

# SIBUTRAMINE ANTINOCICEPTIVE EFFECT IN FEMALE RODENTS IS NOT DEPENDENT ON CATECHOLAMINERGIC SIGNALING

Maria Luisa Azevedo de Oliveira Sales<sup>1</sup>, Karolinne Souto de Figueiredo<sup>1</sup>, Juvenia Bezerra Fontenele<sup>2</sup>, Glaucé Socorro de Barros Viana<sup>1</sup>, Francisco Hélder Cavalcante Félix<sup>3</sup>

<sup>1</sup>Department of Biophysiology and Pharmacology, Faculdade de Medicina de Juazeiro do Norte.

<sup>2</sup>Department of Pharmacy, Faculdade de Farmácia, Odontologia e Enfermagem, Universidade Federal do Ceará.

<sup>3</sup>Pediatric Hemato-oncology Service, Hospital Infantil Albert Sabin

Correspondence to: Francisco Hélder Cavalcante Félix, Pediatric Cancer Center, Hospital Infantil Albert Sabin, Alberto Montezuma, 350 - Vila União - 60410-770 - Fortaleza - CE – Brazil, e-mail: [helderfelix@hias.ce.gov.br](mailto:helderfelix@hias.ce.gov.br)

**Running title:** Sibutramine analgesia not reverted by catecholamine block

Pages: 16; Abstract word count: 127; Text word count: 2443

Figures: 03; References: 15

## Authorship:

All authors have read and approved this manuscript.

# **Abstract:**

Sibutramine has a mechanism of action similar to that of antidepressants used as analgesics (like duloxetine). Limited data exists regarding the analgesic action of sibutramine. We tested increasing doses of p.o. sibutramine (0.1, 0.5, 1.5, 5.0 mg/kg) in the writhing test in female mice and in the plantar thermal hyperalgesia induced by carrageenan in female rats. The results showed a statistically significant ( $p < 0.001$ ) dose-response antinociceptive effect of sibutramine in these models, with a maximum effect comparable to the effect of a high dose of ASA (200 mg/kg) in mice and amitriptyline (10mg/kg) or indomethacin (10mg/kg) in rats. Rotarod test with sibutramine-treated rats ruled out motor impairment. Sibutramine-induced antinociception was not reverted by reserpine or yohimbine pre-treatment, suggesting that catecholamines and  $\alpha$ -2-adrenoceptors are not involved in this effect.

**Keywords:** sibutramine; analgesia; thermal hyperalgesia; catecholamines; chronic pain

## Introduction:

Sibutramine is a serotonin (5HT) and norepinephrine (NE) reuptake inhibitor (SNRI) approved for the treatment of obesity. It induces significant weight reduction in experimental animals and human patients (Finer, 2002; Brown, 2001). Antidepressant drugs with a similar mechanism of action, like venlafaxine and duloxetine show analgesic action in animal models and humans, both in acute and chronic pain (Jones, 2005; Arnold, 2007). Duloxetine have been approved for clinical use in chronic pain syndromes (Russell, 2008). The possibility that sibutramine, despite its lack of antidepressant effect, could have analgesic properties is supported by at least one publication using an experimental animal model of pain and two non-controlled, non-randomized reports from clinical usage in humans (Gray, 1999; Palangio, 2002). It could be possible that sibutramine could be used to treat painful conditions. There are scanty pre-clinical and clinical reports of analgesic efficacy of sibutramine in published literature.

We sought to test sibutramine analgesic effect in widely used animal models of nociception: writhing test in female mice and plantar thermal hyperalgesia test (Hargreaves, 1988) in female rats, and to evaluate catecholaminergic and alpha-2-adrenergic participation in the latter model with reserpine and yohimbine pre-treatment. We also wanted to rule out motor impairment or sedation with a rotarod test.

