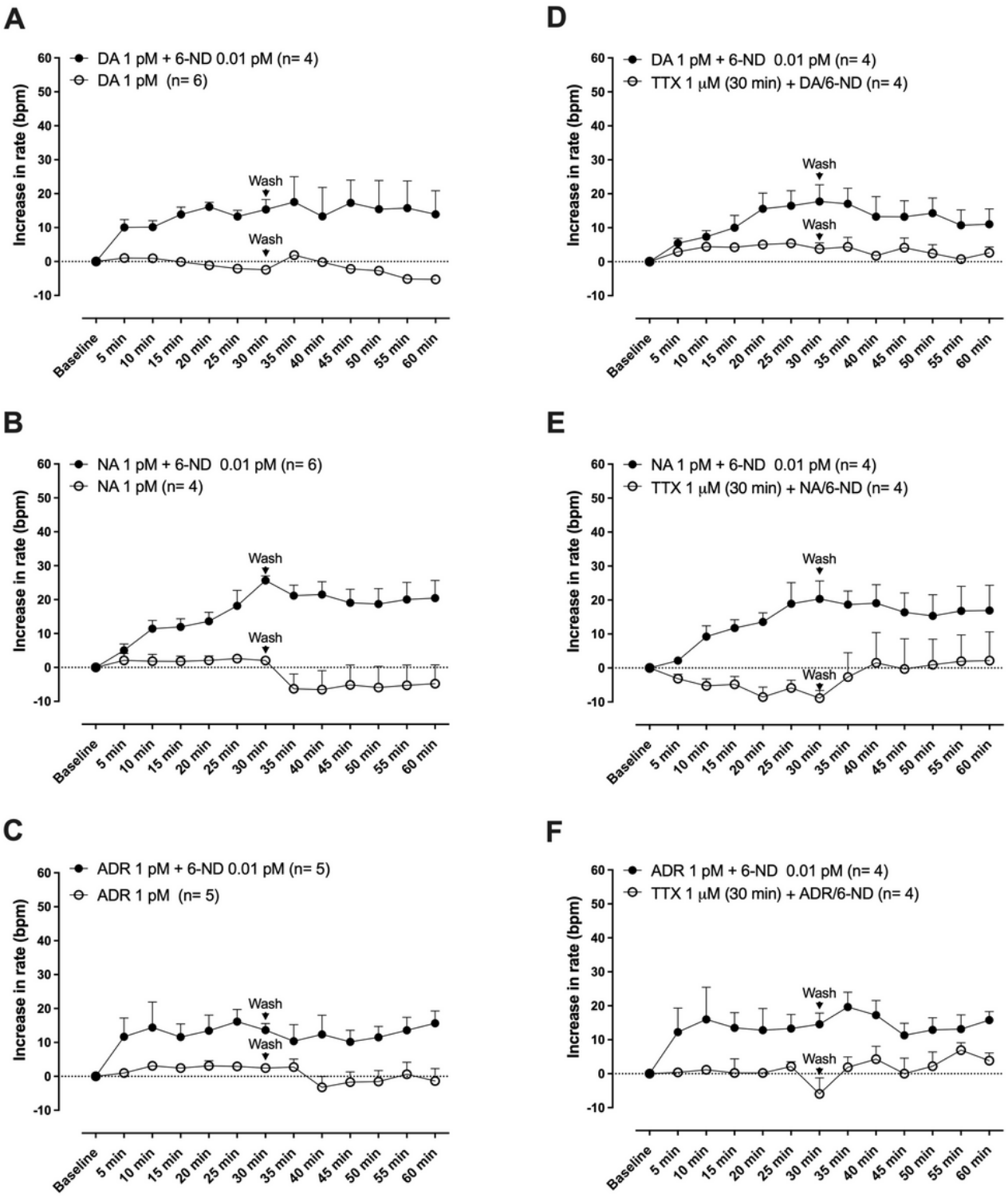

Figure 1

Interactions of 6-nitrodopamine (6-ND) with dopamine (DA), noradrenaline (NA), and adrenaline (ADR) in the rat isolated atrium. Interaction 6-ND (0.01 pM) of the rat isolated atrium with dopamine (A), noradrenaline (B), or adrenaline (C). Pre-treated with tetrodotoxin (TTX 1 mM, 30 min) in the interaction 6-ND (0.01 pM) of the rat isolated atrium with dopamine (D), noradrenaline (E), and adrenaline (F). Data represent the mean ± standard error of the mean (SEM).

