

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

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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

1 **Introduction:**

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

13 We sought to test sibutramine analgesic effect in widely used animal models of
14 nociception: writhing test in female mice and plantar thermal hyperalgesia test (Hargreaves,
15 1988) in female rats, and to evaluate catecholaminergic and alpha-2-adrenergic participation in
16 the latter model with reserpine and yohimbine pre-treatment. We also wanted to rule out motor
17 impairment or sedation with a rotarod test.

18 **Material and Methods:**

19 **Animals:**

20 Inbred female Swiss mice weighing between 18-30 g and female Wistar rats weighing a
21 mean of 200 g were divided in groups of 6-10 animals each. Mice were housed in groups up to
22 17 per cage and rats were housed in groups up to six per cage in a large colony room on a 12:12-
23 h light/dark cycle (lights on 06:00 h), with food and water provided ad libitum. Each animal was

24 used only once. Test sessions were performed between 08:00 and 18:00 h and animals habituated
25 to the laboratory for a week prior to the tests. Food and water were withdrawn for 4 h before
26 drug administrations. Estrous cycle was confirmed in each animal prior to the test performing.
27 All experiments were conducted in accordance with the brazilian regulations of animal care and
28 experimentation covered by CONCEA documentations, equivalent to the *Guide to the Care and*
29 *Use of Experimental Animals* (Vol. 1, 2nd ed., 1993, and Vol. 2, 1984, available from the
30 Canadian Council on Animal Care, Constitution Square, Tower 2, Suite 315, 350 Albert Street,
31 Ottawa, ON K1R1B1, Canada, or on their Web site at www.ccac.ca), and they were approved by
32 the local Institutional Animal Care and Use Committee.

33 **Animal models:**

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

42 **Carrageenan-Induced Thermal Hyperalgesia.** Plantar test was performed as previously
43 described (Hargreaves, 1988). Briefly, each rat was placed in a Plexiglas cubicle with a glass
44 floor through which an infrared photo beam was shown onto the plantar surface of the hind paws
45 and the latency to withdrawal from the thermal stimulus was determined. The intensity of the
46 infrared photo beam from the plantar reflex device (Plantar Test, Ugo Basile) was adjusted to

47 produce a withdrawal latency in untreated rats (SHAM) of approximately 10-12 s (mean 11 s).
48 The response latency was determined using a timer linked to the photodiode motion sensors in
49 the plantar reflex device. Response latency was defined as the time from the onset of exposure to
50 the infrared photo beam to the cessation of the photo beam when the photodiode motion sensors
51 detected the withdrawal response of the paw of the rat. Groups of 10 rats were injected s.c. with
52 λ -carrageenan (100 μ l of a 1.5% solution) into the plantar surface of the right hind paw at time
53 zero followed immediately by a p.o. dose of vehicle or a dose of drug, 120 min before plantar
54 test. The drugs used were sibutramine hydrochloride monohydrate in increasing doses of 0.1,
55 0.5, 1.5, and 5.0 mg/kg; amitriptyline 10 mg/kg and indomethacin 10 mg/kg. Three groups were
56 pre-treated with reserpine (Sigma), 5.0 mg/kg, dissolved in 0.5 ml of glacial acetic acid and 9.5
57 ml of saline and administered i.p. (0.2ml/animal), 6 h before plantar test. Three groups were pre-
58 treated with yohimbine 2.0 mg/kg dissolved in saline and administered i.p. (0.2 ml/animal) at the
59 same time of oral drugs administration. Then, reserpine or yohimbine pre-treated animals were
60 treated with sibutramine hydrochloride 1.5 mg/kg, amitriptyline 10 mg/kg, or vehicle in a single
61 p.o. dose. Doses were selected from usual dosing in published literature and from our initial
62 experiments with sibutramine (data not reported). Oral doses of drugs were given through a
63 metal rodent feeding tube in a volume of less than 1.0ml, after fasting as described.