## Material and Methods:

### Animals:

Inbred female Swiss mice weighing between 18-30 g and female Wistar rats weighing a mean of 200 g were divided in groups of 6-10 animals each. Mice were housed in groups up to 17 per cage and rats were housed in groups up to six per cage in a

large colony room on a 12:12-h light/dark cycle (lights on 06:00 h), with food and water provided ad libitum. Each animal was used only once. Test sessions were performed between 08:00 and 18:00 h and animals habituated to the laboratory for a week prior to the tests. Food and water were withdrawn for 4 h before drug administrations. Estrous cycle was confirmed in each animal prior to the test performing. All experiments were conducted in accordance with the Brazilian regulations of animal care and experimentation covered by CONCEA documentations, equivalent to the *Guide to the Care and Use of Experimental Animals* (Vol. 1, 2nd ed., 1993, and Vol. 2, 1984, available from the Canadian Council on Animal Care, Constitution Square, Tower 2, Suite 315, 350 Albert Street, Ottawa, ON K1R1B1, Canada, or on their Web site at [www.ccac.ca](http://www.ccac.ca)), and they were approved by the local Institutional Animal Care and Use Committee.

### **Animal models:**

**Writhing Test in Mice.** Separate groups of 6 mice were administered vehicle or a dose of drug p.o. followed 55 min later by an i.p. injection of 0.55% acetic acid. Each mouse was then placed in an individual clear plastic observation chamber, and the total number of writhes made by each mouse was counted between 5 and 20 min after acetic acid administration (60–75 p.o. min after vehicle or dose of drug). Data are expressed as the mean number of writhes during the 15-min observation period. A group was treated with aspirin (ASA) 200 mg/kg and 4 other groups were treated with increasing doses of sibutramine hydrochloride monohydrate (0.1, 0.5, 1.5, and 5.0 mg/kg).

**Carrageenan-Induced Thermal Hyperalgesia.** Plantar test was performed as previously described (Hargreaves, 1988). Briefly, each rat was placed in a Plexiglas

cubicle with a glass floor through which an infrared photo beam was shown onto the plantar surface of the hind paws and the latency to withdrawal from the thermal stimulus was determined. The intensity of the infrared photo beam from the plantar reflex device (Plantar Test, Ugo Basile) was adjusted to produce a withdrawal latency in untreated rats (SHAM) of approximately 10-12 s (mean 11 s). The response latency was determined using a timer linked to the photodiode motion sensors in the plantar reflex device. Response latency was defined as the time from the onset of exposure to the infrared photo beam to the cessation of the photo beam when the photodiode motion sensors detected the withdrawal response of the paw of the rat. Groups of 10 rats were injected s.c. with  $\lambda$ -carrageenan (100  $\mu$ l of a 1.5% solution) into the plantar surface of the right hind paw at time zero followed immediately by a p.o. dose of vehicle or a dose of drug, 120 min before plantar test. The drugs used were sibutramine hydrochloride monohydrate in increasing doses of 0.1, 0.5, 1.5, and 5.0 mg/kg; amitriptyline 10 mg/kg and indomethacin 10 mg/kg. Three groups were pre-treated with reserpine (Sigma), 5.0 mg/kg, dissolved in 0.5 ml of glacial acetic acid and 9.5 ml of saline and administered i.p. (0.2ml/animal), 6 h before plantar test. Three groups were pre-treated with yohimbine 2.0 mg/kg dissolved in saline and administered i.p. (0.2 ml/animal) at the same time of oral drugs administration. Then, reserpine or yohimbine pre-treated animals were treated with sibutramine hydrochloride 1.5 mg/kg, amitriptyline 10 mg/kg, or vehicle in a single p.o. dose. Doses were selected from usual dosing in published literature and from our initial experiments with sibutramine (data not reported). Oral doses of drugs were given through a metal rodent feeding tube in a volume of less than 1.0ml, after fasting as described.

**Rotarod Test.** The effects of sibutramine on motor performance were evaluated using a Rotarod. Groups of 10 animals were given 3 initial training trials of 120 s, approximately 10 min apart, to maintain posture on a Rotarod (model 7650; Ugo Basile, Comerio, Italy), 3 cm in diameter, and rotating at a constant 12 revolutions/ min. The day after the initial training trials, a 60 s test trial was conducted, 2 hours after administration of sibutramine in a single p.o. dose of 5 mg/kg, or vehicle. The number of falls of each animal was recorded.

### **Statistical analyses:**

We used the number of animals per group necessary to obtain  $\alpha = 0.05$  (or less) with  $\beta = 0.8$  for an effect size between groups of at least 0.5 (calculation performed with R, package pwr). The results (expressed in mean and standard deviation of mean) between all groups were compared using one-way analysis of variance (degrees of freedom – DF, and F values – F, were reported), with Tukey’s multiple comparison as a post hoc test if significant ( $p < 0.05$ ). Anderson-Darling test for the composite hypothesis of normality was performed. Rotarod results were compared with Fischer’s exact test of independence. The statistical packages used were GraphPad Prism 5.0 (La Jolla, CA – USA) and R 2.X (R Foundation for Statistical Computing, Vienna, Austria).

