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ARTICLE

Raphe obscurus neurons participate in thermoregulation in rats

Neurônios do núcleo obscuro da rafe participam da termorregulação em ratos

Melissa Araújo Ulhoa[1], Nyam Florencio da Silva[1], José Guilherme Pinheiro Pires[2,3], Henrique de Azevedo Futuro Neto[1,2,4]

ABSTRACT

In mammalian, several evidences suggest that central serotonin participates in thermoregulation. Nucleus raphe obscurus (NRO), a serotonergic nucleus, has been recognized to be the source of generation of various hemodynamic patterns in different behavioral conditions, but its involvement in thermoregulation is unclear. In the present study, extracellular action potentials of NRO neurons were recorded in anesthetized rats, which were submitted to cold and warm stimuli in the tail. The firing rate of the neurons was compared before and after each stimulation. It was found that 59% of the neurons submitted to a cold stimulus trial had a significant increase in their firing frequency, while 48% of the neurons submitted to warm stimulation trial were inhibited. The opposite responses in neuronal activity of NRO units to cooling or heating suggest that these cells are involved in producing the homoeothermic vascular adaptations secondary to changes in cutaneous temperature in the rat tail.

Key words: nucleus raphe obscurus, serotonin, serotonergic neurons.

RESUMO

A termorregulação em mamíferos envolve a participação da serotonina. O núcleo obscuro da rafe (NRO), que é serotoninérgico, participa do controle autonômico, mas seu envolvimento na termorregulação é incerto. Neste estudo, registramos potenciais de ação extracelulares de neurônios do NRO em ratos anestesiados nos quais a cauda foi submetida a estímulos de calor ou frio. A frequência de disparo dos neurônios foi comparada antes e depois dos estímulos. O grupo controle não apresentou modificação da frequência de disparo, enquanto que 59% dos neurônios registrados em animais submetidos a estímulo de frio tiveram sua frequência aumentada. Por outro lado, 48% dos animais submetidos a estímulo de calor tiveram sua frequência de disparo diminuída. As respostas opostas da frequência de disparo em neurônios de animais submetidos à estimulação com frio e calor sugere que estes neurônios estejam envolvidos na geração de respostas hemodinâmicas, que são coerentes com a termorregulação nesta espécie.

Palavras-Chave: núcleo obscuro da rafe, serotonina, neurônios serotoninérgicos.

The thermoregulatory mechanism constitutes a complex and integrated system. The "main controller" is the hypothalamus, which comprises many different sub-areas; it receives inputs either from temperature sensitive neurons within the hypothalamus itself to monitor core temperature or from receptors in the skin which monitor the changes in external temperature. Nevertheless, the final drive or command to the vascular smooth muscle is not controlled by the hypothalamus but by a complex neuronal circuitry that comprises brainstem and spinal cord nuclei[1].

In rats, non-evaporative heat loss occurs mainly through the tail[2], the blood vessels vasodilate when the hypothalamic preoptic area is warmed[3]. The efferent signals for this response originate in heat-sensitive neurons, and the descending pathway passes through the medial forebrain bundle[4]. Blood flow to the rat tail is determined by the level of activity in its sympathetic postganglionic vasoconstrictor fibers[5]. These are supplied by preganglionic sympathetic neurons situated mainly in the intermediolateral cell column of the first and second lumbar segments[6,7].

[1]Departamento de Morfologia, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo (UFES), Vitória ES, Brazil;

[2]Curso de Medicina da Faculdade Brasileira (UNIVIX), Vitória ES, Brazil;

[3]Faculdade de Medicina, Centro Universitário do Espírito Santo (UNESC), Colatina ES, Brazil;

[4]Escola Superior de Ciências da Saúde da Santa Casa de Misericórdia de Vitória (EMESCAM), Vitória ES, Brazil.

Correspondence: José Guilherme Pinheiro Pires; Departamento de Morfologia, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo; Avenida Marechal Campos 1.468; 29042-751 Vitória ES - Brasil; E-mail: jgppires@hotmail.com

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These spinal cord sympathetic neurons are strongly modulated by neurons located at the brainstem and suprasegmental areas, particularly the raphe neurons[8]. The raphe nuclei are a group of structures distributed in the midline of the brainstem from the midbrain to the caudal pole of the medulla[9]. These nuclei have the highest density of serotonergic neurons in the central nervous system[10]. Classically the caudal raphe nuclei (magnus, pallidus and obscurus) are involved in several physiological and pathological processes. These nuclei have been implicated in homeostatic circuitry and regulate life-sustaining respiratory and thermoregulatory networks[11]. A recent study found that caudal raphe nuclei are related to processing thermogenesis, cardiovascular and gastric functions, by neuronal connections from the hypothalamus[12].