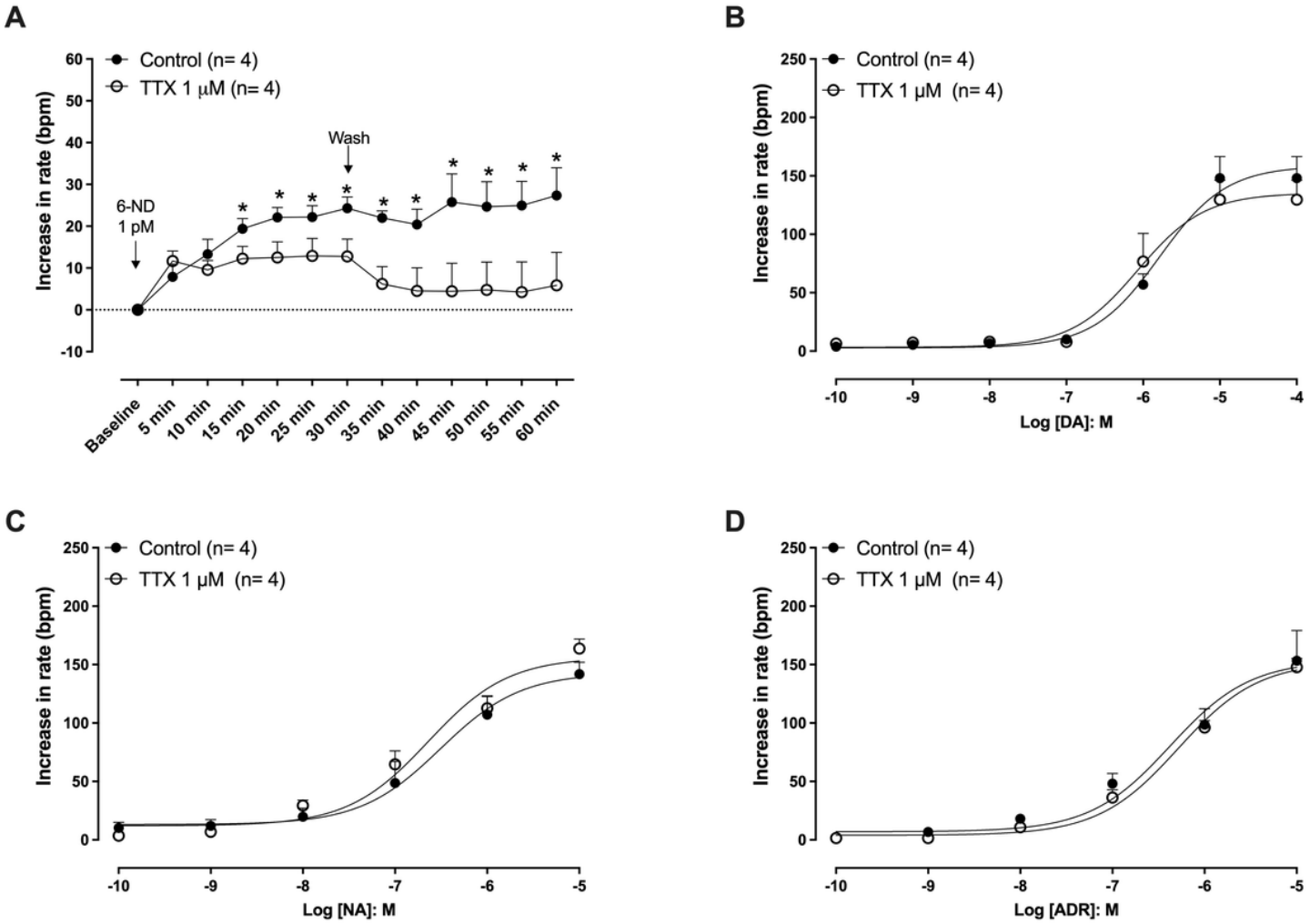

Figure 2

Effect of tetrodotoxin on the positive chronotropic effect of catecholamines in the rat isolated atrium. Pre-treated with tetrodotoxin (TTX 1 mM, 30 min) in the chronotropic effect of 6-nitrodopamine (6-ND, 1 pM; A) and connection-response curve dopamine (B), noradrenaline (C), or adrenaline (C) on the rat isolated atrium. Data represent the mean ± standard error of the mean (SEM). *P < 0.05 compared with respective control values