### **Results:**

#### **Writhing Test in Mice.**

The number of writhes for control group was  $59.5 \pm 14.1$ , whereas it was  $27.5 \pm 3.4$  in ASA-treated animals and  $30.5 \pm 14.4$  (0.1 mg/kg),  $29.5 \pm 12.1$  (0.5 mg/kg),  $25 \pm 8.4$  (1.5 mg/kg) and  $21.8 \pm 6.8$  (5.0 mg/kg) in sibutramine-treated mice ( $p < 0.001$ , DF = 5, F = 9.8, ANOVA). All treated groups had mean number of writhes significantly

different from control (fig.1). Although there was no significant difference between treated groups, sibutramine treatment induced a clear dose-response effect.

### **Carrageenan-Induced Thermal Hyperalgesia.**

Reaction time for the control was  $6.0 \pm 1.6$  s. Sibutramine showed an acute analgesic dose-response effect in the plantar test. Reaction times were  $6.6 \pm 2.5$  s (0.1 mg/kg),  $8.8 \pm 2.7$  s (0.5 mg/kg),  $14.9 \pm 4.0$  s (1.5 mg/kg) and  $16.7 \pm 5.8$  s (5 mg/kg) ( $p < 0.001$ ,  $DF = 4$ ,  $F = 17.7$ , ANOVA). Mean reaction times of groups treated with sibutramine 1.5 and 5 mg/kg ( $p < 0.001$ ) were significantly different from control (fig.2B). Amitriptyline 10 mg/kg prolonged reaction time to  $11.1 \pm 4.7$  s ( $p < 0.05$ ), as well as indomethacin 10 mg/kg, prolonging reaction time to  $12.7 \pm 5.9$  s ( $p < 0.01$ ). Sibutramine 1.5 mg/kg did not modify significantly plantar test results when administered to rats without inflammatory stimulus ( $11.9 \pm 3.3$  s;  $p = 0.99$  compared to SHAM) (fig.2A). Pre-treatment with reserpine 5 mg/kg or yohimbine 2 mg/kg did not modify the antinociception induced by sibutramine 1.5 mg/kg, p.o. (fig.3) but did inhibit the antinociceptive effect of amitriptyline 10 mg/kg p.o. in the plantar test (data not shown). The results of incremental sibutramine doses were used to make a sigmoid log dose-response curve with  $R^2 = 0.94$  and  $ED_{50} = 0.72$  mg/kg (95% confidence interval = 0.16 – 3.22). Runs test showed that the results did not deviate significantly from the model ( $p = 1.00$ ).

### **Rotarod Test.**

All animals treated with sibutramine or vehicle were able to complete the Rotarod test without falls.

### **Discussion:**

Our results confirm that sibutramine has analgesic potential and showed a dose-response antinociceptive effect in the writhing test in mice, with a maximum effect equivalent to a high dose of ASA. It also showed anti-hyperalgesic effect in the plantar thermal hyperalgesia test model in rats, seemingly as potent as a high dose of amitriptyline or indomethacin. This action seems not to be dependent on central nervous system effects, like sedation or motor skill impairment, once animals did not show any alteration in rotarod test.

Animal models of nociception are usually performed with parenteral drug administration for convenience. However, most often the clinical usage of analgesic drugs is orally. Sibutramine hydrochloride monohydrate achieves pharmacological active concentrations when given orally to rodents, with a  $T_{max}$  of  $1 \pm 0.7$  h and a  $t_{1/2}$  of  $2.5 \pm 1.7$  h (Brown, 2001; Li, 2010). Based on this, we planned an interval of 2 h after oral administration of sibutramine to ensure pharmacologically active concentrations of the drug when the plantar test was done. Yohimbine timing of administration was planned to achieve receptor loading at sibutramine  $T_{max}$ , thus affecting its pharmacological action. Yohimbine dose was chosen based in another report that showed  $\alpha_2$ -adrenoceptors blockade could revert sibutramine anti-nociception (Gray, 1999). Although reserpine pretreatment is usually done for 24-48h in daily doses, this procedure yielded much motor impairment, precluding us to use this paradigm in plantar test (personal data, not published). Alternatively, we used a single dose 6 h before the nociception test, resulting in a significant reversal of amitriptyline antinociception (data not shown). Early works showed that doses as low as 1.0 mg/kg in animals have pharmacological action beginning 10-30 min after administration, blunting cardiovascular responses from 3-4 h and lasting



