Effects of sibutramine on anxiety-related behaviours in rats

Salim Demétrio Jorge a, Roger Luís Henschel Pobbe a, Vanessa de Paula Soares a, Ana Maria de Oliveira b, Hélio Zangrossi, Jr a,∗

a Department of Pharmacology, School of Medicine, University of São Paulo. Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, São Paulo, Brazil
b Laboratory of Pharmacology, Faculty of Pharmaceutical Science, University of São Paulo. Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, São Paulo, Brazil

Accepted 4 May 2004

Abstract

Sibutramine is an anorexiant drug that inhibits the reuptake of noradrenaline and serotonin, a pharmacological property shared with drugs clinically effective in treating anxiety pathologies. However, the effects of this compound on experimental and clinical anxiety have not been assessed yet. In this study, we evaluated the effects of sibutramine on anxiety-related behaviours which have been related to specific anxiety disorders. Acute injection of sibutramine (5, 10 or 20 mg kg−1; intraperitoneally) in male Wistar rats impaired inhibitory avoidance in the elevated T-maze (ETM) and in the light/dark transition test, indicative of an anxiolytic effect. The drug also inhibited one-way escape in the ETM. Sibutramine, however, was ineffective in changing rat performance in the elevated plus-maze. Therefore, sibutramine decreased the expression of defensive behaviours that have been associated with generalized anxiety disorder (inhibitory avoidance) and with panic disorder (one-way escape). Yet, in contrast to what has been reported with drugs such as the tricyclic anti-depressants that also inhibit monoamine reuptake, the anxiolytic effects of sibutramine were revealed after a single administration.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Sibutramine; Generalized anxiety disorder; Panic disorder

1. Introduction

Sibutramine was developed by the Knoll Pharmaceuti-
cals research group as a putative anti-depressant drug [1]. The pharmacological profile of this compound motivated such expectation since as the tricyclic anti-depressants imipramine and amitriptyline, sibutramine was shown to block the neuronal reuptake of serotonin and noradrenaline [2–5]. Moreover, early pharmacological findings showed that sibutramine lacked antagonistic activity against cholin-
ergic, histaminergic and alpha1-adrenergic receptors, the proven causes of most of the undesirable side effects of the tricyclic anti-depressants [2,3,6]. However, although preliminary animal experimentation data supported the anti-depressant effect of sibutramine [2], less promising results were subsequently obtained in clinical trials [6,7]. Curiously, during clinical investigation, it was observed that the drug induced noticeable and dose-dependent weight loss in the depressed patients [6]. On the basis of these results, efforts were directed at disclosing the potential of sibutramine as an anti-obesity drug. Indeed, the anorexi-
ant effect of this drug and its low side effects were later attested in many studies (for a review, see [7]), leading to the approval of sibutramine as an anti-obesity agent by the American Food and Drug Administration (FDA) in 1998.

Clinical evidence demonstrates that drugs that block monoamine reuptake such as the tricyclic anti-depressants are also effective in treating anxiety pathologies such as generalized anxiety disorder (GAD) and panic disorder (PD) [8,9]. Considering the pharmacological profile of sibutramine, it is surprising that no controlled study has been performed thus far to investigate the effect of this compound on anxiety in healthy individuals or in patients with anxiety disorders. It is also of note that a search of the published literature has failed to reveal studies examining the effects of sibutramine in animal models of anxiety. The aim of this study was to investigate the effects of sibutramine in different animal models of anxiety. The tests selected were: the elevated plus-maze, the light/dark transition test and the elevated T-maze (ETM). Whereas the defensive responses generated by the former two tests have been frequently associated with GAD, the ETM has been developed
to separate GAD- and PD-related behaviours in the same animal (for further details of these tests see [10,11]).

2. Methods

2.1. Animals

Male Wistar rats weighing 250–300 g were housed in groups of 6 under a 12:12 h light/dark cycle (lights on at 07:00 h) at 22 ± 1 °C. Food and water were freely available throughout the experiment. The experiments were performed in compliance with the recommendations of Brazilian Society of Neuroscience and Behaviour (SBNNeC), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Drug

Sibutramine hydrochloride (Knoll Pharmaceutical, UK) was dissolved in saline and administered intraperitoneally (volume of 1 ml kg\(^{-1}\)). The drug was prepared immediately before the test.