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

70 Statistical analyses:

71 We used the number of animals per group necessary to obtain $\alpha = 0.05$ (or less) with
72 $\beta = 0.8$ for an effect size between groups of at least 0.5 (calculation performed with R,
73 package pwr). The results (expressed in mean and standard deviation of mean) between all
74 groups were compared using one-way analysis of variance (degrees of freedom – DF, and F
75 values – F, were reported), with Tukey’s multiple comparison as a post hoc test if significant ($p <$
76 0.05). Anderson-Darling test for the composite hypothesis of normality was performed. Rotarod
77 results were compared with Fischer’s exact test of independence. The statistical packages used
78 were GraphPad Prism 5.0 (La Jolla, CA – USA) and R 2.X (R Foundation for Statistical
79 Computing, Vienna, Austria).

80 Results:**81 Writhing Test in Mice.**

82 The number of writhes for control group was 59.5 ± 14.1 , whereas it was 27.5 ± 3.4 in
83 ASA-treated animals and 30.5 ± 14.4 (0.1 mg/kg), 29.5 ± 12.1 (0.5 mg/kg), 25 ± 8.4 (1.5 mg/kg)
84 and 21.8 ± 6.8 (5.0 mg/kg) in sibutramine-treated mice ($p < 0.001$, DF = 5, F = 9.8, ANOVA). All
85 treated groups had mean number of writhes significantly different from control (fig.1). Although
86 there was no significant difference between treated groups, sibutramine treatment induced a clear
87 dose-response effect.

88 Carrageenan-Induced Thermal Hyperalgesia.

89 Reaction time for the control was 6.0 ± 1.6 s. Sibutramine showed an acute analgesic
90 dose-response effect in the plantar test. Reaction times were 6.6 ± 2.5 s (0.1 mg/kg), 8.8 ± 2.7 s
91 (0.5 mg/kg), 14.9 ± 4.0 s (1.5 mg/kg) and 16.7 ± 5.8 s (5 mg/kg) ($p < 0.001$, DF = 4, F = 17.7,
92 ANOVA). Mean reaction times of groups treated with sibutramine 1.5 and 5 mg/kg ($p < 0.001$)

93 were significantly different from control (fig.2B). Amitriptyline 10 mg/kg prolonged reaction
94 time to 11.1 ± 4.7 s ($p < 0.05$), as well as indomethacin 10 mg/kg, prolonging reaction time to
95 12.7 ± 5.9 s ($p < 0.01$). Sibutramine 1.5 mg/kg did not modify significantly plantar test results
96 when administered to rats without inflammatory stimulus (11.9 ± 3.3 s; $p = 0.99$ compared to
97 SHAM) (fig.2A). Pre-treatment with reserpine 5 mg/kg or yohimbine 2 mg/kg did not modify the
98 antinociception induced by sibutramine 1.5 mg/kg, p.o. (fig.3) but did inhibit the antinociceptive
99 effect of amitriptyline 10 mg/kg p.o. in the plantar test (data not shown). The results of
100 incremental sibutramine doses were used to make a sigmoid log dose-response curve with $R^2 =$
101 0.94 and $ED_{50} = 0.72$ mg/kg (95% confidence interval = $0.16 - 3.22$). Runs test showed that the
102 results did not deviate significantly from the model ($p = 1.00$).

103 **Rotarod Test.**

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

106 **Discussion:**

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

113 Animal models of nociception are usually performed with parenteral drug administration
114 for convenience. However, most often the clinical usage of analgesic drugs is orally. Sibutramine
115 hydrochloride monohydrate achieves pharmacological active concentrations when given orally to

116 rodents, with a T_{\max} of 1 ± 0.7 h and a $t_{1/2}$ of 2.5 ± 1.7 h (Brown, 2001; Li, 2010). Based on this,
117 we planned an interval of 2 h after oral administration of sibutramine to ensure
118 pharmacologically active concentrations of the drug when the plantar test was done. Yohimbine
119 timing of administration was planned to achieve receptor loading at sibutramine T_{\max} , thus
120 affecting its pharmacological action. Yohimbine dose was chosen based in another report that
121 showed $\alpha 2$ -adrenoceptors blockade could revert sibutramine anti-nociception (Gray, 1999).
122 Although reserpine pretreatment is usually done for 24-48h in daily doses, this procedure yielded
123 much motor impairment, precluding us to use this paradigm in plantar test (personal data, not
124 published). Alternatively, we used a single dose 6 h before the nociception test, resulting in a
125 significant reversal of amitriptyline antinociception (data not shown). Early works showed that
126 doses as low as 1.0 mg/kg in animals have pharmacological action beginning 10-30 min after
127 administration, blunting cardiovascular responses from 3-4 h and lasting 24 h after dose (Alper,
128 1963). *In vivo* brain microdialysis experiments have shown that 5 mg/kg i.p. reserpine induced
129 an almost complete depletion of striatum dopamine at 180 min after dose that persisted for more
130 than 6 h (Kannari, 2000).

