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Involvement of β_3-adrenergic receptors in the control of food intake in rats

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Abstract

This study examined the food intake changes evoked by intracerebroventricular (*icv*) injection of a selective agonist (BRL37344, 2 and 20 nmol) or antagonist (SR59230A, 10 and 50 nmol) of β_3-adrenergic receptors in 24-h fasted rats (adult male Wistar rats, 200-350 g, N = 6/treatment). The animals were also pretreated with saline *icv* (SAL) or SR59230A (50 nmol) followed by BRL37344 (20 nmol) or SAL in order to determine the selectivity of the effects evoked by BRL37344 on food intake or the selectivity of the effects evoked by SR59230A on risk assessment (RA) behavior. The highest dose of BRL37344 (N = 7) decreased food intake 1 h after the treatment (6.4 ± 0.5 g in SAL-treated *vs* 4.2 ± 0.8 g in drug-treated rats). While both doses of SR59230A failed to affect food intake (5.1 ± 1.1 g for 10 nmol and 6.0 ± 1.8 g for 50 nmol), this treatment reduced the RA frequency (number/30 min) (4 ± 2 for SAL-treated *vs* 1 ± 1 for 10 nmol and 0.5 ± 1 for 50 nmol SR59230A-treated rats), an ethological parameter related to anxiety. While pretreatment with SR59230A (7.0 ± 0.5 g) abolished the hypophagia induced by BRL37344 (3.6 ± 0.9 g), BRL37344 suppressed the reduction in RA frequency caused by SR59230A. These results show that the hypophagia caused by BRL37344 is selectively mediated by β_3-adrenergic receptors within the central nervous system. Moreover, they suggest the involvement of these receptors in the control of anxiety.

Key words: Food intake; CNS; Intracerebroventricular injection; β_3-adrenergic receptor

Introduction

The initial evidence that the noradrenergic circuit is involved in the control of food intake was indicated by the decrease in feeding behavior evoked by the administration of 6-hydroxydopamine into the hypothalamus (1). Studies using a push-pull cannula have shown noradrenaline (NA) release in the paraventricular nucleus during food consumption (2,3). In addition, NA microinjection into hypothalamic nuclei such as the ventromedial hypothalamus and the paraventricular nucleus has been reported to increase food intake in satiated rats (4-6), whereas injections into more lateral sites including the perifornical region decreased feeding (7).

The effects on feeding evoked by NA could be mediated by α_1- and α_2-adrenoceptors present in the hypothalamus. The medial hypothalamus, especially the ventromedial area, was reliably sensitive to the α-agonists, which increased food intake (8). In other brain areas, such as the paraventricular nucleus of the hypothalamus, while the injection of clonidine, an α_2-adrenergic agonist, stimulated food intake (9,10), the activation of α_1-adrenoceptors suppressed it (11,12). NA could also modulate feeding by acting through either β_1- or β_2-adrenergic receptors. Intracerebroventricular (*icv*) injection of salbutamol, a β_2-adrenergic receptor agonist, decreases food intake in rats (13). Stimulation of β_2-adrenergic receptors in the perifornical lateral hypothalamus suppressed milk intake in pups (14). In the same perifornical site, the food intake of hungry rats was reliably suppressed by the α-antagonist phentolamine, and the β-agonist isoproterenol (8). The lateral hypothalamus was found to be reliably sensitive, in the same way as the perifornical area, to the β-receptor drugs; the agonist, isoproterenol, suppressed food intake and the antagonist, propranolol, produced a very small but reliable enhancement of food intake (8).

The hypothesis that β_3-agonists may be inhibitors of food intake is supported by the finding that injections of BRL37344 into the third ventricle of obese rats produce a significant and dose-related depression in food intake (13). A small reduction in food intake was also observed in lean rats at 6 h (13). The idea that suppression of food

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intake is mediated by β-adrenergic receptors is supported by the fact that this hypophagic response was blocked by propranolol, a β-adrenergic antagonist (13,15).

