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Multiple effects of sibutramine on ejaculation and on vas deferens and seminal vesicle contractility

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ABSTRACT

Sibutramine is an inhibitor of norepinephrine and 5-HT reuptake largely used in the management of obesity. Although a fairly safe drug, postmarketing adverse effects of sibutramine were reported including abnormal ejaculation in men. This study investigates the effects of sibutramine on ejaculation and vas deferens and seminal vesicle contractility. Adult male rats received sibutramine (5; 20; or 50 mg kg^{-1}, ip) and after 60 min were exposed to receptive females for determination of ejaculation parameters. The vasa deferentia and seminal vesicles of untreated rats were mounted in isolated organ baths for recording of isometric contractions and HEK293 cells loaded with fluorescent calcium indicator were used to measure intracellular Ca^{2+} transients. Sibutramine 5 and 20 mg kg^{-1} reduced ejaculation latency whereas 50 mg kg^{-1} increased ejaculation latency. Sibutramine 3 to 30 μM greatly increased the sensitivity of the seminal vesicle and vas deferens to norepinephrine, but at concentrations higher than 10 μM there were striking depressions of maximal contractions induced by norepinephrine, carbachol and $CaCl_2$. In HEK293 cells, sibutramine 10 to 100 μM inhibited intracellular Ca^{2+} transients induced by carbachol. Depending on the doses, sibutramine either facilitates or inhibits ejaculation. Apart from its actions in the central nervous system, facilitation of ejaculation may result from augmented sensitivity of smooth muscles to norepinephrine while reductions of intracellular Ca^{2+} may be involved in the delayed ejaculation observed with high doses of sibutramine.

Introduction

Sibutramine is a non-selective inhibitor of the neuronal reuptake of norepinephrine and serotonin (5-hydroxytryptamine, 5-HT) currently used in the management of obesity and overweight (Padwal and Majumdar, 2007). The efficacy of sibutramine for treatment of obesity and overweight is related to increases in the monoaminergic neurotransmission, especially the noradrenergic, in the centers controlling satiety in the central nervous system (Jackson et al., 1997).

Although sibutramine is a fairly safe drug, postmarketing adverse effects were reported including abnormal ejaculation in men (FDA-Medwatch, 2000, available in http://www.fda.gov/medwatch/safety/1999/nov99.htm#meridi). It is well established that 5-HT modulates negatively ejaculation through activation of inhibitory efferent pathways (for review, see Giuliano, 2007). In fact, drugs that increase serotonergic neurotransmission, such as the antidepressants inhibitors of the neuronal reuptake of monoamines, delay ejaculation.

Interestingly, the potencies of the inhibitors of the neuronal reuptake of monoamines as antidepressants and as inhibitors of ejaculation are not necessarily coincident, suggesting that additional mechanisms may be involved, including peripheral actions such as direct inhibition of the contractions of the smooth muscles of the vas deferens (Seo et al., 2001).

Ejaculation is comprised of two coordinated phases, emission and ejection (for review see McMahon et al., 2004). The sympathetic nervous system innervates the structures involved in emission, including the vasa deferentia and seminal vesicles along with the ejaculatory ducts, the bladder neck, the prostate and other structures in the perineal floor. Emission starts with bladder neck closure, and then propulsive contractions of the smooth muscles of the vas deferens and prostate expel their contents into the prostatic urethra; this is accompanied by forcible expulsion of the contents of the seminal vesicles. Norepinephrine released from sympathetic varicosities innervating these organs has an important role in the contractions of these smooth muscles by activating α_1-adrenoceptors. Finally, the ejaculate is expelled from the urethra in a series of spurts caused by rhythmic contractions of the ischiocavernosus, bulbospongiosus and other associated perineal muscles as a consequence of activation

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of somatic fibers arising from S2 to S4 level of the sacral medulla (McMahon et al., 2004). In fact, the sympathetic nervous system has paramount importance in ejaculation and a α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor triple knockout mouse has severe fertility impairment because emission is drastically impaired in despite of producing viable sperm (Sanbe et al., 2007).

The present study characterizes the effects of sibutramine on ejaculation in male rats and investigates in details the multiple and complex actions of sibutramine in the contractility of the vas deferens and seminal vesicle *in vitro*.

Methods

General. The experimental procedures were approved by the Ethics Committee for the Use of Experimental Animals from UNESP — Botucatu and are in accordance with the Guide for the Care and Use of Laboratory Animals (NIH). Wistar rats (males, 16 to 20 weeks old, 250 to 380 g; virgin females, 14 to 16 weeks old, 200 to 220 g) were kept in a controlled environment with temperature at 25 ± 1 °C; humidity of 55 ± 5%; 12-h light/dark cycle (lights on at 6:00 a.m.) and had free access to regular lab chow (estrogen-free) and tap water.

Determination of ejaculation parameters. Male Wistar rats were previously tested for sexual behaviour. If the male did not mount or intromit within 10 min, this male was considered sexually inactive and not used (Agmo, 1997; Gerardin et al., 2005). Thus, sexually experienced male rats received sibutramine 5; 20; or 50 mg kg^{-1}, or vehicle (saline) ip, during the dark phase of the cycle. After 60 min, the rats were individually placed into a Plexiglass cage under red-light illumination, and after 5 min, a receptive female in natural oestrus phase (exhibiting lordosis) was introduced. The following parameters were recorded: intromission and ejaculation latencies and number of intromissions until the first ejaculation.