2.3. Apparatus

2.3.1. Elevated T-maze

The elevated T-maze was made of wood and had three arms of equal dimensions (50 cm × 12 cm). One arm was enclosed by 40-cm high walls and was arranged perpendicularly to two opposite open arms. The entire apparatus was elevated 50 cm above the floor. To prevent falls, the open arms were surrounded by a Plexiglas rim 1 cm high.

2.3.2. Open field

The open field test, used to measure locomotion after testing in the elevated T-maze, was a wooden square arena (60 cm × 60 cm), with 30-cm high walls.

2.3.3. Light/dark transition model

The apparatus used was a box made of wood with overall dimensions of 48 cm × 24 cm × 27 cm (length, width, height) and a grid floor composed by bars spaced 5 cm apart. The box was further divided by a barrier possessing a doorway (10 cm × 10 cm), which rats could cross into two chambers of equal measures (24 cm × 24 cm × 27 cm): one painted black, not illuminated, and one painted white and illuminated with a 60-lux light source.

2.3.4. Elevated plus-maze

The elevated plus-maze was made of wood and had four arms of equal dimensions (50 cm × 10 cm). Two arms were enclosed by 40-cm high walls and were arranged perpendicularly to two opposite open arms. The entire apparatus was elevated 50 cm above the floor. To prevent falls, the open arms were surrounded by a Plexiglas rim 1 cm high.

The elevated plus-maze and T-maze were in a different room from the open field and the light/dark transition test. In both rooms, illumination was provided by an incandescent lamp of 60 W in the ceiling. Environmental temperature was kept at 22 ± 1 °C with an air conditioner that also produced background noise.

2.4. Procedure

Independent groups of rats were tested in each of the anxiety models described below.

2.4.1. Elevated T-maze

Starting on the third day after their arrival at the laboratory, animals were gently handled by the experimenter for 5 min on 2 consecutive days. Twenty-four hours before the test, each animal was pre-exposed to one of the open arms of the model for 30 min. A wood barrier mounted on the border of the maze central area, and the open arm’s proximal end isolated this arm from the rest of the maze. It has been shown that this pre-exposure, by shortening latencies to withdrawal from the open arm during the test, renders the escape task more sensitive to the effects of anti-panic drugs [12,13].

On the test day, animals were randomly allocated to different treatment groups and injected ip with one of the doses of sibutramine (5, 10 or 20 mg kg\(^{-1}\); \(n = 9\), for each group) or saline (\(n = 9\)). Thirty minutes later, each animal was placed at the distal end of the enclosed arm of the ETM facing the intersection of the arms. The baseline latency was defined as the time(s) required for the rat to exit this arm (defined as all the four paws outside the arm). The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30 s intervals. Following avoidance training (30 s), rats were placed at the end of the open arm where they had been previously exposed and the latency to leave this arm with the four paws was recorded for three consecutive times (escape 1, 2 and 3) with 30 s intertrial intervals. A cut off time of 300 s was established for the avoidance and escape latencies. Immediately after being tested in the ETM, each animal was placed for 5 min in the open field for the evaluation of locomotor activity. The total distance travelled was analyzed by a video-tracking system (Ethovision; Noldus, Holland).

2.4.2. Light/dark transition model

Animals were handled as described before. On the test day, rats were injected i.p. with sibutramine (5, 10 or 20 mg kg\(^{-1}\); \(n = 9\), for each group) or saline (\(n = 9\)). Thirty minutes later, each animal was placed in the middle of the lit compartment, facing the doorway separating the two compartments. After the first transition from the lit to the dark compartment, the behaviour of the animals was recorded for an additional 5-min period using a video camera connected to a VHS recorder; for measurements of two parameters: total number of transitions between the two compartments and time spent in the lit compartment.
2.4.3. Elevated plus-maze
Animals were handled as described before. On the test day, rats were injected ip with sibutramine (5, 10 or 20 mg kg\(^{-1}\); \(n = 11-12\)) or saline (\(n = 14\)). Thirty minutes later, each animal was placed in the centre of the maze, facing one of the enclosed arms. The number of entries into and time spent in the open and enclosed arms were scored for 5 min.
All experimental sessions were conducted between 13:00 and 17:00 h.