Since BRL37344 has a high affinity for β_3-adrenergic receptors (K_i values of 287, 1750, and 1120 nM for β_3-, β_1-, and β_2-receptors, respectively) but also interacts with β_2-adrenoceptors at a much lower affinity (16) and that propranolol is a non-selective antagonist of β-adrenergic receptors, the suppressive effects of food intake induced by BRL37344 may occur due to interactions with β_2- but not with β_3-adrenergic receptors. In the present study, we used a potent and selective β_3-adrenoceptor antagonist (SR59230A, IC_{50} values of 40, 408, and 648 nM for β_3-, β_1-, and β_2-receptors, respectively), in order to identify the β-receptor subtype found in the central nervous system that mediates the effects of BRL37344 on food intake.

Material and Methods

Animals

Adult male Wistar rats weighing approximately 200-350 g were supplied by the animal breeding division of Universidade Federal de Santa Catarina. The animals were housed in a polypropylene box (49 x 34 x 16 cm) kept at a constant temperature of 21 ± 2°C, on a 12:12-light-dark cycle (lights on at 6:00 h) with free access to food (CR-1, Nuvilab, Brazil) and water. The animals were housed in groups of 5 per cage until the beginning of the experiments. After surgery, rats were housed individually. The experimental procedures used in this study are in accordance with the ethical principles of animal experimentation of the Brazilian College of Animal Experimentation and were approved by the Ethics Committee for the Use of Animals (CEUA) of Universidade Federal de Santa Catarina. All experiments were carried out between 8:00 and 13:00 h.

Stereotaxic surgery

The animals were anesthetized with a mixture of xylazine hydrochloride (13 mg/kg) and ketamine hydrochloride (87 mg/kg) injected intraperitoneally and placed in a stereotaxic apparatus (Insight Instruments, Brazil) for the implantation of a unilateral stainless steel guide cannula (30 G, 15 mm length), aimed at the right lateral cerebral ventricle according to the coordinates described by Paxinos and Watson (17). The cannula was anchored to the skull with dental cement and the whole implant stabilized with jeweler screws and more dental cement. A removable stylet was introduced to keep the cannula free from blockages until the day of the experiment.

Drug injections

The drugs used were BRL37344 (a β_3-adrenergic receptor agonist at doses of 2 and 20 nmol), purchased from Sigma, USA, and SR59230A (a β_3-adrenergic receptor antagonist at doses of 10 and 50 nmol) obtained from Tocris Bioscience, USA, both dissolved in saline, which was also used as vehicle (VEH) in the control experiments. Injections were made through an inner cannula (33G), which extended 2 mm beyond the tip of the guide cannula connected by polyethylene tubing to a Hamilton microsyringe (1 µL) fitted to an injection pump. The injected volumes (1 µL) were administered over a period of 60 s and a further 60 s were allowed for the solution to diffuse from the cannula.

Experimental procedures

All experiments were carried out 7 days after surgery on rats submitted to an overnight fast and with free access to water.

Experiment 1. The aim of this experiment was to investigate the separate effects of the agonist or the antagonist of β_3-adrenergic receptors on ingestive and non-ingestive behaviors. To this end, during the experimental session, naive fasting rats were treated *icv* with VEH (N = 6), BRL37344 at doses of 2 nmol (N = 8) or 20 nmol (N = 7), and SR59230A at doses of 10 nmol (N = 6) or 50 nmol (N = 6).

Experiment 2. In order to determine the selectivity of the effects evoked by the adrenergic agonist on food intake or the selectivity of the effects evoked by the antagonist on risk assessment behavior, naive fasting rats (N = 6/treatment) were treated *icv* with VEH 10 min before the *icv* injection of VEH or of the β_3-adrenergic agonist (BRL37344 at a dose of 20 nmol). Another group was injected *icv* with the antagonist of the β_3-adrenergic receptor (SR59230A at a dose of 50 nmol) administered 10 min before VEH or BRL37344. After the injections, each animal was placed in the feeding recording chamber to evaluate ingestive behavior.