131 The exclusive use of male animal models when investigating diseases more frequent in
132 females, like painful syndromes, has been criticized. Research regarding gender differences in
133 pain sensation has increased substantially in recent years, after the publication of influential
134 reviews in the 90s. The relationship between sex and pain is not simple; nevertheless, most
135 population-based studies have found higher prevalence in women than in men. (Fillingim, 2009)
136 The prevalence of most common forms of pain is higher among women than men, and women
137 report greater pain after invasive procedures than men, though these findings are less consistent.
138 Additionally, compared with men, women display enhanced sensitivity to most forms of

139 experimentally induced pain (with the exception of ischemic pain). (Fillingim, 2009) It has been
140 also widely demonstrated that gender can modify the response to painful stimuli in experimental
141 animals. Once SNRI are more often used for painful syndromes such as neuropathic pain and
142 migraine, diseases more often affect women, we believe that using females animals as
143 experimental models will lead to more appropriate results.

144 The writhing model in mice is the most sensitive animal model to detect analgesic action
145 of drugs. The effect of sibutramine in this model confirms its analgesic effect. However, the
146 writhing test is not specific, and its usefulness to investigate mechanisms of actions and dose-
147 response relation is limited. Thus, we chose the plantar test to do both tasks. Unexpectedly, the
148 analgesic effect of sibutramine was not reverted by pre-treatment with reserpine or yohimbine,
149 which may indicate that it does not depend on general catecholaminergic signaling and
150 specifically on α 2-adrenoceptors. Since sibutramine induced marked analgesia in single-dose 5
151 mg/kg reserpine pre-treated animals, one can rule out the confounding factor of regulation of
152 cutaneous vasoconstrictor tone and skin temperature by the sympathetic nervous system. A
153 previous publication had showed analgesic effects of sibutramine in a non-thermal pain evoked
154 animal model, reverted by α 2-adrenoceptors blockade (Gray, 1999).

155 There has been considerable discussion at the experimental level about the nature and
156 underlying mechanisms of antidepressant analgesia. It has been demonstrated that
157 antidepressants exert their antinociceptive effect via noradrenergic and opioidergic
158 neurotransmission both in spinal and supraspinal sites (Gray, 1999). In the last decade, little was
159 added to this paradigm and it has been repeatedly demonstrated that antidepressant-induced
160 analgesia can be reverted by opioidergic antagonism (Sawynok, 2001) and depends partially on
161 α 2A-adrenoceptors of spinal location but also on a variety of individual drug effects that goes

162 from serotonin (5HT) descending spinal signaling through peripheral ion channel blocking
163 (Sawynok, 2001). Amitryptiline analgesia is lost in $\alpha 2$ -adrenoceptors knock-out animals,
164 indicating that this receptor class is the main responsible for mediating tricyclic antidepressants
165 antinociception (Ozdogan, 2004). Venlafaxine, a SNRI with a similar mechanism of action than
166 that of sibutramine, has been shown to induce $\alpha 2$ -adrenoceptors-dependent analgesia (Sawynok,
167 2001); whereas duloxetine (other SNRI) had no analgesic effect in mice models where $\alpha 2$ -
168 adrenoceptor agonists show marked antinociception (Jones, 2005). However, both drugs as well
169 as sibutramine were shown to increase extracellular NE in various regions of rat central nervous
170 system (Invernizzi, 2004). Taken together, these data suggest that sibutramine could have its
171 analgesic effect explained by spinal $\alpha 2$ -adrenoceptor activation, at least partially. This is not
172 upheld by our results, indicating that other neural networks may be more important for
173 sibutramine-related acute analgesia in the plantar test. One possibility, although speculative, is
174 that sibutramine mechanism of action could be dependent on gender. It is more likely, however,
175 that different animal models of nociception have different contributions of signaling pathways.
176 Whereas sibutramine effect in mechanical hyperalgesia could have a prominent participation of
177 descending spinal adrenergic signaling (Gray, 1999), this could not be the case for thermal
178 hyperalgesia (our results), indicating the possible participation of supraspinal, perhaps
179 opioidergic, signaling.