2.5. Statistical analysis
Avoidance and escape data in the ETM were analyzed by split-plot analysis of variance (ANOVA) with treatment as the independent factor and trials (baseline, avoidance 1 and 2 or escape 1, 2 and 3) as the dependent factor. In case of a significant effect of treatment or of treatment versus trials interaction, data was analyzed by one-way ANOVA followed by the Duncan post-hoc test. Behavioural data from the other tests were analyzed by one-way ANOVA followed by the Duncan post-hoc test. In all cases, a value of \(P \leq 0.05\) was considered significant.

3. Results

3.1. Elevated T-maze
As illustrated in the upper panel of Fig. 1, treatment with sibutramine impaired inhibitory avoidance performance in the elevated T-maze. Split-plot ANOVA showed a significant effect of treatment \([F(3, 32) = 6.51; P < 0.01]\), trials \([F(2, 64) = 23.67; P < 0.001]\) and treatment by trial interaction \([F(6, 64) = 5.42; P < 0.001]\). The Duncan post-hoc test showed that in avoidance 1 and avoidance 2, the three doses of sibutramine decreased the latency to leave the enclosed arm, when compared to the control group (\(P < 0.05\)).

The lower panel of Fig. 1 shows the effect of sibutramine administration on one-way escape. Split-plot ANOVA revealed a significant effect of treatment \([F(3, 32) = 5.10; P = 0.01]\), but not of trials \([F(2, 64) = 0.02; \text{NS}\] or a treatment by trial interaction \([F(6, 64) = 1.57; \text{NS}\]. The Duncan post-hoc test showed that animals treated with 10 and 20 mg kg\(^{-1}\) of sibutramine had longer latencies (\(P < 0.05\)) to leave the open arm than the control group in the three trials. Animals treated with 5 mg kg\(^{-1}\) of sibutramine had longer escape 1 and 2 latencies.

Treatment with sibutramine did not alter \([F(3, 35) = 0.96; \text{NS}\] locomotor activity in the open field (Table 1).

3.2. Light/dark transition
As illustrated in the upper panel of Fig. 2, sibutramine increased \([F(3, 32) = 5.58; P < 0.01]\) the number of transitions between the two compartments of the light/dark transition model. This effect was observed with all three doses used.

The percentage of time spent in the lit compartment (see lower panel of Fig. 2) was also increased by sibutramine \([F(3, 32) = 7.92; P < 0.001]\). Again, the three doses used contributed to this effect.

3.3. Elevated plus-maze
Fig. 3 shows that sibutramine did not alter the percentage of entries into the open arms \([F(3, 45) = 1.57; \text{NS}\] and the percentage of time spent in these arms \([F(3, 45) = 0.98; \text{NS}\].

<table>
<thead>
<tr>
<th>Sibutramine (mg kg(^{-1}))</th>
<th>Distance travelled (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.7 ± 3.0</td>
</tr>
<tr>
<td>5</td>
<td>27.2 ± 1.9</td>
</tr>
<tr>
<td>10</td>
<td>26.5 ± 2.7</td>
</tr>
<tr>
<td>20</td>
<td>26.4 ± 2.6</td>
</tr>
</tbody>
</table>
Fig. 2. Effects (mean ± S.E.M.) of acute administration of sibutramine on the behaviour of rats tested in the light/dark transition model. The upper panel shows the number of transitions between the two compartments and the lower panel shows the percentage of time spent by the animals in the lit compartment. N = 9 for each group.

4. Discussion

The results of the present study showed that sibutramine decreased the expression of anxiety-related behaviours in rats submitted to the ETM and to the light/dark transition model of anxiety. The drug, however, failed to change anxiety indexes in the elevated plus-maze.

In the ETM, sibutramine impaired inhibitory avoidance and one-way escape from the open arm, defensive behaviours which have been related to GAD and PD, respectively [12,14–16]. Since sibutramine had no significant effect in the open field, it is reasonable to affirm that the effects obtained in the ETM are not due to motor alteration. This conclusion is also supported by the fact that although the motor demand of inhibitory avoidance and one-way escape are nearly the same, the drug shortened avoidance while lengthening escape latency.