Food intake behavior

Immediately after the injection, the rats were placed in a recording chamber constructed with transparent glass (49 x 34 x 32 cm), containing food and tap water (in a bottle placed outside the test cage with a spout projecting through the wall of the cage). The session was recorded by a webcam perpendicularly located 60 cm above the cage floor, for subsequent detailed behavioral analysis by the Etholog 2.2 software (18). The back and lateral walls, as well as the floor cage, were coated with black adhesive plastic paper. In order to facilitate behavioral recording, the front wall of the test cage had a mirror with the same dimensions arranged at a 45° angle in relation to the vertical plane. This mirror arrangement also prevented the animal from seeing its reflection on the mirror. At the end of the recording period, any food that occasionally spilled on the cage floor was recovered and weighed with the food that remained in the feeder. The difference between food or water weight at the beginning and at the end of the recording period was taken to be the amount of food or water consumed. During the 60-min experimental session, the following variables were evaluated: latency to start feeding, feeding duration (amount of time

the animal mouth was either touching or chewing a food pellet), feeding frequency, grooming duration (defined as paw strokes over the face or licking of the paws or body), locomotion duration (defined as front-to-back cage crossings), rearing duration (defined as front paws raised from the cage floor and either placed on the side of the cage or placed in front of the body), stretched-attend postures (SAP) duration (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion) (19), and resting time (tucking head against chest without movement for >5 s). After this period, the animal was returned to the housecage and the consumption of food and water continued to be monitored at 1, 2, 3, and 4 h after injection.

Histological analysis

At the end of the experiments, the rats were anesthetized deeply and Evans blue dye (1% in water, 1 µL) was injected through the guide cannula to confirm the injection in the lateral ventricles. The animals were then perfused transcardially with 0.9% saline, followed by 10% formalin. The brains were removed and subsequently cut with a vibratome on the transverse plane (100 µm). Sections were stained with cresyl violet and the cannula sites were examined and documented with a camera lucida attached to a light microscope. Data from rats with the cannula not in the *icv* space were not included in the analysis.

Statistical analysis

The food intake data were analyzed by one-way analysis of variance (ANOVA) for repeated measures. The other ingestive and non-ingestive data were evaluated by one-way ANOVA. All of these tests were followed, when appropriate, by the *post hoc* Duncan test, and $P < 0.05$ was accepted as being statistically significant in these procedures.

Results

Experiment 1 - *icv* injections of a β_3-agonist or antagonist

Data analysis of food intake by one-way ANOVA for repeated measures revealed a significant interaction between treatment and time [$F(6,48) = 3.73$; $P = 0.0039$]. The *icv* treatment with 20 nmol BRL37344 decreased food intake 1 h after injection in fasting rats. While the VEH-treated animals ingested 6.4 ± 0.5 g, the drug-injected rats ingested 4.2 ± 0.8 g (Figure 1). This hypophagic response was not accompanied by changes in the latency to start feeding, feeding duration, or feeding frequency (Table 1). Food intake remained unchanged for 3 h after the *icv* injection of 2 nmol BRL37344; however, an increase in food intake was observed 4 h after drug administration. While the VEH-treated animals ingested 0.1 ± 0.1 g, the drug-injected ones ingested 3.3 ± 0.6 g (Figure 1). One-way ANOVA indicated that feeding behavior remained unchanged after *icv* injection of both doses of β_3-adrenergic receptor antagonist (SR59230A) in fasting rats [$F(2,15) = 1.14$; $P = 0.34$; Figure 2 and Table 1]. Food consumption evoked by 10 nmol SR59230A was 5.1 ± 1.1 g and that evoked by 50 nmol was 6.0 ± 1.8 g. The *icv* treatment with both doses of SR59230A decreased the risk assessment frequency (Table 2). Drinking and non-ingestive behaviors such as frequency and durations of rearing, grooming, immobility, and locomotor behaviors were not affected by *icv* treatment with either dose of BRL37344 and SR59230A (data not shown).