180 We conclude that sibutramine, a drug used for weight lossing that has a similar
181 mechanism of action to that of SNRI antidepressants, has analgesic effect in writhing test in
182 female mice and in the plantar thermal hyperalgesia in female rats. Furthermore, this effect is
183 independent from catecholaminergic signaling and specifically $\alpha 2$ -adrenoceptors. More research

184 is needed to clarify its mechanisms of action and its possible dependence on gender. It may be
185 additionally tested in other pre-clinical models.

186 **Conflicts of interest:**

187 There are not potential sources of conflicts of interest.

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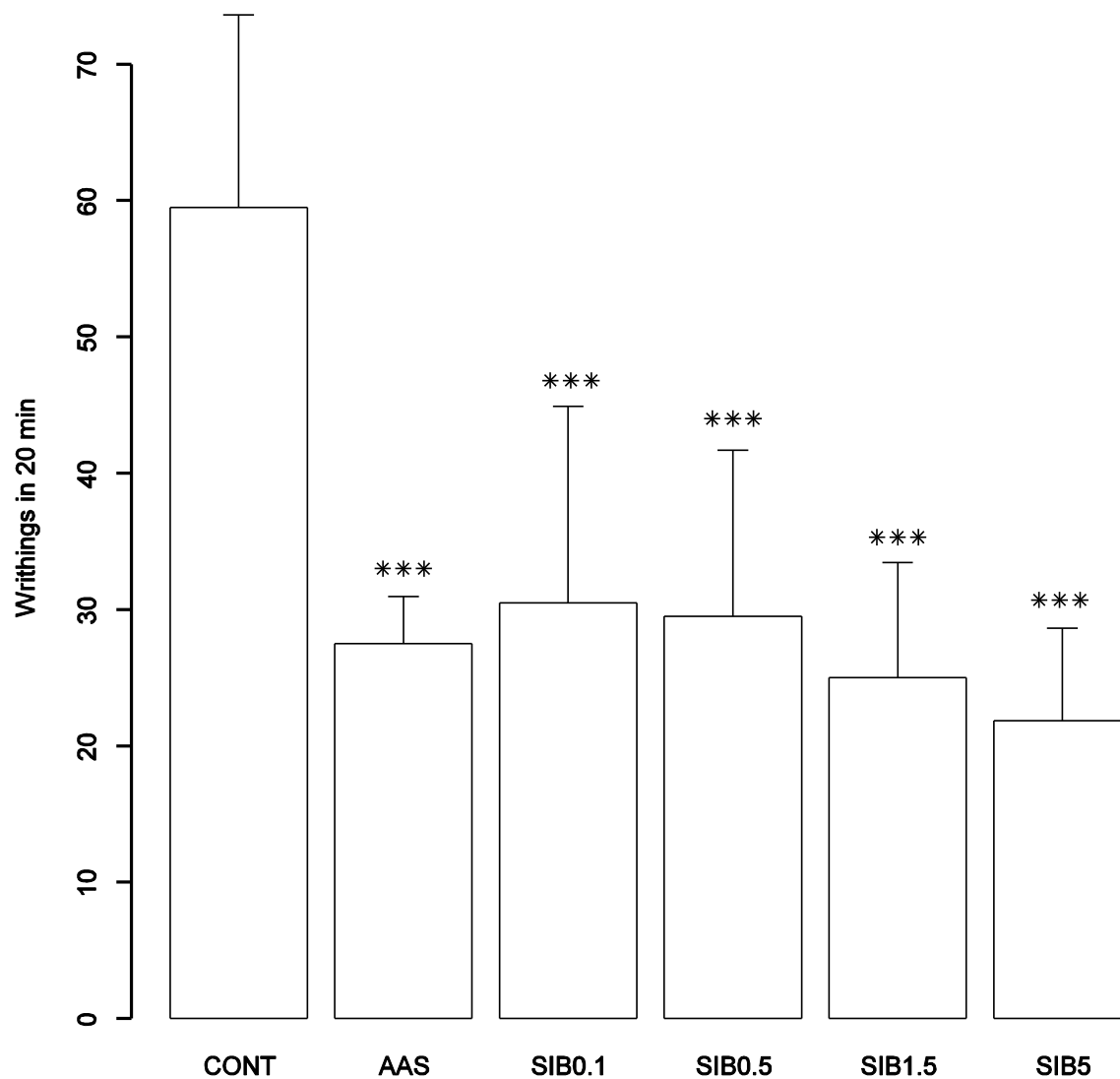