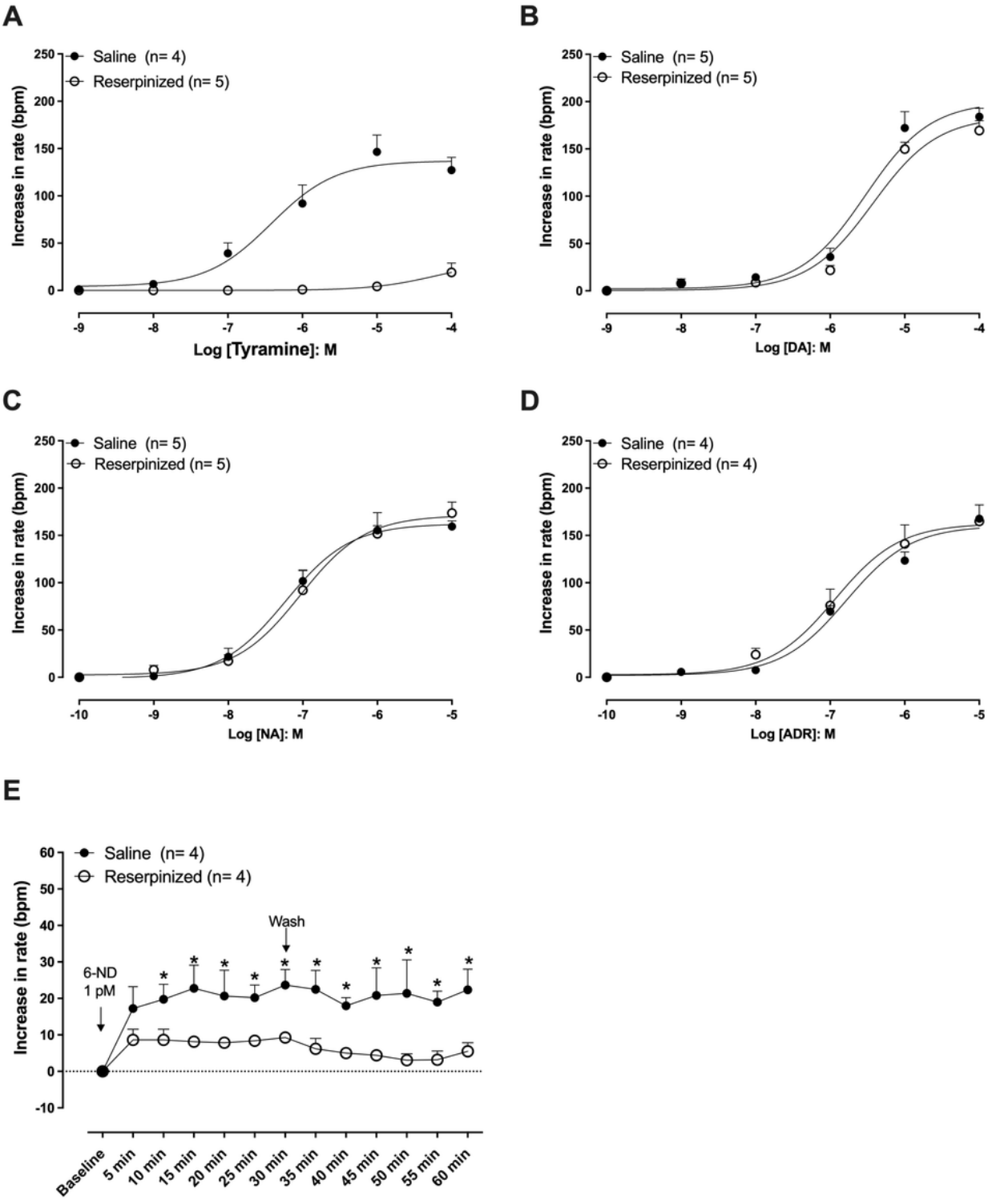

Figure 3

Effect of reserpine treatment on the positive chronotropic effect of catecholamines in the rat isolated atrium. In rats were treated with reserpine and saline chronotropic effect of chronotropic effect of connection-response curve tyramine (A), dopamine (B), noradrenaline (C), adrenaline (D) or single concertation of 6-nitrodopamine (6-ND, 1 pM; E) on the rat isolated atrium. Data represent the mean ± standard error of the mean (SEM). *P < 0.05 compared with respective control values.

