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Influence of acute treatment with sibutramine on the sympathetic neurotransmission of the young rat vas deferens

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ABSTRACT

The effects of acute treatment with sibutramine on the peripheral sympathetic neurotransmission in vas deferens of young rats were still not evaluated. Therefore, we carried out this study in order to verify the effects of acute sibutramine treatment on the neuronal- and exogenous agonist-induced contractions of the young rat vas deferens. Young 45-day-old male Wistar rats were pretreated with sibutramine 6 mg/kg and after 4 h the vas deferens was used for experiment. The acute treatment with sibutramine was able to increase the potency (pD_2) of noradrenaline and phenylephrine. Moreover, the efficacy (E_{max}) of noradrenaline was increased while the efficacy of serotonin and nicotine were decreased. The maximum effect induced by a single concentration of tyramine was diminished in the vas deferens from treated group. Moreover, the leftward shift of the noradrenaline curves promoted by uptake blockers (cocaine and corticosterone) and β-adrenoceptor antagonist (propranolol) was reduced in the vas deferens of treated group. The initial phasic and secondary tonic components of the neuronal-evoked contractions of vas deferens from treated group at the frequencies of 2 Hz were decreased. Moreover, only the initial phasic component at 5 Hz was diminished by the acute treatment with sibutramine. In conclusion, we showed that the acute treatment with sibutramine in young rats was able to affect the peripheral sympathetic nervous system by inhibition of noradrenaline uptake and reduction of the neuronal content of this neurotransmitter, leading to an enhancement of vas deferens sensitivity to noradrenaline.

1. Introduction

Overweight and obesity among children and adolescents is considered a global public health epidemic (Kanekar and Sharma, 2010). Moreover, strong evidences suggest that the pharmacological intervention in obese youngster could avoid several comorbidities in their adulthood (Viner et al., 2009). In this context, sibutramine has been described in clinical trials as a valid therapeutic option for the management of obesity among children and adolescents (Kanekar and Sharma, 2010).

Sibutramine is an anti-obesity drug that exerts its pharmacological actions by inhibiting the reuptake of serotonin and noradrenaline in the central nervous system, mainly in the hypothalamus (Fletcher et al., 2010) leading to the energy expenditure increase and food intake decrease by enhancing satiety (Finer, 2002). Sibutramine is commonly considered a safe and well tolerated agent, but it may cause several side effects on cardiovascular system (Scheen, 2010) and on male reproduction, both related to the peripheral sympathomimetic actions of this drug (Padwal and Majumdar, 2007; Nojimoto et al., 2009; Borges et al., 2013). Because of the cardiovascular risk, in 2010 sibutramine was withdrawn from European and North American market by European Medicines Agency (EMEA) and Food and Drug Administration (FDA) (Scheen, 2011). However, it is still marketed in several countries, including Brazil (Mariotti et al., 2013).

The indirect peripheral sympathomimetic actions of sibutramine on reproductive system is manifested by the greatly increased potency of noradrenaline in inducing contractions of the adult rat vas deferens and seminal vesicle, leading to an abnormal ejaculation (Nojimoto et al., 2009). Additionally, sibutramine promoted an acceleration of sperm transit time in epididymal cauda, and a decrease of the quality and quantity of sperm reserves in adult rodents (Bellentani et al., 2011; Borges et al., 2013).

Previous study from our laboratory showed that the acute sibutramine treatment increase the calcium-induced contraction by the L-type voltage-gated calcium channels in vas deferens from young rats (Jurkiewicz et al., 2012). Nevertheless, the effect of

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acute sibutramine treatment on the peripheral sympathetic neurotransmission of young rat vas deferens was still not established.

Therefore, due to the few studies that emphasize the in vivo acute treatment with sibutramine in young animals, and its consequences on the peripheral sympathetic neurotransmission, we carried out a study in order to further evaluate the effects of acute sibutramine treatment on the neuronal and exogenous agonist-induced contractions of the young rat vas deferens. In the present work we have analyzed the effects of acute treatment with sibutramine in the contractions of young rat vas deferens induced by noradrenaline in the absence or presence of uptake blockers (cocaine and corticosterone) and β-adrenoceptor antagonist (propranolol), phenylephrine, serotonin, nicotine and tyramine or by endogenous neurotransmitters released from nerve terminals by electrical field stimulation (EFS).

2. Materials and methods

2.1. Animals and treatment

Young male Wistar, 45 days-old rats, from our colony (INFAR) were treated with a single dose of sibutramine 6 mg/kg (diluted in distilled water) by oral gavage or distilled water (control group). The dose of 6 mg/kg was chosen following previous studies in the literature that used similar doses to study the effects of sibutramine in rats, mainly in peripheral nervous system (Nojimoto et al., 2009; Bellentani et al., 2011; Francia-Farje et al., 2010). Four hours after acute treatment, rats were killed by decapitation, followed by the removal and isolation of vas deferens for functional experiments. The 4 h interval of treatment was chosen based on the half-life of sibutramine and the formation of its active metabolites, that for rodents have plasmatic pick between 2 and 3 h (Noh et al., 2010), presenting a high plasmatic concentration in the moment of the experiment. All experimental procedures were approved by Ethics Committee of Unifesp (protocol number 0015/13).