24 h after dose (Alper, 1963). *In vivo* brain microdialysis experiments have shown that 5 mg/kg i.p. reserpine induced an almost complete depletion of striatum dopamine at 180 min after dose that persisted for more than 6 h (Kannari, 2000).

The exclusive use of male animal models when investigating diseases more frequent in females, like painful syndromes, has been criticized. Research regarding gender differences in pain sensation has increased substantially in recent years, after the publication of influential reviews in the 90s. The relationship between sex and pain is not simple; nevertheless, most population-based studies have found higher prevalence in women than in men. (Fillingim, 2009) The prevalence of most common forms of pain is higher among women than men, and women report greater pain after invasive procedures than men, though these findings are less consistent. Additionally, compared with men, women display enhanced sensitivity to most forms of experimentally induced pain (with the exception of ischemic pain). (Fillingim, 2009) It has been also widely demonstrated that gender can modify the response to painful stimuli in experimental animals. Once SNRI are more often used for painful syndromes such as neuropathic pain and migraine, diseases more often affect women, we believe that using females animals as experimental models will lead to more appropriate results.

The writhing model in mice is the most sensitive animal model to detect analgesic action of drugs. The effect of sibutramine in this model confirms its analgesic effect. However, the writhing test is not specific, and its usefulness to investigate mechanisms of actions and dose-response relation is limited. Thus, we chose the plantar test to do both tasks. Unexpectedly, the analgesic effect of sibutramine was not reverted by pre-treatment with reserpine or yohimbine, which may indicate that it does not depend on

general catecholaminergic signaling and specifically on  $\alpha 2$ -adrenoceptors. Since sibutramine induced marked analgesia in single-dose 5 mg/kg reserpine pre-treated animals, one can rule out the confounding factor of regulation of cutaneous vasoconstrictor tone and skin temperature by the sympathetic nervous system. A previous publication had showed analgesic effects of sibutramine in a non-thermal pain evoked animal model, reverted by  $\alpha 2$ -adrenoceptors blockade (Gray, 1999).

There has been considerable discussion at the experimental level about the nature and underlying mechanisms of antidepressant analgesia. It has been demonstrated that antidepressants exert their antinociceptive effect via noradrenergic and opioidergic neurotransmission both in spinal and supraspinal sites (Gray, 1999). In the last decade, little was added to this paradigm and it has been repeatedly demonstrated that antidepressant-induced analgesia can be reverted by opioidergic antagonism (Sawynok, 2001) and depends partially on  $\alpha 2A$ -adrenoceptors of spinal location but also on a variety of individual drug effects that goes from serotonin (5HT) descending spinal signaling through peripheral ion channel blocking (Sawynok, 2001). Amitriptyline analgesia is lost in  $\alpha 2$ -adrenoceptors knock-out animals, indicating that this receptor class is the main responsible for mediating tricyclic antidepressants antinociception (Ozdogan, 2004). Venlafaxine, a SNRI with a similar mechanism of action than that of sibutramine, has been shown to induce  $\alpha 2$ -adrenoceptors-dependent analgesia (Sawynok, 2001); whereas duloxetine (other SNRI) had no analgesic effect in mice models where  $\alpha 2$ -adrenoceptor agonists show marked antinociception (Jones, 2005). However, both drugs as well as sibutramine were shown to increase extracellular NE in various regions of rat central nervous system (Invernizzi, 2004). Taken together, these data suggest that sibutramine

could have its analgesic effect explained by spinal  $\alpha 2$ -adrenoceptor activation, at least partially. This is not upheld by our results, indicating that other neural networks may be more important for sibutramine-related acute analgesia in the plantar test. One possibility, although speculative, is that sibutramine mechanism of action could be dependent on gender. It is more likely, however, that different animal models of nociception have different contributions of signaling pathways. Whereas sibutramine effect in mechanical hyperalgesia could have a prominent participation of descending spinal adrenergic signaling (Gray, 1999), this could not be the case for thermal hyperalgesia (our results), indicating the possible participation of supraspinal, perhaps opioidergic, signaling.

