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Involvement of pre- and post-synaptic serotonergic receptors of dorsal raphe nucleus neural network in the control of the sweet-substance-induced analgesia in adult *Rattus norvegicus* (Rodentia, Muridae)

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Abstract

In order to investigate the effects of monoaminergic mechanisms of the dorsal raphe nucleus on the elaboration and control of sweet-substance-induced antinociception, male albino Wistar rats weighing 180–200 g received sucrose solution (250 g/L) for 14 days as their only source of liquid. After the chronic consumption of sucrose solution, each animal was pretreated with unilateral microinjection of methiothepin mesylate (5.0 μg/0.2 μL), or methysergide maleate (5.0 μg/0.2 μL) in the dorsal raphe nucleus. Each rat consumed an average of 15.6 g sucrose/day. Their tail withdrawal latencies in the tail-flick test were measured immediately before and after this treatment. An analgesia index was calculated from the withdrawal latencies before and after the pharmacological treatment. The blockade of serotonergic receptor in the dorsal raphe nucleus with methysergide after the chronic intake of sucrose decreased the sweet-induced antinociception. However, microinjections of methiothepin in the dorsal raphe nucleus did not cause a similar effect on the tail-flick latencies after the chronic intake of sucrose solution, increasing the sweet-substance-induced analgesia. These results indicate the involvement of serotonin as a neurotransmitter in the sucrose-produced antinociception. Considering that the blockade of pre-synaptic serotonergic receptors of the neural networks of the dorsal raphe nucleus with methiothepin did not decrease the sweet-substance-induced antinociception, and the central blockade of post-synaptic serotonergic receptors decreased the sucrose-induced analgesia, the modulation of the release of serotonin in the neural substrate of the dorsal raphe nucleus seems to be crucial for the organization of this interesting antinociceptive process.

Keywords: Sweet-substance-induced analgesia; Tail-flick test; Serotonin; 5-HT_2 post-synaptic receptor; 5-HT_1 pre-synaptic receptors; Dorsal raphe nucleus

There is evidence that the consumption of sweet palatable solutions produces analgesia in animals [3,9,18,20,29] and in men [1,8]. It has been reported that intra-oral infusion of a small amount of sucrose solution into the mouth of 10-days-old rat pups rapidly increased the latency of paw withdrawal in a hot-plate test [7]. In addition, rats drinking sucrose for relatively long periods of time show an increase in the nociceptive thresholds in the tail-flick test [20,27]. Although we must consider the evidence that the consumption of sucrose solutions or sweet-food intake can change the nociceptive sensitivity by the activation of the opioid mechanism [13,21], we cannot dismiss the possibility of the involvement of other neurotransmitters, such as serotonin and other chemical mediators, in this process. In fact, the involvement of the acetylcholine-mediated mechanism in the sucrose-induced antinociception has recently been reported [20], and peripheral administration of serotonergic antagonists also decreased the sweet-substance-induced antinociception [27]. Considering that the dorsal raphe nu-

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cleus is an important source of serotonin-containing neurons involved in antinociceptive processes [4,5,30,35], the aim of the present work was to investigate the involvement of the neural networks of the dorsal raphe nucleus in the sweet-substance-induced antinociception.

Male Wistar rats from the Animal Facility of the Campus of the University of São Paulo at Ribeirão Preto were used. All experiments were performed in accordance with the recommendations of the SBNeC (Brazilian Society for Neuroscience and Behavior), which is based on the US National Institute of Health-Guide for Care and Use of Laboratory Animals. The animals, weighing 180–200 g, were housed four to a cage with free access to food and sweetened water (sucrose at 250 g/L) throughout the experiment. All rats had their nociception thresholds compared, using the tail-flick test. Each animal was placed in a restraining apparatus (Insight, São Paulo, Brazil) with acrylic walls, and its tail was placed on a heating sensor (tail-flick Analgesia Instrument; Insight, São Paulo, Brazil). The progressive heat elevation was automatically interrupted at the moment when the animal removed its tail from the apparatus. The current raised the coil's temperature (Ni/Cr alloy; 26.04 cm in length × 0.02 cm in diameter) at a rate of 9 °C/s, starting at room temperature (approximately 20 °C). A small adjustment of the current intensity could be done, when necessary, at the beginning of the experiment (baseline records), aiming the obtainment of three consecutive tail-flick latencies (TFL) between 2.5 and 3.5 s. If the animal did not remove its tail from the heater within 6 s, the apparatus was turned off in order to prevent damage to the skin. Considering that there were no statistical significant differences between the baseline latencies among the experimental groups (Mann–Whitney *U*-test: $U = 29$; $p > 0.05$), all tail-flick latencies were normalized by an index of analgesia (IA) using the formula:

$$\mathrm{IA} = \frac{(\mathrm{TFL\ test}) - (\overline{\mathrm{TFL}}\ \mathrm{control})}{6 - (\overline{\mathrm{TFL}}\ \mathrm{control})}$$