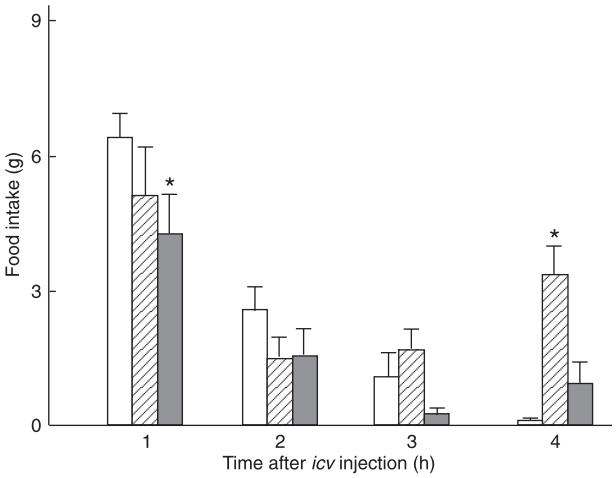

Figure 1. Effects of *icv* injection of BRL37344 [a β_3-adrenergic receptor agonist at doses of 2 (striped bars) and 20 nmol (gray bars)] on food intake of 24-h fasted rats. Vehicle (saline) are open bars. Data are reported as means ± SEM. *$P < 0.05$ compared to the group that received microinjection of vehicle (one-way ANOVA followed by the Duncan *post hoc* test for multiple comparisons).

Table 1. Effects of *icv* injection of BRL37344 (a β_3-adrenergic agonist) or SR59230A (a β_3-adrenergic antagonist) on feeding behavior of 24-h fasted rats.

Treatment	Food intake		
	Latency (s)	Duration (s)	Frequency
Vehicle	363 ± 179	836 ± 163	6 ± 2
BRL37344 (2 nmol)	257 ± 60	445 ± 221	4 ± 1
BRL37344 (20 nmol)	292 ± 111	593 ± 209	5 ± 2
SR59230A (10 nmol)	182 ± 96	648 ± 219	3 ± 1
SR59230A (50 nmol)	99 ± 28	816 ± 277	7 ± 2

Data are reported as means ± SD. The vehicle was saline. Data were analyzed by one-way ANOVA followed by the Duncan *post hoc* test for multiple comparisons.

Experiment 2 - *icv* pretreatment with SR59230A followed by *icv* injection of BRL37344

Data analysis of food intake by one-way ANOVA for repeated measures revealed a significant interaction between treatment and time [F (9,60) = 3.26; P = 0.002]. Previous *icv* injection of SR59230A abolished the hypophagic response evoked by *icv* treatment with 20 nmol BRL37344 1 h after the agonist injection in fasting rats. While the rats treated with the adrenergic agonist ingested 3.6 ± 0.9 g, the animals pretreated with the antagonist followed by the adrenergic agonist ingested 7.2 ± 0.8 g, a food intake response similar to that shown by VEH-treated rats, 8.2 ± 0.9 g (Figure 3). Furthermore, the risk assessment decrease induced by SR59230A was blocked by the presence of the β_3-adrenergic receptor agonist (Table 2).