We conclude that sibutramine, a drug used for weight loss that has a similar mechanism of action to that of SNRI antidepressants, has analgesic effect in writhing test in female mice and in the plantar thermal hyperalgesia in female rats. Furthermore, this effect is independent from catecholaminergic signaling and specifically  $\alpha 2$ -adrenoceptors. More research is needed to clarify its mechanisms of action and its possible dependence on gender. It may be additionally tested in other pre-clinical models.

#### **Conflicts of interest:**

There are not potential sources of conflicts of interest.

## References:

Alper M.H., Flacke W., Kraye O. 1963. Pharmacology of reserpine and its implications for anesthesia. *Anesthesiology* 24: 524-542.

Arnold L.M., Pritchett Y.L., D'Souza D.N., Kajdasz D.K., Iyengar S., Wernicke J.F. 2007. Duloxetine for the treatment of fibromyalgia in women: pooled results from two randomized, placebo-controlled clinical trials. *J. Womens Health (Larchmt)* 16:1145-1156.

Brown M., Bing C., King P., Pickavance L., Heal D., Wilding J. 2001. Sibutramine reduces feeding, body fat and improves insulin resistance in dietary-obese male Wistar rats independently of hypothalamic neuropeptide Y. *Br. J. Pharmacol.* 132:1898-1904.

Fillingim R.B., King C.D., Ribeiro-Dasilva M.C., Rahim-Williams B., Riley J.L., III. 2009. Sex, Gender, and Pain: A Review of Recent Clinical and Experimental Findings. *J. Pain* 10: 447-485.

Finer N. 2002. Sibutramine: its mode of action and efficacy. *Int. J. Obes. Relat. Metab. Disord.* 26 Suppl 4:S29-33.

Gray A.M., Pache D.M., Sewell R.D.E. 1999. Do  $\alpha_2$ -adrenoceptors play an integral role in the antinociceptive mechanism of action of antidepressant compounds? *Eur. J. Pharmacol.* 378:161-168.

Hargreaves K., Dubner R., Brown F., Flores C., Joris J. 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32(1):77-88.

Invernizzi R.W., Garattini S. 2004. Role of presynaptic  $\alpha_2$ -adrenoceptors in antidepressant action: recent findings from microdialysis studies. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28:819-827.

Jones C.K., Peters S.C., Shannon H.E. 2005. Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. *J. Pharmacol. Exp. Ther.* 312:726-732.

Kannari K., Tanaka H., Maeda T., Tomiyama M., Suda T., Matsunaga M. 2000. Reserpine Pretreatment Prevents Increases in Extracellular Striatal Dopamine Following L-DOPA Administration in Rats with Nigrostriatal Denervation. *J. Neurochem.* 74: 263-269.