Regarding thermoregulation, a multiple-input system operates within the spinal cord, raphe nuclei and locus subcoeruleus, all involved in generating afferent thermal signals and modulating efferent vasomotor thermoregulatory responses[1,13]. Projections from medullary raphe cells to the thoracolumbar intermediolateral cell column are implicated in the sympathetic control of physiological functions such as brown adipose metabolism and cutaneous vasoconstriction, therefore these cells are implicated in temperature control[14].

The main serotonergic nuclei involved in thermoregulation are raphe pallidus (rostral pole) and raphe magnus. Raphe pallidus and magnus nuclei receive projections from the dorsomedial hypothalamus and warm-sensitive preoptic neurons, and contains spinally projecting premotor neurons that provide the excitatory drive to spinal circuits controlling the activity of thermogenic effectors and heat loss controlling cutaneous vasoconstriction[1]. Dense projections of these nuclei have been described to the spinal cord neurons that innervate the vessels of the tail of the rat, which is a major organ of heat loss in this species. Sympathetic premotor neurons controlling the tail circulation are located in the rostral medullary raphe[15-21].

On the other hand, nucleus raphe obscurus (NRO) has been implicated in generating sympathetic patterns of activity in different behavioral situations as paradoxical sleep[22] and in response to nociceptive stimulation[23]. Therefore this nucleus has the ability to mediate hemodynamic changes in different behavioral situations, so it is reasonable to speculate its participation in the vasomotor adaptations induced by thermoregulatory mechanisms. Hence, the present work was designed to evaluate the participation of NRO neurons in thermoregulatory functions in the rat.

METHODS

The experiments were performed on anesthetized, spontaneously breathing, male Wistar rats of 250–300 g body weight (n=24 overall; 4 animals in the time-matched control group and 10 animals per experimental group), obtained from the breeding stock of the *Universidade Federal do Espírito Santo* (UFES). All procedures were conducted in accordance with the Biomedical Research Guidelines for Care and Use of Laboratory Animals, as stated by the Federation of the Brazilian Societies of Experimental Biology (FeSBE). The experimental protocol was approved by the Animal Use Committee at *Escola Superior de Ciências da Santa Casa de Misericórdia* (nº 021/2007 — CEUA/EMESCAM, Vitória, ES, Brazil). Anesthesia was induced with halothane and maintained with urethane (1.2 g/kg, i.v.) and supplementary doses of urethane administered as required. A tracheotomy was performed in all animals and respiratory frequency was continuously monitored. The femoral artery was cannulated for the measurement of blood pressure by means of a pressure transducer (Viggo-Spectramed, P23XL) and the heart rate was electronically derived from the blood pressure signal using a rate meter (Biotach, Gould 13-64616-66). These parameters were continuously monitored. The left femoral vein was cannulated for drug administration. Rectal temperature was maintained between 37–37.5°C with a thermostatically controlled heating blanket (Harvard).

The animal's head was positioned in a stereotaxic head holder (Stoelting). The dorsal surface of the medulla was exposed by a drilled hole for electrode insertions. We employed stainless steel electrodes, etched electrolytically and insulated with resin except for the tip (stainless steel needles, Darning nº 8; 4.5 cm length, 0.1 cm diameter and 50 μm tip diameter). Extracellular action potentials were recorded with an AC amplifier (NL 104, NeuroLog, Digitimer) connected to a high-impedance headstage (NL 100). The recording electrodes were positioned in the following stereotaxic coordinates, midline -11.8 mm AP and 8.0 to 8.5 mm from brainstem surface[24]. The amplified signals were filtered (NL 126: low pass filter 5 to 20 Hz, high pass filter 900 to 1,000 Hz, with a 60 Hz band notch), connected to an audio amplifier, were processed using a spike trigger (NL 200) and a rate meter (NL 256) for subsequent analysis. The processed data was digitalized (Biopac MP 100, acquisition frequency 2,000 Hz) and stored on a hard disk.