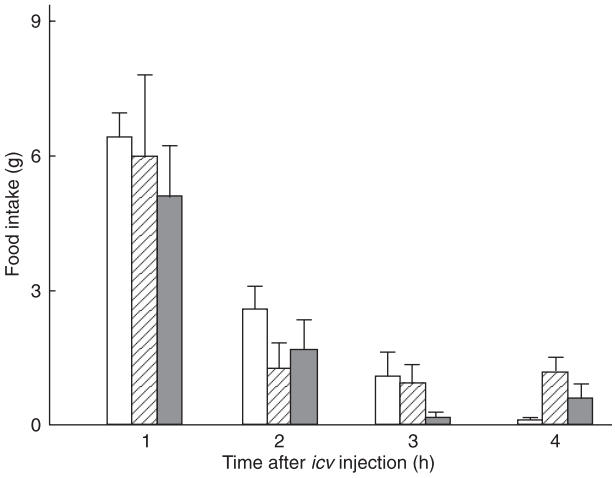

Figure 2. Effects of *icv* injection of SR59230A [β_3-adrenergic receptor antagonist at doses of 10 (gray bars) and 50 nmol (striped bars)] on food intake of 24-h fasted rats. Vehicle (saline) are open bars. Data are reported as means ± SEM.

Table 2. Effects of *icv* injection of BRL37344 (a β_3-adrenergic agonist) or SR59230A (a β3-adrenergic antagonist) on the frequency of risk assessment behavior in 24-h fasted rats.

Treatment	Risk assessment (stretched-attend posture)
	Frequency (number/30 min)
Vehicle	4 ± 2
BRL37344 (2 nmol)	3 ± 1
BRL37344 (20 nmol)	3 ± 1
SR59230A (10 nmol)	1 ± 1*
SR59230A (50 nmol)	0.5 ± 1*
Vehicle + vehicle	3 ± 1
SR59230A (50 nmol) + vehicle	0.5 ± 1*
Vehicle + BRL37344 (20 nmol)	2.5 ± 1
SR59230A + BRL37344	2 ± 1

Data are reported as means ± SD. The vehicle was saline. The table shows that the presence of a β_3-adrenergic receptor agonist prevented the risk assessment decrease induced by the β_3-receptor antagonist. The stretched-attend posture is defined as an exploratory posture in which the body is stretched forward and then retracted to the original position without any forward locomotion (19). *P < 0.05 compared to the group that received microinjection of vehicle (one-way ANOVA followed by the Duncan *post hoc* test for multiple comparisons).

Discussion

The results of this study revealed that food intake during refeeding after 24 h of fasting decreased after *icv* injection of BRL37344 at a dose of 20 nmol, corroborating previous data in the literature (13,15) for lean rats. The β_3-adrenergic receptor agonist, BRL37344, caused a longer and deeper decrease in feeding when injected *icv* or peripherally in obese rats (13,15).

The present data also showed that the hypophagic response induced by BRL37344 is mediated by specific β_3-receptors located in the central nervous system, since *icv* injection of the permeable selective antagonist of adrenergic receptors SR59230A abolished the reduction of food intake caused by BRL37344. Previous studies (13) have indicated that *icv* treatment with propranolol, a nonselective antagonist of β-adrenergic receptors, suppressed the hypophagia induced by *icv* injection of BRL37344 only in obese rats and not in lean rats, which differs from the results obtained in the present study. The reasons for this

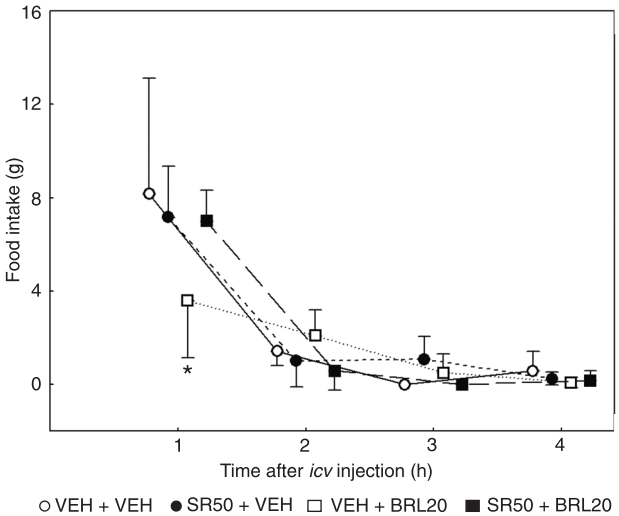

Figure 3. Effects of *icv* injection of 20 nmol BRL37344 (BRL20, a β_3-adrenergic receptor agonist) in rats fasted for 24 h and pretreated with the antagonist of the β_3-adrenergic receptor [SR59230A (SR50) at a dose of 50 nmol] or vehicle (VEH, saline). Data are reported as means ± SEM. *P < 0.05 compared to the group that received microinjection of vehicle (one-way ANOVA followed by the Duncan *post hoc* test for multiple comparisons).