Figure 1: Sibutramine inhibition of acetic acid-induced abdominal writhes in mice. Separate groups of 6 mice were administered vehicle or a dose of drug p.o. followed 55 min later by an i.p. injection of 0.55% acetic acid. The total number of writhes made by each mouse was counted between 5 and 20 min 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).

*** $p < 0.001$

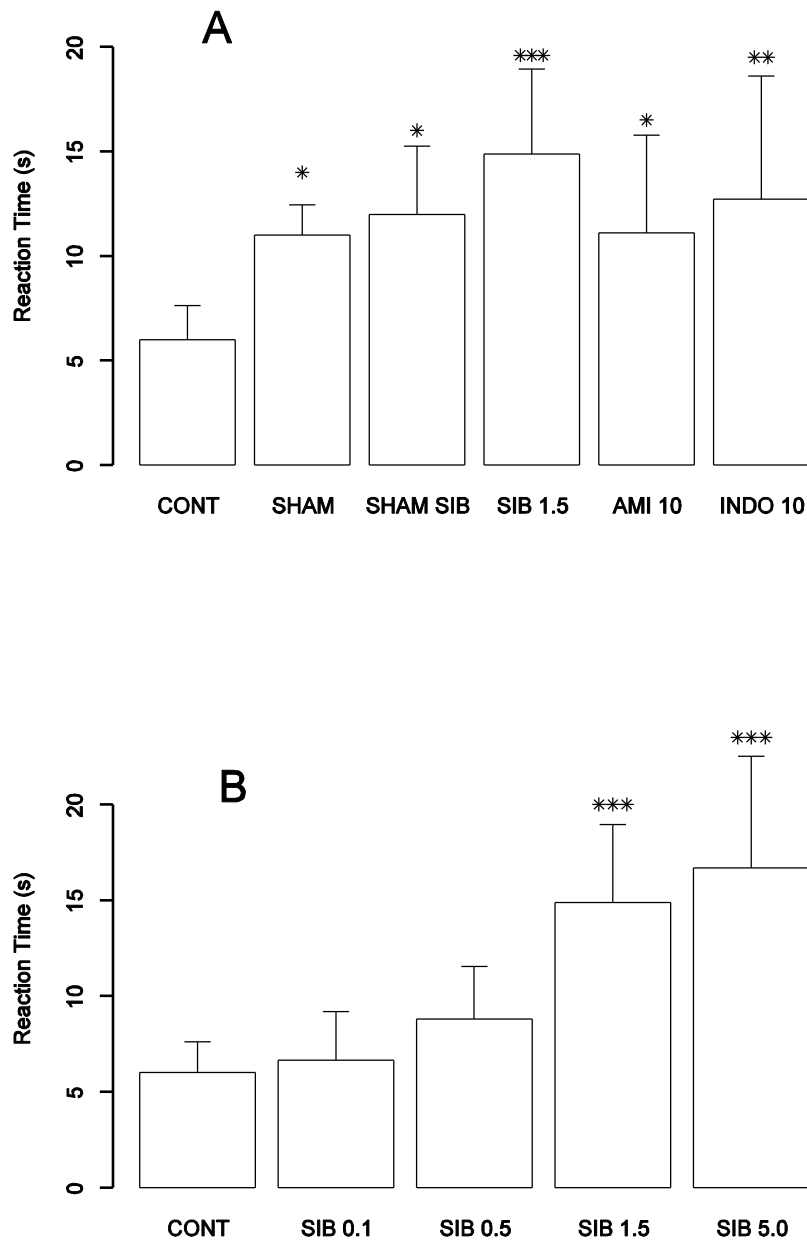


Figure 2: Sibutramine reversal of carrageenan-induced acute thermal hyperalgesia. Plantar test was performed as described