Neurons at the rostral raphe nuclei, which include the nucleus obscurus, are mainly but not exclusively serotonergic. Serotonergic raphe neurons were described as displaying firing rates inferior than 10 Hz, while higher frequencies are associated to firing of non-serotonergic neurons. Since we were interested in the effects of 5-hydroxytrypyamine (5-HT) neurons, we limited our analysis to the effects of temperature on NRO cells firing at 10 Hz or less[25].

After recording stabilization the animals were submitted to cold and warm stimuli. All stimulation was done in the rat tail. Cold and warm stimuli were obtained with the application of rubber condoms filled with cold (13 to 15°C) or warm water (40 to 45°C). The condoms filled with water were kept in

cold and hot baths up to the moment of the stimulation, so as to assure maintenance of a constant temperature. In a time-matched control group (n=4 animals), a rubber condom at room temperature was applied to the tail, in a similar protocol.

Stimulation time was of 30 seconds and intervals between stimuli were at least of 5 minutes. The firing rates of the neurons were compared before and after each stimulation, a new trial was only initiated after the firing rate of the unit returned to pre-trial values. Each experimental session did not last more than 120 minutes.

At the end of the recording period the position of the stainless steel electrode was marked by passing a DC current of 200 μA for 30 seconds (stainless steel electrode anode); in this manner a small deposit of iron was made at the stimulus site. At the end of the experiment the animals were killed, by a lethal anesthetic injection (urethane, i.v.), and the brainstem was removed and placed in 1% potassium ferrocyanide in 10% formaldehyde saline for 7 days, so that the ferrocyanide would cause the conversion of the iron deposit to an identifiable Prussian Blue spot. Brainstem 60 μm thick frontal sections were cut with a freezing microtome (Ernst Leitz, Wetzlar, Germany) and stained with neutral red. Individual maps were drawn for each experiment.

All data are expressed as mean±standard error mean (SEM). Unless otherwise stated, comparisons of the changes in the parameters were carried out by means of a one-way ANOVA of repeated measures, followed by a multiple comparison test (Tukey). To discard the possibility of a chance finding, regarding the firing frequency distribution, it was applied a χ^2 test. Differences were considered significant at $p<0.05$.

RESULTS

A total number of 51 cells were recorded in 24 animals. In the time-matched control group (n=4), 6 cells were recorded and the basal firing rates varied from 2.7 to 9.0 Hz with a mean of 6.2±0.9 Hz. In the experimental groups (n=20), i.e., submitted to thermal stimulation, 45 cells were recorded and the basal firing rate varied from 0.3 to 10.0 Hz with a mean of 6.1±0.4 Hz.

Time-matched control group

In four animals, we stimulated the rat tail with a condom with water at room temperature, therefore no thermal stimuli were applied but just a mild tactile stimulation (Fig 1A). The basal firing rate of the 6 cells varied from 2.7 to 9.0 Hz, with a mean of 6.2±0.9 Hz. The mild tactile stimulation did not induce any significant changes to the basal firing rate of these units, varying from 3.7 to 10.0 Hz with a mean of 6.9±0.9 Hz ($p>0.05$). Fig 2 depicts representative sections with the plotted recording sites.

Neuronal firing rate modifications due to cold stimulation of the rat tail

In 10 animals, the basal firing rate of the 22 cells submitted to cold stimulation of the rat tail varied from 0.3 to 10.0 Hz with a mean of 5.4±0.6 Hz (Fig 1B). Of the total number of cells; 4 (18%) showed no response to the stimulus, with the control firing rate varying from 4.3 to 10.0 Hz with a mean of 6.5±1.2 Hz, and after cold stimulation the firing rate of the units was: 3.7 to 9.5 Hz with a mean of 6.5±1.2 Hz ($p>0.05$). Five cells (23 %) decreased its control firing rate, varying from 2.7 to 9.3 Hz with a mean of 5.7±1.3 Hz, and after cold stimulation the firing rate of the units was: 1.0 to 7.7 Hz with a mean of 3.6±1.2 Hz ($p<0.01$). Whilst 13 (59 %) increased its control firing rate, varying from 0.3 to 10.0 Hz with a mean of 4.9±0.7 Hz, and after cold stimulation the firing rate of the units was: 2.3 to 15.0 Hz with a mean of 9.5±1.3 Hz ($p<0.01$). Fig 2 depicts representative sections with the plotted recording sites.