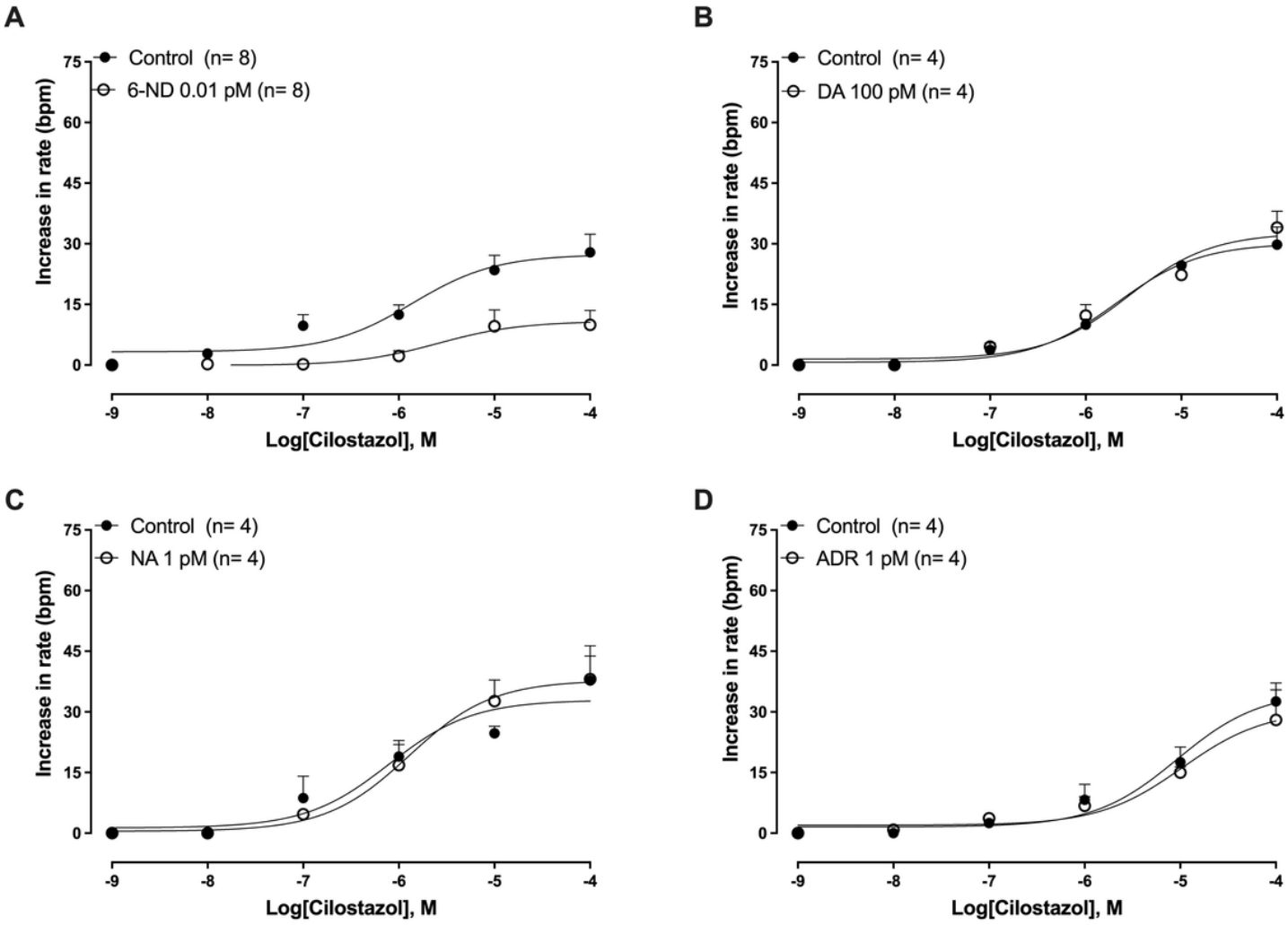

Figure 4

Interactions of catecholamines with the PDE3 inhibitor cilostazol

Cumulative concentration-response curves to cilostazol were performed in rat isolated atrial rate the presence (30 min) of 6-ND (A), dopamine (B), noradrenaline (C) or adrenaline (D). Data represent the mean ± standard error of the mean (SEM).