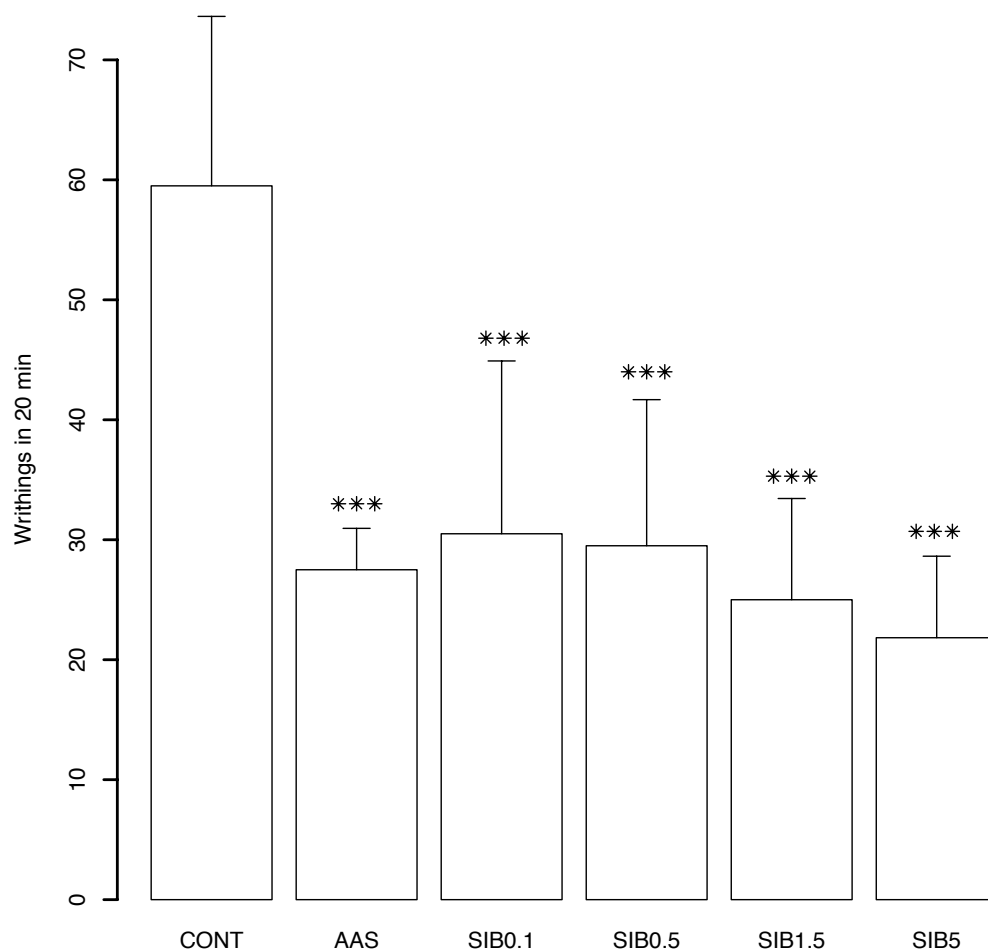
Li D.X., Jang K.Y., Kang W., Bae K., Lee M.H., Oh Y.K., Jee J.P., Park, Y.J., Oh D.H., Seo Y.G., Kim Y.R., Kim J.O., Yong C.S., Choi H.G. 2010. Enhanced Solubility and Bioavailability of Sibutramine Base by Solid Dispersion System with Aqueous Medium. *Biol. Pharm. Bull.* 33: 279-284.

Ozdogan U.K., Lahdesmaki J., Mansikka H., Scheinin M. 2004. Loss of amitriptyline analgesia in  $\alpha_2$ -adrenoceptor deficient mice. *Eur. J. Pharmacol.* 485:193-196.

Palangio M., Flores J.A., Joyal S.V. 2002. Treatment of fibromyalgia with sibutramine hydrochloride monohydrate: comment on the article by Goldenberg et al. *Arthritis Rheum.* 46:2545-2546.

Russell I.J., Mease P.J., Smith T.R., Kajdasz D.K., Wohlreich M.M., Detke M.J., Walker D.J., Chappell A.S., Arnold L.M. 2008. Efficacy and safety of duloxetine for treatment of fibromyalgia in patients with or without major depressive disorder: Results from a 6-month, randomized, double-blind, placebo-controlled, fixed-dose trial. *Pain* 136:432-444.

Sawynok J., Esser M.J., Reid A.R. 2001. Antidepressants as analgesics: an overview of central and peripheral mechanisms of action. *J. Psychiatry Neurosci.* 26:21-29.