2.2. Functional experiments

2.2.1. Standard isolated organ bath preparation

Following isolation, the whole vas deferens was mounted under 1 g tension in a 10 ml organ bath containing a nutrient solution with composition (mM): 138 NaCl; 5.7 KCl; 1.8 $CaCl_2$; 0.36 NaH_2PO_4; 15 $NaHCO_3$ and 5.5 glucose, prepared in glass distilled deionized water bubbled with air, and maintained under 32 °C, pH 7.3 (Picarelli et al., 1962). Changes of isometric tension were digitally recorded by attaching one end of the vas deferens to a force-displacement transducer (CB Science, mod FT 302, USA) connected through a bridge amplifier to a PowerLab recording system (AD Instruments, Castle Hill, Australia), coupled to a computer. Data were stored by means of Chart version 4.2.1 software (AD Instruments, Castle Hill, Australia).

2.2.2. Concentration–response curves for agonists

The vas deferens was mounted as described above. After a stabilization period of 30 min, concentration–response curves were performed for noradrenaline, phenylephrine, serotonin or nicotine by the cumulative addition of the agonists to the organ bath. Moreover, cumulative concentration–response curves for noradrenaline were performed in the absence or presence of a mixture composed by cocaine (6×10^{-6} M), corticosterone (10^{-5} M) and propranolol (10^{-7} M) pre-incubated for 30 min in order to check the participation of noradrenaline uptake system (neuronal and extra-neuronal uptake) and β-adrenoceptors. Furthermore, the effect of a single concentration of tyramine (10^{-4} M, for 3 min) was performed to evaluate the release of noradrenaline from sympathetic nerve endings (Langeloh and Trendelenburg, 1987).

2.2.3. Neurogenic contractions

For the study of nerve-mediated contractions the vas deferens was mounted as described above and placed between two parallel platinum electrodes connected to an electrical stimulator Grass S88 (Grass, USA) inducing an electrical field stimulation (EFS) (Vladimirova et al., 1994). After an equilibration period of 30 min, the tissues were subjected to electrical field stimulation of 60 V, 1 ms duration, and 2 and 5 Hz, by means of a Grass S88 stimulator (Grass USA). For each frequency, the initial phasic (purinergic contraction) and the secondary tonic (noradrenergic contraction) components were measured (Fig. 1) (Westfall and Westfall, 2001; Burnstock and Verkhratsky, 2010).

2.3. Pharmacological parameters

The pharmacological parameters E_{max} (efficacy, indicated by the maximum contraction induced by a full agonist) (Jurkiewicz and Jurkiewicz, 1976) and pD_2 (potency, indicated by the negative Log of EC_{50}) (Jurkiewicz et al., 1977), were calculated to allow comparisons between curves noradrenaline, phenylephrine, serotonin and nicotine from control and treated groups. In addition, the concentration-ratio ($CR = EC_{50}a/EC_{50}b$; the ratio between EC_{50} of the agonist in the absence of blockers and EC_{50} of the agonist in the presence of blockers) was measured to quantify the potentiation of noradrenaline concentration–response curves after the addition of the noradrenaline system blockers and β-adrenoceptor antagonist, as described above (Section 2.2.2).

2.4. Drugs and reagents

Drugs were obtained from the following sources: sibutramine hydrochloride monohydrate, tyramine hydrochloride, (−)-nicotine, (±)-norepinephrine (+)-bitartarate salt, serotonin hydrochloride,

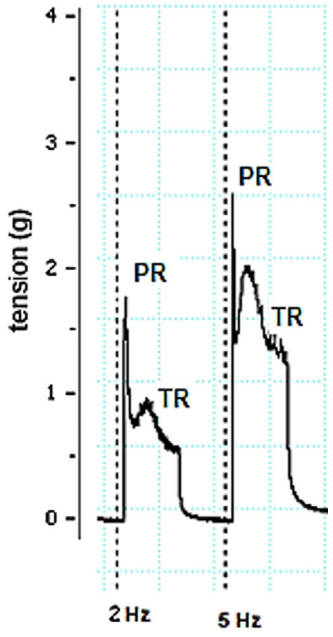

Fig. 1. Record of the contractile response of young rat vas deferens induced by electrical field stimulation (EFS) at frequencies of 2 and 5 Hz. The neuronal-evoked vas deferens contraction at 2 and 5 Hz is composed by an initial rapid component (PR: initial phasic response) followed by a maintained component (TR: secondary tonic response).