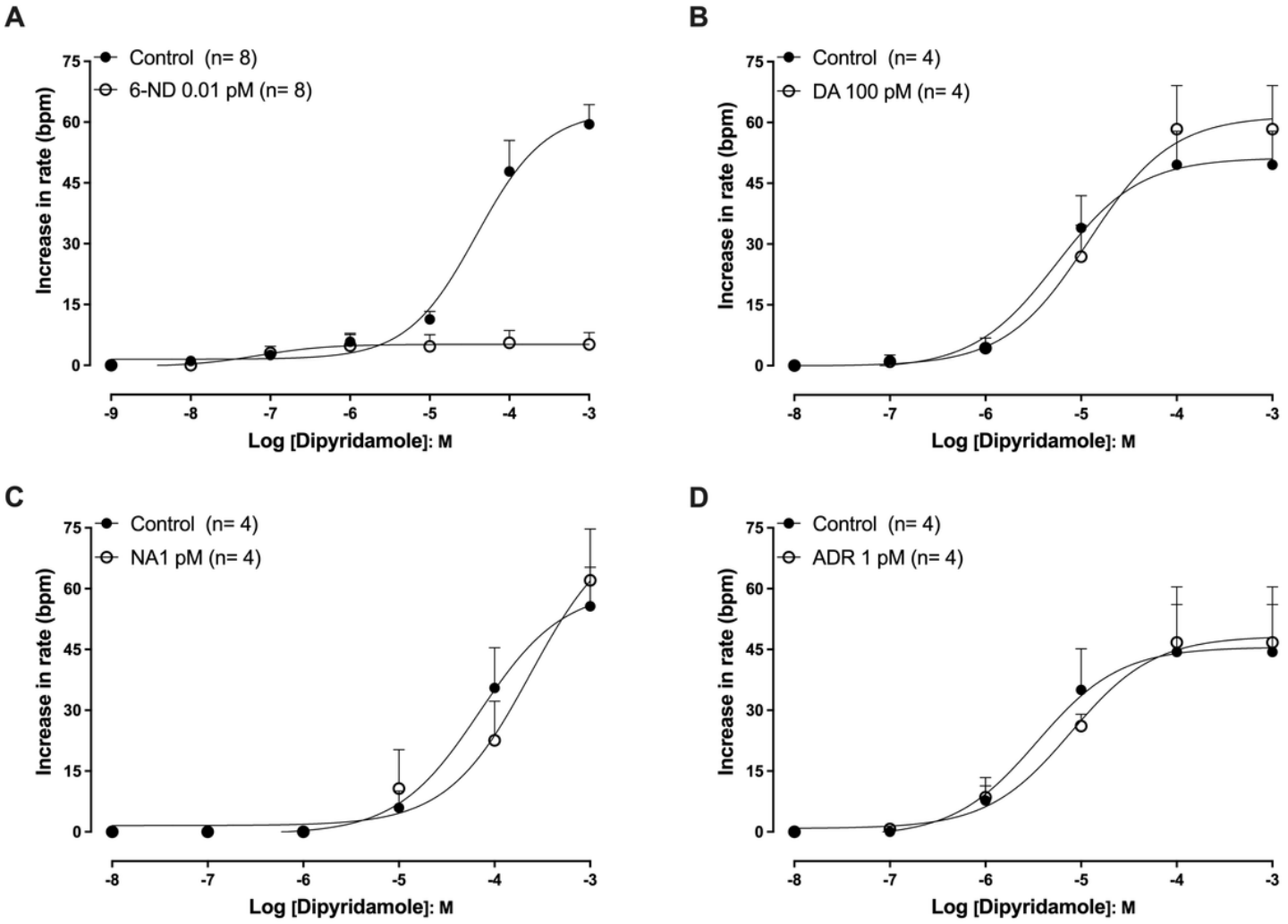

Figure 5

Interactions of catecholamines with the PDE3 inhibitor dipyridamole.

Cumulative concentration-response curves to dipyridamole were performed in rat isolated atrial rate the presence (30 min) of 6-ND (A), dopamine (B), noradrenaline (C) or adrenaline (D). Data represent the mean ± standard error of the mean (SEM).