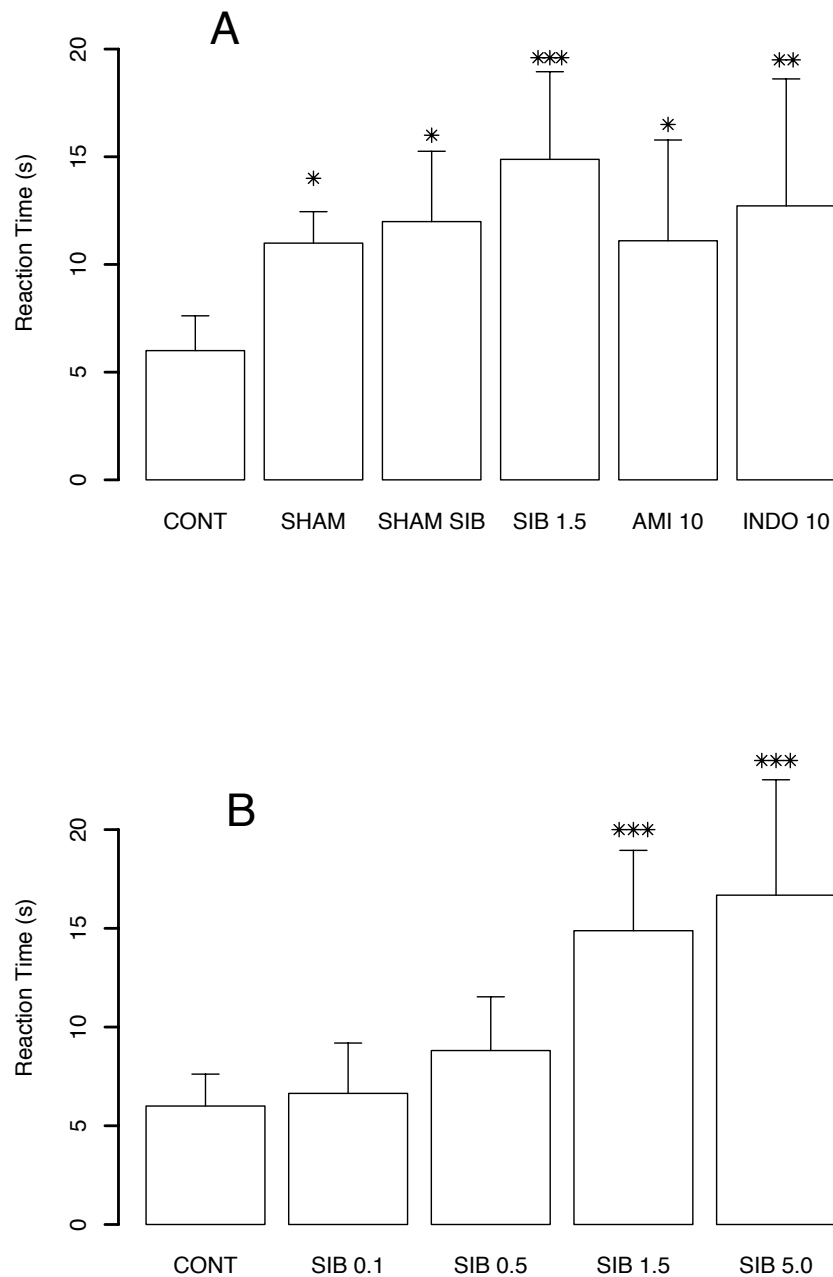
**Figure 1: Sibutramine inhibition of acetic acid-induced abdominal writhes in mice.**

Separate groups of 6 mice were administered vehicle or a dose of drug p.o. followed 55 min later by an i.p. injection of 0.55% acetic acid. The total number of writhes made by each mouse was counted between 5 and 20 min after acetic acid administration. Data are expressed as the mean number of writhes during the 15-min observation period. A group was treated with aspirin (ASA) 200 mg/kg and 4 other groups were treated with increasing doses of sibutramine hydrochloride monohydrate (0.1, 0.5, 1.5, and 5.0

mg/kg). Statistical significant difference from control is depicted when present (One-way ANOVA with Tukey as post-hoc test).