discrepancy may be due to the fact that in our study we used a selective antagonist of the β_3-receptor, propranolol, which is a weak antagonist of β_3-adrenergic receptors, but is an effective antagonist of β_1- and β_2-adrenergic receptors. Furthermore, our investigation was conducted in male rats and not in females. This evidence suggests that the changes in ovarian hormone secretion during the estrous cycle could modify the influence of β_3-adrenergic receptors on food intake.

Several studies have shown that fluctuations in ovarian hormones during the estrous cycle affect food intake. A reduction in food intake occurs during estrus relative to diestrus in rats with a normal estrous cycle (20-22). In addition, these hormones also affect the influence of serotonin or neuropeptides on food intake (23). Similarly, ovarian hormones could affect food intake in response to BRL37344. In females, the hypophagic response induced by BRL37344 occurred later, 6 h after the *icv* injection of BRL37344 (13). In the present study, the reduction of food intake was observed 1 h after *icv* treatment with BRL37344. Thus, our results indicate that activation of β_3-receptors in the central nervous system also results in inhibition of food intake in non-obese rats.

Studies carried out by Grujic et al. (24) demonstrated that transgenic mice without β_3-receptors show no reduction in food intake when treated with β_3-adrenergic agonists. The hypophagic effect could be restored by the expression of β_3-receptors in white and brown adipose tissues but not in brown adipose tissue alone. This result led to the suggestion that white adipose tissue releases an inhibitory factor in response to stimulation of β_3-receptors in brown adipose tissue that reduces food intake. Studies by Mohamed-Ali et al. (25) have attributed to interleukin-6 a partial contribution to the anorectic effect induced by activation of the β_3-adrenergic receptor. Thus, the activation of β_3, through the activation of the sympathetic nervous system, could activate the β_3-receptor in white adipose tissue and cause the secretion of interleukin-6, which in turn would trigger hypophagia. However, the hypophagic response induced by *icv* injection of BRL37344 could also contribute to the activation of central circuits that trigger satiety. This suggestion is reinforced by the finding that *icv* injection of a β_3-adrenergic receptor agonist increases the expression of the Fos protein in neurons of the paraventricular and ventromedial nuclei of the hypothalamus and those found in the lateral hypothalamic area and dorsal regions of the hypothalamus involved in the control of food intake (26). Although there was no change in the latency to start eating or the duration and frequency of this response after *icv* injection of the drug, satiety signals from the periphery (27) could be operating on the central circuits, inducing slower food consumption, causing the animal to eat a smaller amount of food.

Food consumption by animals that received an *icv* injection of 2 nmol BRL37344 was not significantly affected for 3 h after *icv* injection; however, treatment with the β_3-adrenergic agonist at this dose caused an increase in food consumption 4 h after administration. This increase was also blocked by pretreatment with a selective antagonist of the β_3-receptor. This result indicates that this increase is selectively mediated by the β_3-adrenergic receptor subtype found in the central nervous system. The reasons for the effect of β_3-receptor activation on food intake are unclear but the effect could be mediated by a signal of slower action such as a chemical endocrine hormone. Evidence in the literature indicates that treatment with a β_3-agonist suppresses both serum leptin and the expression of leptin mRNA in white adipose tissue (28,29). Although the hypophagia induced by treatment with a β_3-adrenergic receptor agonist is not associated with a reduction in leptin levels (28), the decrease in blood leptin concentration could contribute to the increase in food consumption induced by the lower dose of BRL37344. Since rearing, grooming, immobility, and locomotion behaviors remained unchanged after *icv* treatment with the β_3-adrenergic receptor agonist, we suggest that the hypophagic effects triggered by the administration of BRL37344 cannot be attributed to motor abnormalities. Water intake was not affected by the activation of β_3-receptors, indicating that β_3-receptors in the central nervous system are involved only in the neural control of food intake.

