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Vas deferens smooth muscle responses to the nitric oxide-independent soluble guanylate cyclase stimulator BAY 41-2272

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ABSTRACT

The nitric oxide–cGMP signaling pathway modulates the ejaculatory functions. The nitric oxide (NO)-independent soluble guanylate cyclase haem-dependent stimulator BAY 41-2272 potently relaxes different types of smooth muscles. However, no study investigated its effects in vas deferens smooth muscle. Therefore, we designed experiments to evaluate the in vitro relaxing responses of vas deferens to BAY 41-2272. The effects of prolonged oral intake with BAY 41-2272 in vas deferens contractions of rats treated chronically with the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) were also investigated. BAY 41-2272 (0.001–100 μM) produced concentration-dependent relaxations in the prostatic and epididymal portions of vas deferens, an effect markedly reduced by the soluble guanylate cyclase inhibitor ODQ (100 μM). BAY 41-2272 significantly increased cGMP levels that were fully prevented by ODQ. In separate protocols, rats received L-NAME (20 mg/rat/day) concomitantly with BAY 41-2272 (10 mg/kg/day, 4 weeks), after which vas deferens contractions to electrical-field stimulation and noradrenaline were achieved. Electrical-field stimulation (1–32 Hz) evoked frequency-dependent contractions that were significantly enhanced in L-NAME-treated rats. Co-treatment with BAY 41-2272 fully reversed the increased contractile responses in L-NAME group. Noradrenaline (0.01–100 μM)-induced contractions were also greater in L-NAME-treated rats, and that was normalized by BAY 41-2272. In conclusion, BAY 41-2272 potently relaxes in vitro rat vas deferens smooth muscle and elevates the cGMP levels in an ODQ-sensitive manner. Moreover, prolonged oral intake with BAY 41-2272 restores the enhanced contractile vas deferens activity in rats treated with L-NAME. NO-independent soluble guanylate cyclase stimulators may be an alternative treatment for premature ejaculation.

1. Introduction

Premature ejaculation is defined as a persistent or recurrent ejaculation with minimal initial stimulation before, during or shortly after penetration, and most often before the subject wishes (Giuliano and Hellstrom, 2008). It is one of the most common male sexual disorders and has been estimated to occur in 21% of men in the general community, and is frequently present together with erectile dysfunction (Jannini and Lenzi, 2005; Jannini et al., 2005). Clinical studies demonstrated a beneficial effect of the phosphodiesterase-5 inhibitor sildenafil as a monotherapy or in combination or in with a serotonin selective reuptake inhibitor in the treatment of premature ejaculation (Abdel-Hamid, 2004; Chen et al., 2007; Wang et al., 2006).

Ejaculation consists of two main phases, namely the emission and expulsion phases (Andersson and Abdel-Hamid, 2011). The emission phase consists of sympathetic efferent fibers that cause sequential contractions of the epididymis, vas deferens, seminal vesicles and prostate, with closure of the bladder neck. The expulsion phase is initiated somatically from the sacral spinal cord via the pudendal nerve, causing rhythmic contractions of the bulbocavernosus muscles and other associated perineal muscles, which force the ejaculate through the distal urethra. Ejaculatory disorders may be caused by an association between hyperexcitable ejaculatory reflex and by alterations in the contractile mechanisms of vas deferens smooth muscle that occurs by releases of noradrenaline and ATP through stimulation of post-synaptic α_1-adrenoceptor and purinergic $P2X_1$ receptors, respectively (Burnstock, 2009; Buvat, 2011; Michel, 2007). Moreover, nitric oxide (NO) has been shown to play a critical role in the smooth muscle relaxation pathway by activating the soluble guanylyl cyclase to generate cGMP, which, in turn, promotes relaxation of different types of smooth muscle (Meldrum et al., 2010), including vas deferens smooth muscle (Mancina et al., 2005; Schultz et al., 1977). Intracellular cGMP is rapidly inactivated to 5′GMP by phosphodiesterases

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(Carson and Lue, 2005). Human vas deferens expresses a cGMP-related phosphodiesterase activity that mostly corresponds to PDE5, and is largely reduced by sildenafil and tadalafil (Mancina et al., 2005).

A previous study reported that mice lacking the gene for endothelial NO synthase ($eNOS^{-/-}$) need less stimulation to elicit ejaculation compared with wild mice (Kriegsfeld et al., 1999). Moreover, a recent study showed that the prostatic portion of vas deferens smooth muscle from rats treated chronically with the NO synthase inhibitor L-NAME displays enhanced purinergic and adrenergic contractions, indicating that NO deprivation is a useful model to study premature ejaculation (Gur et al., 2010). However, the role of NO–cGMP signaling pathway in vas deferens neurotransmission is still conflicting (Postorino et al., 1998; Ventura et al., 1998; Vladimirova et al., 1994).

The compound BAY 41-2272 has been reported as a potent NO-independent soluble guanylate cyclase haem-dependent stimulator (Evgenov et al., 2006; Stasch et al., 2001). This compound directly stimulates soluble guanylate cyclase, converts GTP into the second messenger cGMP and increases the sensitivity of the enzyme to NO (Stasch and Hobbs, 2009). BAY 41-2272 potently relaxes vascular (Baracat et al., 2003; Bischoff et al., 2003; Kalsi et al., 2003; Teixeira et al., 2006a,b) and non-vascular smooth muscle in vitro (Báu et al., 2010). Moreover, prolonged treatment with BAY 41-2272 ameliorate the erectile dysfunction (Claudino et al., 2011) and overactive bladder (Mónica et al., 2011) seen in L-NAME-treated rats. However, no study exists investigating the effects of NO-independent soluble guanylate cyclase agonist such as BAY 41-2272 in vas deferens smooth muscle in healthy or pathological conditions. In the present study we hypothesized that BAY 41-2272 counteracts the enhanced vas deferens contractions in L-NAME-treated rats. Therefore, we designed experiments to evaluate the relaxing responses of vas deferens smooth muscle to BAY 41-2272. The effects of prolonged oral intake with BAY 41-2272 in the enhanced vas deferens contractions in rats treated chronically with the NO synthase inhibitor L-NAME were also investigated. Since vas deferens smooth muscle contractions are greater in the epididymal portion compared with the prostatic portion (Kato et al., 1995), we have also compared the contractile responses to EFS and noradrenaline in both of these portions.