\*\*\*  $p < 0.001$



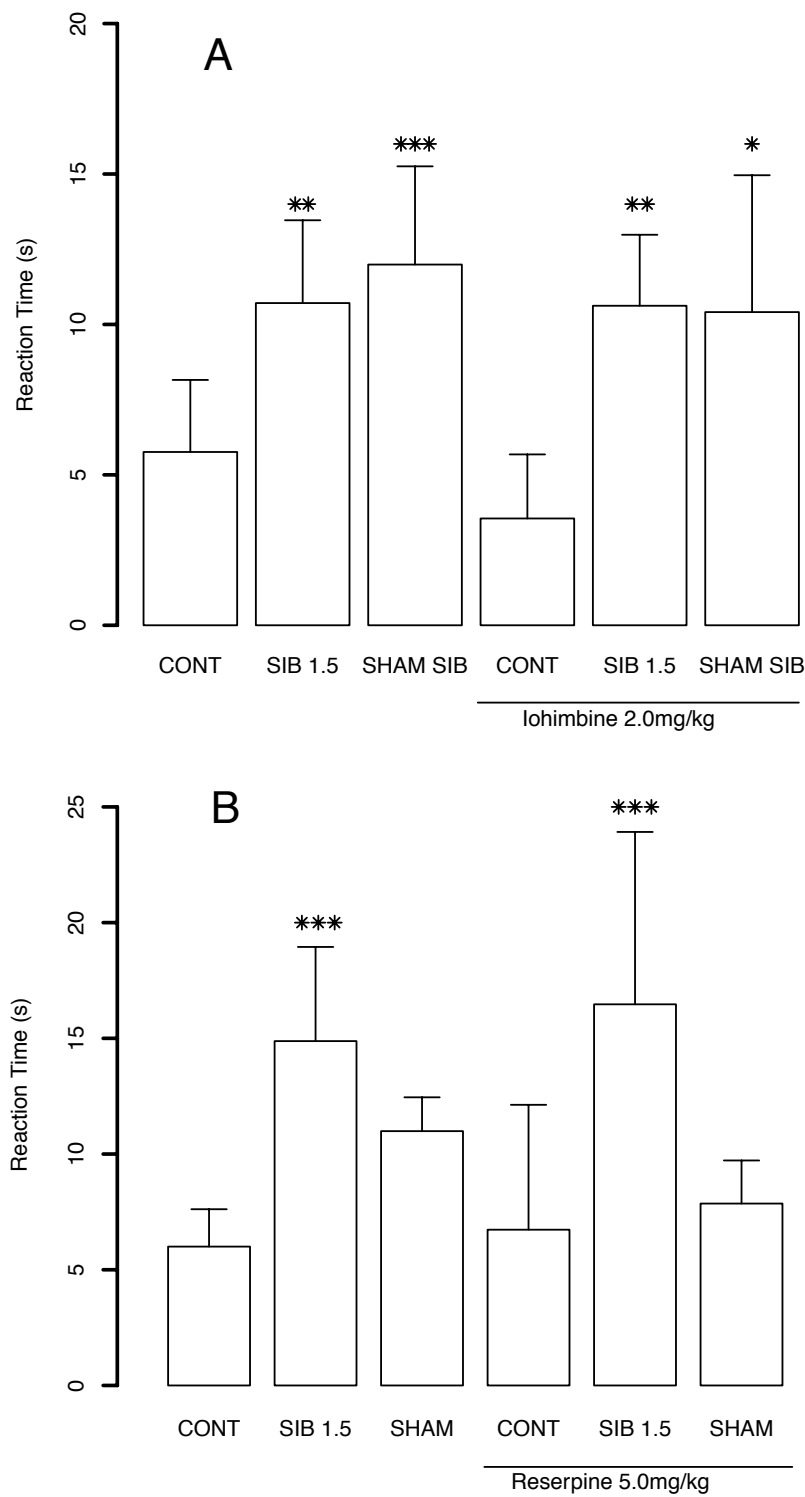


**Figure 2: Sibutramine reversal of carrageenan-induced acute thermal hyperalgesia.**

Plantar test was performed as described in methods. SHAM group (SHAM) had no pharmacological treatment. Control group had a 1.0 mg carrageenan plantar injection in

right hindpaw 2 h before the test. Treatment groups (SIB 0.1, SIB 0.5, SIB 1.5, SIB 5.0, AMI 10, and INDO 10) were treated with sibutramine 0.1 to 5.0 mg/kg (B), amitriptyline 10 mg/kg or indomethacin 10 mg/kg (A) at the same time of carrageenan injection. A group had sibutramine 1.5 mg/kg treatment but no inflammatory stimulus (SHAM SIB). Reaction time to thermal stimulus (mean  $\pm$  SD) was plotted. Statistical significant difference from control is depicted when present (One-way ANOVA with Tukey as post-hoc test).

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$



**Figure 3: Neither reserpine nor yohimbine did inhibit sibutramine effect.** Plantar test was performed as described in methods. Animals were pre-treated with reserpine (Sigma), 5.0 mg/kg, administered i.p. (0.2 ml/animal), 6 h before plantar test (B), or with yohimbine 2.0 mg/kg at the same time of p.o. drug treatment (A). Treatment groups (SIB 1.5) were treated with sibutramine 1.5 mg/kg at the same time of carrageenan injection. Reaction time to thermal stimulus (mean  $\pm$  SD) was plotted. Statistical significant difference from control is depicted when present (One-way ANOVA with Tukey as post-hoc test).

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$