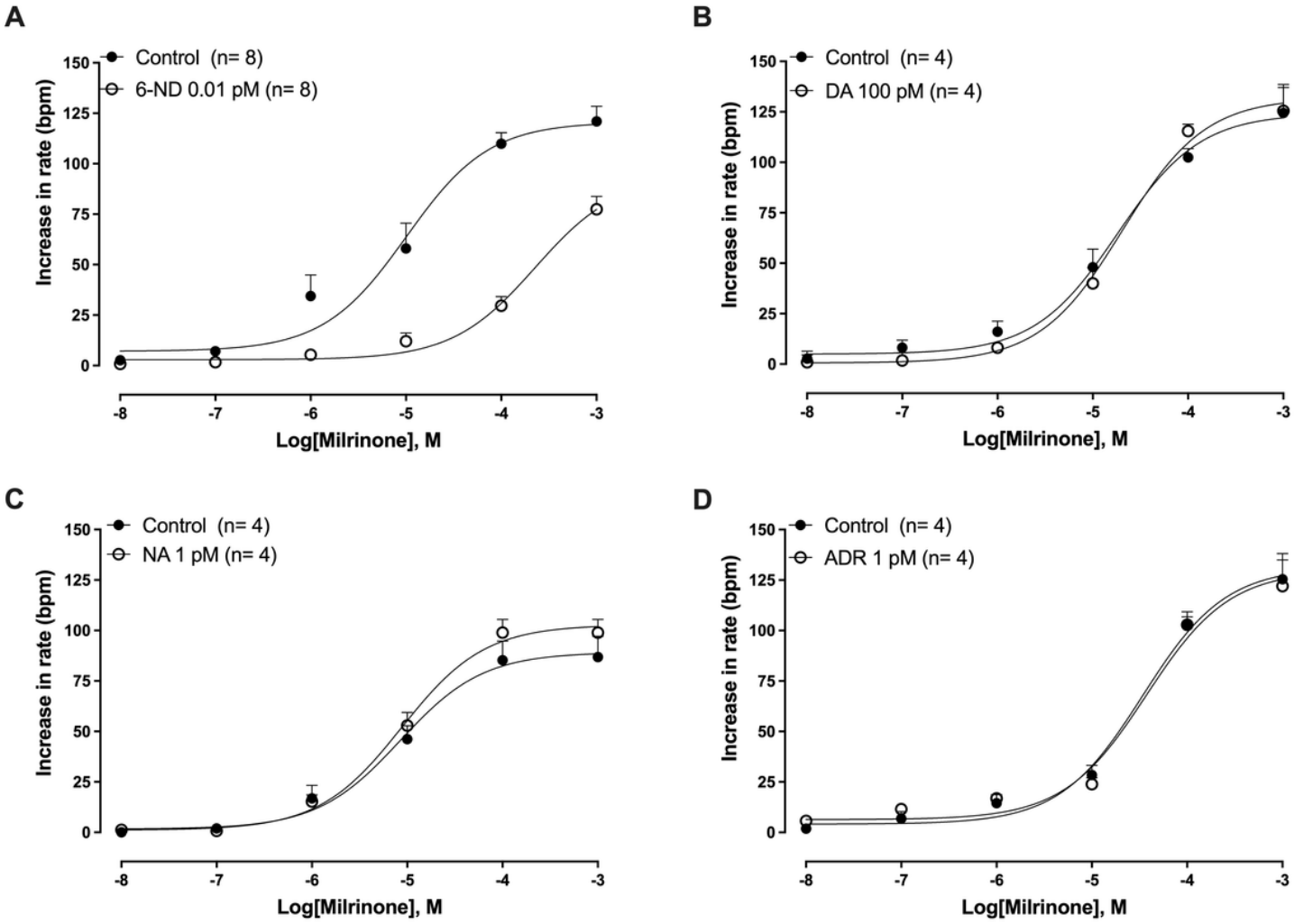

Figure 6

Interactions of catecholamines with the PDE3 inhibitor milrinone.

Cumulative concentration-response curves to milrinone were performed in rat isolated atrial rate the presence (30 min) of 6-ND (A), dopamine (B), noradrenaline (C) or adrenaline (D). Data represent the mean ± standard error of the mean (SEM).