2. Material and methods

2.1. Animals

All experimental procedures were conducted in accordance with institutional guidelines, and they were approved by the Ethical Principles in Animal Research by the Brazilian College for Animal Experimentation (COBEA). Male wistar rats (weighing 250–300 g) were provided by Central animal House Services (CEMIB) of University of Campinas (UNICAMP, São Paulo). The animals were housed 5 per cage on 12 h light–dark cycle, and fed a standard chow diet with water ad libitum.

2.2. Functional studies in vas deferens strips

The animals were anesthetized with halothane and exsanguinated. The vas deferens was removed and immediately placed in chilled Krebs solution of the following composition (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$ $7H_2O$, 1.17 and $CaCl_2$ $2H_2O$, 2.5. Epididymal and prostatic portions of vas deferens were surgically dissected free (length, 1 cm, each) and each strip was mounted under resting tension of 10 mN in 10-ml organ chambers containing Krebs solution at 37 °C (pH 7.4) and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. Isometric force was recorded using a PowerLab 400™ data acquisition system (Software Chart, version 6.0, AD Instrument, MA, USA). The tissues were allowed to equilibrate for 1 h before starting the experiments. To verify the viability of the preparations, a high potassium chloride (KCl) solution (80 mM) was added to the organ baths at the end of the equilibration period.

The adrenergic contractile responses were assessed by cumulative concentration–response curves to noradrenaline (NA; 0.01 to 100 μM) in tissues pretreated with 17-β-estradiol (10 μM) to inhibit the extraneuronal uptake for catecholamines.

Electrical-field stimulation (EFS) was applied in strips placed between two platinum ring electrodes connected to a grass S88 stimulator (Astro-Med Industrial Park, RI, USA), and EFS was conducted at 50 V, 1 ms pulse width and trains of stimuli lasting 45 s at varying frequencies (1–32 Hz) in epididymal and prostatic portions.

2.3. Determination of cGMP levels

Vas deferens smooth muscle strips were equilibrated for 30 min in warmed and oxygenated Krebs solution. Tissues from control rats were stimulated for 15 min with BAY 41-2272 (10 μM) in the absence and in the presence of the sGC inhibitor ODQ (100 μM, 30 min). Next, tissues were immediately frozen in liquid nitrogen. Frozen tissues were pulverized, homogenized in trichloroacetic acid (TCA, 5% wt/vol), centrifuged for 10 min at 4 °C at 1500 *g* and the supernatant was collected. The pellet was dried and weighted. TCA was extracted from the supernatant with three washes of water saturated ether. Preparation of tracer, samples, standards and incubation with antibody were performed as described in commercially available kits (Cayman Chemical Cyclic GMP EIA kit, Ann Arbor, MI, USA). The assays were performed in duplicates, and the pellet weight was used to normalize the data that were expressed as pmol/mg tissue.

2.4. Experimental design with chronic L-NAME

Animals received L-NAME and/or BAY 41-2272 during 4 weeks, as follows ($n = 4$–5 each group): (1) Control: rats that received tap water alone with vehicle (80% DMSO); (2) L-NAME: rats that received L-NAME (20 mg/rat/day, given in the drinking water plus daily oral gavage of 80% DMSO); (3) L-NAME + BAY 41-2272: rats that received concomitantly L-NAME (20 mg/rat/day; given in the drinking water) and BAY 41-2272 (10 mg/kg/day; dissolved in 80% DMSO, given by daily oral gavage); and (4) BAY 41-2272: rats that received BAY 41-2271 alone (10 mg/kg/day, dissolved in 80% DMSO, given by daily oral gavage).

L-NAME was dissolved in the drinking water at a concentration of 400 mg/L to give a daily intake of 20 mg/rat/day. Rats belonging to Control and L-NAME groups also received daily oral gavage of 80% DMSO (vehicle for BAY 41-2272). The volume of water drunk by each rat was approximately 50 ml/rat/day. Doses of L-NAME and BAY 41-2272 were chosen according to our previous experience in giving these drugs alone or concomitantly to rats (Claudino et al., 2011).

2.5. Drugs and chemicals

17-β-estradiol, dimethylsulphoxide (DMSO), L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochrolide), noradrenaline and sodium nitroprusside were purchased from Sigma Chem. Co. (St Louis, MO, USA). 5-cyclopropyl-2-[1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl]pyrimidin-4-ylamine (BAY 41-2272) was provided by Pharma Research Center, Bayer AG (Wuppertal, Germany). All reagents used were of analytical grade. Stock solutions were prepared in deionized water or DMSO and stored in aliquots at −20 °C, and dilutions were prepared immediately before use.

2.6. Statistical analysis

Data are expressed as means ± S.E.M. of *n* experiments. The program Instat (GraphPad software) was used for statistical analysis. One way analysis of variances (ANOVA) followed by a Tukey's test