The *icv* injection of the β_3-antagonist SR59230A alone did not involve changes in food consumption. This result differs from that obtained by Tsujii and Bray (15), which indicated an increase in food intake after injection of propranolol alone in the third ventricle of non-obese fasting rats. Since propranolol is a nonselective antagonist of β-adrenergic receptors, this effect on food intake can be attributed to a tonic inhibitory influence exerted by the activation of β_1- or β_2-adrenergic receptors. The present data exclude the involvement of β_3-receptors in this tonic inhibitory control of food intake

Except for the frequency of risk assessment behavior, non-ingestive behaviors and water intake were not affected by the *icv* injection of the β_3-receptor antagonist SR59230A. The frequency of risk assessment decreased after treatment with SR59230A, and this effect was reversed by the presence of the BRL37344 agonist. A body of evidence indicates that the behavior of risk assessment can be an indicator of the degree of anxiety in rats. Risk assessment is closely related to fear and anxiety in potentially dangerous situations (19,30) and is a common response of rats to noxious stimuli evoked in a non-social context (31). The conduct of risk assessment also shows a high correlation with the release of corticosterone (19), being sensitive to the effects of anxiogenic and anxiolytic drugs (32,33). Rodents continue to exhibit the behavior of risk assessment even after they leave to avoid an unprotected area, suggesting that this defensive attitude may be more sensitive to drugs that modulate anxiety than to the spatial-temporal measures

related to avoidance (19,32,33). Additionally, the evaluation of the frequency of risk assessment during the recording of feeding behavior confirmed the anxiolytic effect induced by injection of the AMPA receptor antagonist in the nucleus accumbens shell of rats exposed to the elevated plus-maze (34). On the basis of these considerations, our data suggest that β_3-receptors were active, inducing anxiety in rats starved for 24 h, and that the β_3-adrenergic receptor blockade caused this anxiolytic effect. However, recent studies (35,36) have shown that systemic administration or direct administration of a β_3-agonist into the basolateral amygdala reduces the display of anxiety-related behavior in different models of anxiety assessment, contrary to the data obtained in the present study. These contradictory data could be attributed to the route of drug administration (directly into the brain parenchyma or systemically in the investigation of Silberman et al. (35) and Stemmelin et al. (36), respectively), nutritional status of the animal (free access to food in the experiments conducted by Silberman et al. (35) and Stemmelin et al. (36)), or different drugs (SR58611A in the study by Stemmelin et al. (36)). To confirm the anxiolytic effect induced by the β_3-adrenergic receptor antagonist, it would be important to test 24-h fasted animals treated *icv* with SR59230A in the elevated plus-maze, a classic procedure for the study of anxiety.

The β_3-adrenergic receptor subtype is found mainly in peripheral tissues, but small amounts are found in the human and rat brain (37,38). Initially, the existence of β_3-adrenergic receptors in the brain was questioned, since classical binding studies failed to detect its presence in nervous tissue. However, some experiments with RT-PCR revealed the existence of mRNA for the β_3-adrenoceptor in different brain regions of rats and humans, including the hippocampus, hypothalamus, amygdala and cerebral cortex, areas known to be involved in modulating anxiety-related behavior and the control of food intake (39,40).

The present study confirms that the activation of β-adrenergic receptors found in the central nervous system causes hypophagia in non-obese rats. This response is selectively mediated by the β_3-adrenergic receptor subtype since pretreatment with an antagonist of this adrenergic receptor subtype (SR59230A) suppressed the reduction of food intake caused by BRL37344, the β_3-adrenergic receptor agonist.

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