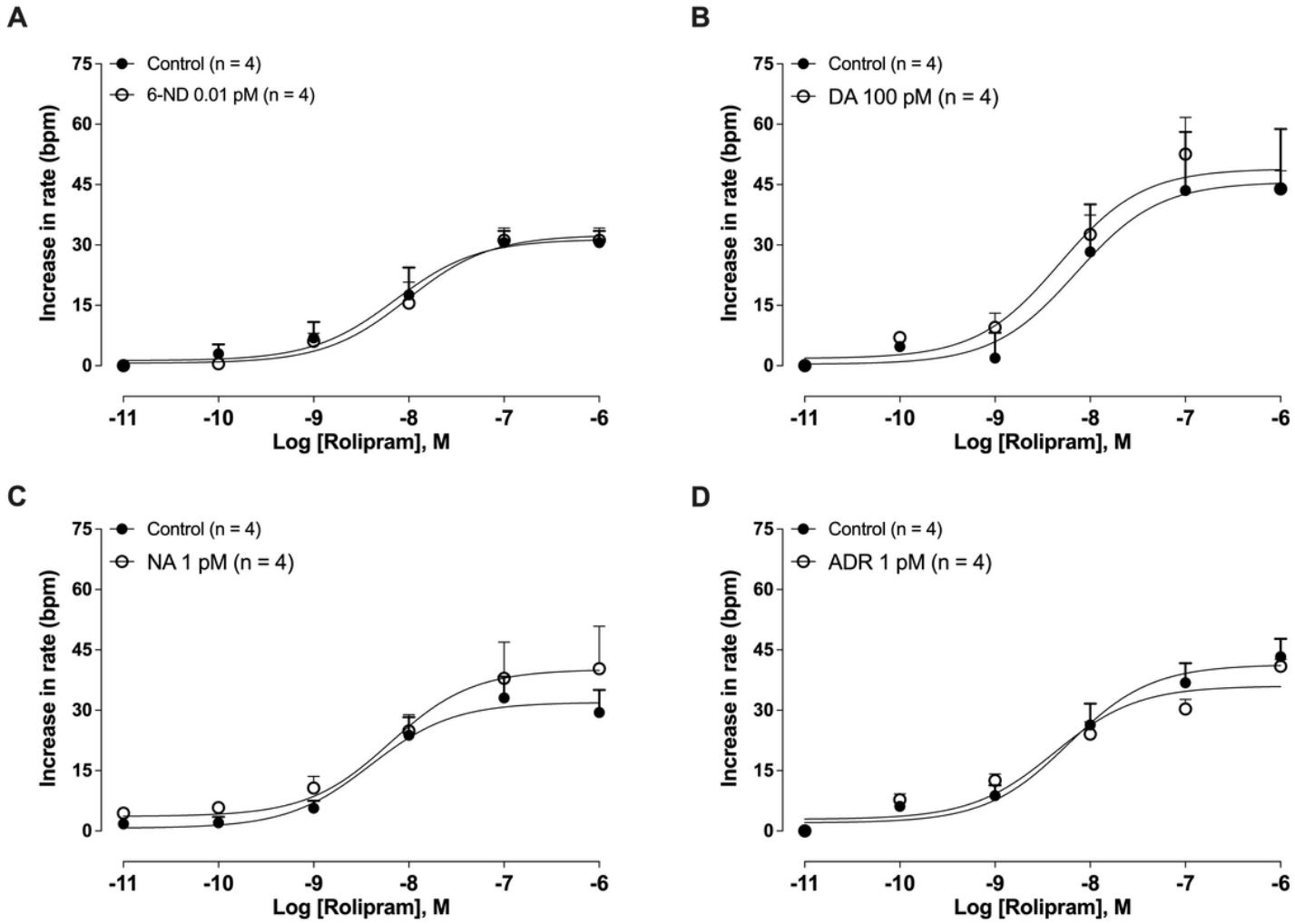

Figure 7

Interactions of catecholamines with the PDE4 inhibitor rolipram.

Cumulative concentration-response curves to rolipram were performed in rat isolated atrial rate the presence (30 min) of 6-ND (A), dopamine (B), noradrenaline (C) or adrenaline (D). Data represent the mean ± standard error of the mean (SEM).