In vitro contraction studies. Male Wistar rats were killed by decapitation and selected tissues were carefully excised and prepared for digital recording of isometric contractions as follows: the vas deferens (epididymal portion) and seminal vesicle were cleaned of adherent tissues and secretions and mounted in organ baths under 9.8 mN tension in a nutrient solution with the following composition (mM): NaCl 138; KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15, dextrose 5.5, prepared in glass-distilled, de-ionised water, maintained at 30 °C (vas deferens) or 32 °C (seminal vesicle), pH 7.4, and continuously bubbled with 95%O_2/5%CO_2. This nutrient solution is a modified Tyrode's solution optimized to minimize erratic "spontaneous" contractions and along with the respective temperatures, improves the contractions of these two smooth muscles (Zuleika P. Picarelli, personal communication). Changes in vas deferens and seminal vesicle tension were recorded using FORT10 isometric force transducers (WPI, USA) connected to Transbridge 4M Transducer Amplifier (WPI, USA) connected to a PC based MP100 System and analysed off-line using AcqKnowledge version 3.5.7 software (Biopac Systems Inc., U.S.A.).

After a 30 min stabilization period, tissues were challenged with 80 mM KCl. This procedure was repeated until reproducible contractions were obtained. Then, a concentration–response curve to norepinephrine, methoxamine, carbachol, or KCl was constructed by adding cumulative concentrations of the drugs. For the concentration–response curves to $CaCl_2$, tissues were incubated in a nominal Ca^{2+}-free nutrient solution (same composition as above, except that $CaCl_2$ was omitted) with high K^+ (80 mM, a submaximal concentration in the vas deferens) as a depolarizing agent obtained by the addition of 74.3 mM KCl, 60 s before the concentration–response curve. After washing and relaxation, sibutramine 3 to 100 μM or nifedipine (10 to 300 nM, under subdued light) was incubated with the tissues. After at least 30 min, a concentration–response curve to the agonist was then obtained in the presence of sibutramine. Preliminary experiments showed that at least five consecutive concentration–response curves to the agonists tested are similar in respect to sensitivity and maximal response in both vas deferens and seminal vesicle. Sibutramine triggered erratic contractile activity in the vas deferens, which was quantified by the sum of any phasic or tonic activity above base line determined at 20 ms intervals during 5 min (15,000 samples) divided by the vas deferens wet weight. For normalization purposes, these measurements were taken 1 min after the addition of sibutramine since there was a delay of up to 50 s for the appearance of contractions.

Intracellular Ca^{2+} measurements. HEK293 cells were propagated in Dulbecco's modified Eagle's medium with sodium pyruvate supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin at 37 °C in a humidified atmosphere with 5% CO_2. Cells were seeded the night before the experiment at a concentration of 50,000 cells/100 μl per well of black walled, clear bottomed, 96-well microplates. Then, the cells were incubated at 37 °C for 60 min with the appropriate calcium indicator; FlexStation Calcium Assay Kit® and 2.5 mM probenecid. Cells loaded with this kit did not require wash step. The final volume in each well was 200 μl. The cells were incubated with sibutramine (3 to 100 μM) for 30 min. The plates were then placed into the FlexStation system (Molecular Devices, USA) to monitor fluorescence before and after the addition of carbachol, 10 μM. The samples were excited at 485 nm, and emission was detected at 525 nm with a 515 nm cutoff filter. Samples were read at 1.52 s intervals for 120 s (total of 79 reads/well). Responses were measured as peak fluorescence intensity minus basal fluorescence intensity, calculated within SoftMax®Pro.

Drugs and reagents. Drugs and reagents were obtained from the following sources: calcium chloride anhydrous (Vetec Quimica Fina Ltda, Brazil); carbamylcholine chloride (carbachol), methoxamine hydrochloride, nifedipine and norepinephrine [L-(−)-norepinephrine bitartrate salt monohydrate] all from Sigma-Aldrich, St. Louis, MO).

Prazosin hydrochloride (RBI/Sigma, Natick, MA); sibutramine hydrochloride monohydrate (Jiangyin Eas, China); FlexStation Calcium Assay Kit® (Molecular Devices, USA); Dulbecco's modified Eagle's medium; fetal bovine serum, streptomycin and penicillin all from Invitrogen, USA. All other salts and reagents were analytical grade.

Results

Effects of sibutramine on ejaculation

Sibutramine 5 and 20 mg kg^{-1}, usual hypophagic doses in rats (Jackson et al., 1997), reduced the latency for the first ejaculation by approximately 48% (Table 1). The reduction in the latency for ejaculation was also accompanied by a reduction in the number of intromissions until the first ejaculation. However, sibutramine 50 mg kg^{-1} increased the latency for the first ejaculation by approximately 50% (Table 1).

Table 1
Ejaculation parameters in male rats treated with vehicle (saline, ip) or increasing doses of sibutramine.

	Sibutramine (mg kg^{-1}, ip)			
	0 (vehicle)	5	20	50
Latency for first intromission (s)	10.4 ± 2.4	6.6 ± 1.2	13.4 ± 4.1	7.4 ± 1.4
Latency for first ejaculation (s)	372 ± 62	195 ± 32*	195 ± 29*	558 ± 62*
Number of intromissions until ejaculation	14.4 ± 1.7	9.7 ± 1.4*	9.0 ± 0.8*	10.7 ± 1.0*

Values represent the mean ± s.e.mean of observations made in 6 male rats per group.
* Different from the respective value found in rats treated with vehicle ($p<0.05$, ANOVA followed by Newman–Keuls).