in methods. SHAM group (SHAM) had no pharmacological treatment. Control group had a 1.0 mg carrageenan plantar injection in right hindpaw 2 h before the test. Treatment groups (SIB 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).

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

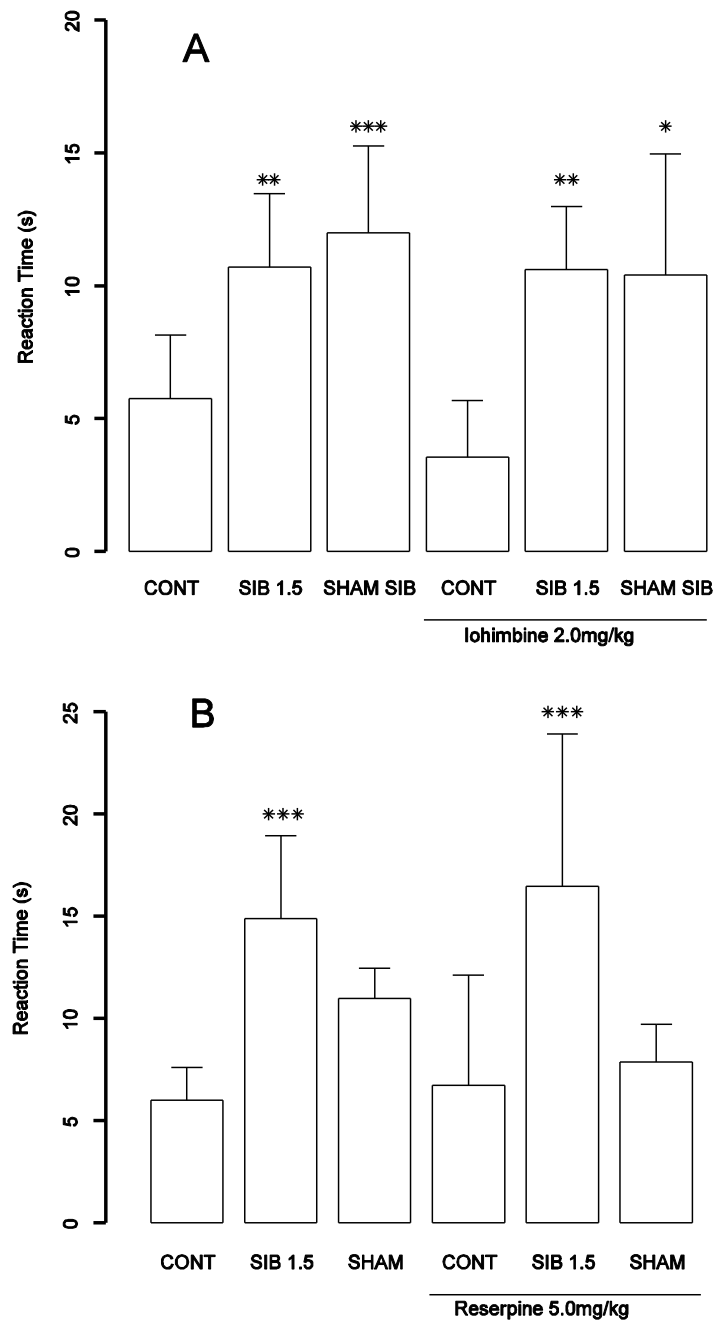


Figure 3: Neither reserpine nor yohimbine did inhibit sibutramine effect. Plantar test was

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).

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