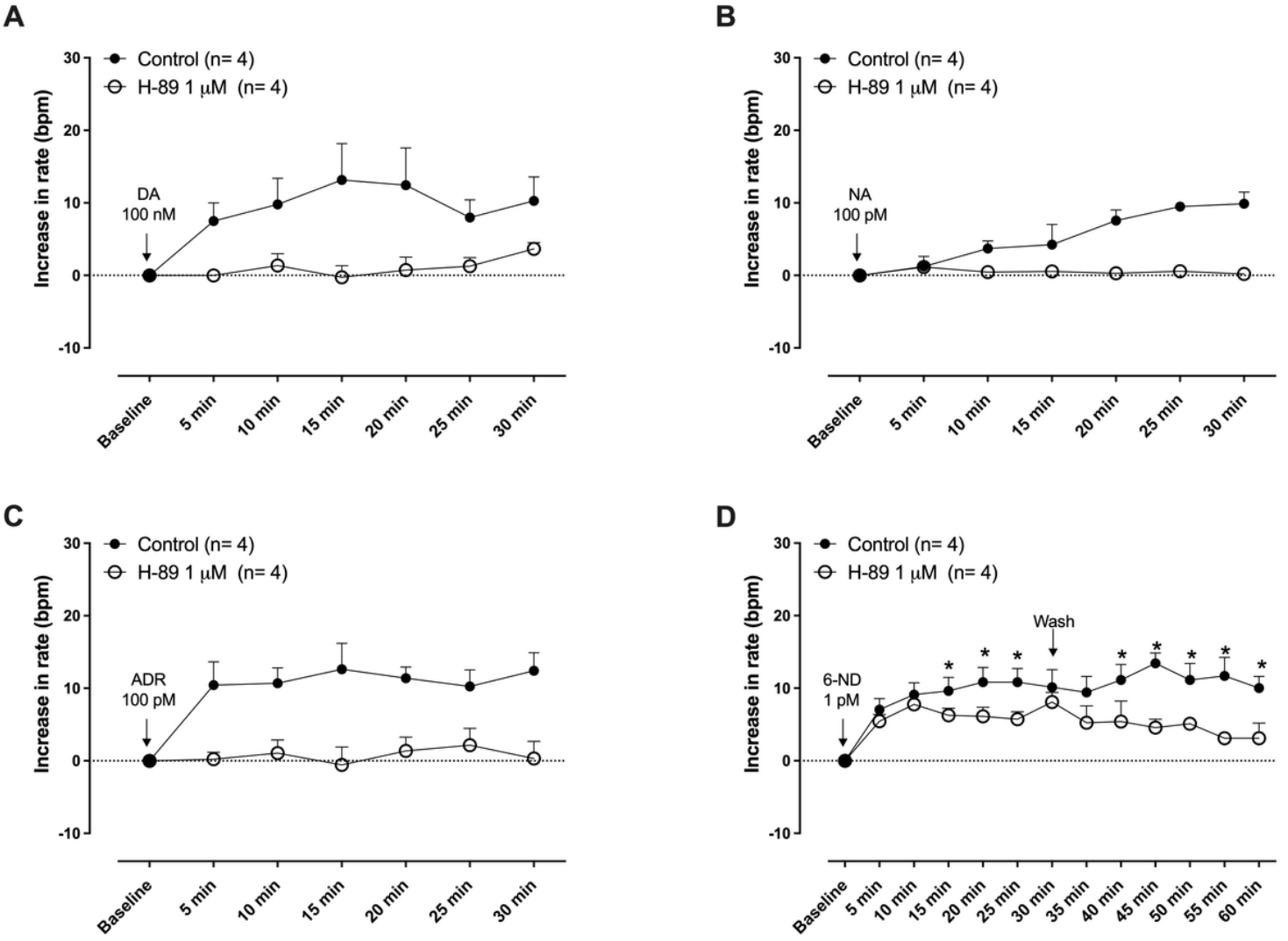

Figure 8

Effect of the protein kinase A inhibitor H-89 on the positive chronotropic effect of catecholamines in the rat isolated atrium. Pre-treated with H-89 (1 mM, 30 min) in the chronotropic effect of single dose 6-nitrodopamine (6-ND, A) and dopamine (B), noradrenaline (C), or adrenaline (C) on the rat isolated atrium. Data represent the mean ± standard error of the mean (SEM). *P < 0.05 compared with respective control values.

Supplementary Files

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