Besides inhibiting inhibitory avoidance in the ETM, sibutramine also impaired avoidance performance in the light/dark transition test, as indicated by the increase in the time spent in the lit box. Previous studies have shown that in both tasks, acute injection of the standard GAD-alleviating benzodiazepine drugs diazepam and chlor-diazepoxide resulted in similar anxiolytic effects [16–18].

On the other hand, the effects of sibutramine contrast with those observed after acute administration of the monoamine reuptake blocker imipramine. This drug, although facilitating inhibitory avoidance in the ETM, an anxiogenic effect [12], was ineffective in changing rat behaviour in the light/dark transition test [17,19]. The anxiogenic effect of imipramine on the ETM inhibitory avoidance turned to anxiolytic after its chronic administration [12], similarly to what has been reported in clinical studies [9]. Therefore, sibutramine may share with drugs such as the benzodiazepines a rapid-onset GAD-relieving property. This conclusion, however, seems to be at odds with the observation that sibutramine did not change anxiety in the elevated plus-maze, a test that reliably detects the anxiolytic effect of clinically relevant benzodiazepine drugs [20].

In defence of the anxiolytic profile of sibutramine in GAD-like behaviours, it has been shown that the elevated plus-maze, while highly predictive for benzodiazepine effects, does not consistently detect the effects of anxiolytics acting on 5-HT neurotransmission [21]. The weak predictability for the latter class of drugs has been attributed to the conflicting anxiety states presumably generated by this test [22,23]. This idea has been based on the observation that when freely exploring the plus-maze rats display multiple defensive responses. For instance, the aversion for open and elevated spaces motivates avoidance of the
open arms, resulting in the preferential exploration of the enclosed arms. However, after an entry is made into the open arm, an escape response is presumably expressed, guiding the animal back to the safe enclosed arm. On the basis of a wealth of evidence showing that 5-HT regulates these types of defensive responses in opposite directions [24–27], the elevated plus-maze as a “mixed” test would show either anxiolytic, anxiogenic or no effects of 5-HT-acting anxiolytics, according to the relative magnitude of the different defensive behaviours expressed on the occasion [23]. In support, it has been reported that the effect of the 5-HT1A receptor agonist 8-OH-DPAT shifts from anxiogenic to anxiolytic when the brightness of the light illuminating the apparatus is increased [23]. Therefore, the ETM, by separating the distinctive anxiety states underlying inhibitory avoidance and escape performance, may have favoured the detection of sibutramine effects.

The most intriguing finding of the present study was that sibutramine consistently impaired one-way escape in the ETM, indicative of an anti-panic activity. Accordingly, inhibition of this response has been observed after injection of clinically relevant anti-panic compounds: the anti-depressants imipramine [12], fluoxetine and clomipramine [13]. The GAD-effective drugs diazepam or buspirone did not interfere with this response [13,15]. However, it is noteworthy that, in contrast to the effect of sibutramine, inhibition of one-way escape was only observed after chronic administration of the three anti-depressants tested to date. Curiously, acute injection of fenfluramine, another appetite suppressant which acts by promoting 5-HT release and by blocking its neuronal reuptake [28], also inhibits one-way escape in the ETM. However, fenfluramine, differently from sibutramine, tended to facilitate inhibitory avoidance, a pro-panic effect [15]. In agreement with the profile of fenfluramine in the ETM, its administration to panic disorder patients submitted to a carbon dioxide (CO2) panic challenge has been shown to increase anxiety and arousal while causing a reduction in the number of panic attacks induced by CO2 [29]. Also, anxiety induced by simulated public speaking in healthy volunteers—a test that has been related to PD [25]—is markedly reduced by d-fenfluramine [30]. Finally, in an open clinical trial, chronic administration of d-fenfluramine reduced the frequency of panic attacks in female patients with a long history of panic disorder [31]. Unfortunately, the cardiopulmonary toxicity of fenfluramine [32], which has led to its withdrawal from the market, has discouraged further investigation on the potential of this drug for treating PD. However, it remains to be explored whether the similar correspondence between fenfluramine profile in the ETM and in clinical assays also stands for sibutramine.

In conclusion, our data indicate the sibutramine may have rapid onset anxiolytic activity, which may be of relevance in generalized anxiety and panic disorders.

Acknowledgements

This work was supported by FAPESP, CAPES and CNPq, Brazil. The authors thank Knoll Pharmaceutical for kindly donating sibutramine.

References