Three baselines of control tail-flick latencies were taken at 5-min intervals. Tail-flick latencies were also measured following chronic (up to 14 days) consumption of sucrose or tap water, and 5 min after the pharmacological pretreatments. The indexes of analgesia were analyzed by the Wilcoxon test. All tests were run using the statistical SPSS software package.

Initially, the animals were submitted to a 3-day habituation period in the animal facility of the Department of Pharmacology of the FMRP-USP. After this procedure, they were submitted to a new habituation period in the restrainer cylinder (Stoelting, IL, USA) for another 3 days (5 min/day in the apparatus). After that procedure, they were anesthetized with thiopental (45 mg/kg, i.p.) and fixed in a stereotaxic frame (David Kopf, USA). A stainless steel guide-cannula (o.d. 0.6 mm, i.d. 0.4 mm) was implanted in the midbrain, aimed at the dorsal raphe nucleus. The upper incisor bar was set at 3.3 mm below the interaural line, in a way that the skull was horizontal between bregma and lambda. The guide-cannula was introduced vertically using the following coordinates, with the bregma serving as the reference for each plane: anteroposterior, −8.0 mm; mediolateral, 0.2 mm; and dorsoventral, 5.0 mm. The guide-cannula was fixed to the skull by means of acrylic resin and two stainless steel screws. At the end of the surgery each guide-cannula was sealed with a stainless steel wire to protect it from obstruction. After a 1-week post-operative period, they were submitted to an experimental situation, which consisted of establishing the baseline in the tail-flick test, followed by the oral administration of sucrose solution, at a concentration of 250 g/L, ad libitum. Finally, the nociceptive thresholds were recorded after the chronic (14 days) consumption of sucrose solution. Animals were given a concentrated (250 g/L) sucrose solution as the only source of liquid instead of a preference paradigm in order to easily compare the previous data from this and others laboratories [3,20,29]. Fourteen days after chronic consumption of sweetened solution, and after the recording of tail-flick latencies, the animals were gently wrapped in a cloth, hand-held and received 5 μg/0.2 μL of methysergide or methiothepin microinjected into the dorsal raphe nucleus (DRN). As the cross-section of the tip of the needle reaches 1 mm below the lower end of the guide-cannula, the central administration of drugs was made in the neural network of the DRN. After 10 min, the effects of drug administrations were then evaluated on the sweet-substance-induced antinociception.

The present results revealed that treatment with sucrose for 14 days causes analgesia (Wilcoxon ranked test: $Z = 2.52$ and 2.80; $p < 0.02$ and $p < 0.01$, respectively, considering methysergide and methiothepin treated group) (Table 1), an effect corroborated by previous studies [20,27,29]. All microinjections of serotonergic antagonists were made in the dorsal raphe nucleus of each Wistar rat, as shown in Fig. 1. The blockade of serotonergic receptors with central administration of methysergide decreased the analgesic effect of sucrose ingestion (Wilcoxon: $Z = 2.52$; $n = 8$; $p < 0.02$), as compared to control (Fig. 2A). However, the pretreatment of the dorsal raphe nucleus with methiothepin did not decrease the tail-

Table 1
Effect of the chronic (14 days) ingestion of sucrose solution (250 g/L), as the only source of liquid, on the nociceptive thresholds of *R. norvegicus* ($n = 8$–10)

Sweet solution	Baseline record (s) (min, 25th, median, 75th, max)	kcal/rat/14 days of intake	Nociceptive threshold (s) (min, 25th, median, 75th, max)
Sucrose (250 g/L) Group 1	2.9, 2.9, 3.0, 3.0, 3.0	172.5	4.0, 4.05, 4.25, 4.6, 5.2*
Sucrose (250 g/L) Group 2	2.9, 2.96, 3.01, 3.24, 3.36	172.5	3.9, 4.4, 4.95, 5.35, 6.0**

The effect of sweet substance intake was evaluated on the tail-flick latencies recorded in grouped caged animals. Data are represented as medians, 25th and 75th percentiles, minimum and maximum. * $p < 0.02$, ** $p < 0.01$ as compared to the control, according to the Wilcoxon ranked test.