Neuronal firing rate modifications due to warm stimulation of the rat tail

In 10 animals, the basal firing rate of the 23 cells submitted to warm stimulation of the rat tail varied from 2.7 to 10.0 Hz with a mean of 6.6±0.5 Hz (Fig 1C). Of the total number of cells; 6 (26%) showed no response to the stimulus, with the control firing rate varying from 3 to 8 Hz with a mean of 6.1±0.9 Hz, and after warm stimulation the firing rate of the units was: 2.7 to 9.3 Hz with a mean of 6.1±0.9 Hz ($p>0.05$). Eleven cells (48%) decreased its control firing rate, varying from 3 to 10 Hz with a mean of 6.8±0.7 Hz, and after warm stimulation the firing rate of the units was: 1.7 to 8.0 Hz with a mean of 4.2±0.6 Hz ($p<0.001$). Whilst 6 (26%) increased its control firing rate, varying from 2.7 to 10.0 Hz with a mean of 6.9±1.1 Hz, and after cold stimulation the firing rate of the units was: 3.3 to 16.0 Hz with a mean of 9.8±1.7 Hz ($p<0.001$). Fig 2 depicts representative sections with the plotted recording sites.

The basal firing rate distribution of neurons in the two experimental groups, i.e. warm or cold stimulation, were compared with a χ^2 test and no significant differences were observed in the distributions, implying that the two neuronal populations recorded from are similar.

DISCUSSION

Thermoregulation is a complex reflex mechanism that is controlled mainly by temperature responsive neurons in the anterior hypothalamus and preoptic area. Nevertheless, this system has its final pathway in brainstem nuclei that control vasomotricity[1]. To our knowledge, this is the first report of the involvement of NRO in this brainstem thermoregulatory circuitry.

Serotonergic neurons in the caudal and rostral raphe nuclei discharge steadily in a state-dependent manner and share common pharmacological properties[26]. Nevertheless, there are differences in the mean firing rate of dorsal and caudal raphe nuclei neurons, being the firing rate of caudal nuclei neurons higher than of the dorsal raphe nuclei neurons[25]. The firing rates distribution of the population of cells recorded from in the present work is compatible with the expected behavior of caudal raphe nuclei neurons.

Cutaneous nociceptors and thermal receptors are structurally similar; they are both free axon terminals and originate in similar neurons in the dorsal root ganglion. Their projection to the spinal cord are mainly circumscribed in the first and second spinal laminas where they make synaptic connections with second order neurons that project to specific thalamic nuclei via anterolateral ascending system[27]. Therefore, there are great similarities between the nociceptive and thermal neural processes starting at the receptor level, projections on the spinal cord, ascending pathways and even in its projection on the primary somatosensory cortex cortical area[27]. In this work, special care was taken so as not to stimulate thermal sensitive nociceptors instead of true thermal sensitive terminals involved in thermoregulation.

As stated above, tail skin cooling in these experiments was within the non-noxious range. Such stimulation elicited an increase in the spontaneous activity of the majority of cells tested (59%). Tail skin heating experiments, conducted as previously stated, induced a decrease in the spontaneous activity of the majority of cells tested (48%). The opposite responses in neuronal activity of raphe obscurus units to cooling or heating suggest that these cells are involved in producing the homeothermic relevant vascular adaptations secondary to changes in cutaneous temperature in the rat tail.

The present results demonstrate a significant number of neurons that have its basal activity modified by thermal stimulation. This may be reinforced by the fact that caudal raphe nuclei have been implicated in the induction of a variety of specific patterns of hemodynamic responses, such as those found in desynchronized sleep[22] and nociception[23].

The "serotonin syndrome" is a rare disorder of 5-HT excess, with findings of hyperthermia, shivering, seizures, coma or even death, which can acutely result from serotonergic drug interactions[28]. This association between 5-HT excess in the central nervous system and hyperthermia is possibly consistent with our findings showing that NRO neurons, which are serotonergic, play a role in thermoregulation. However, the precise mechanisms involved remains to be established.

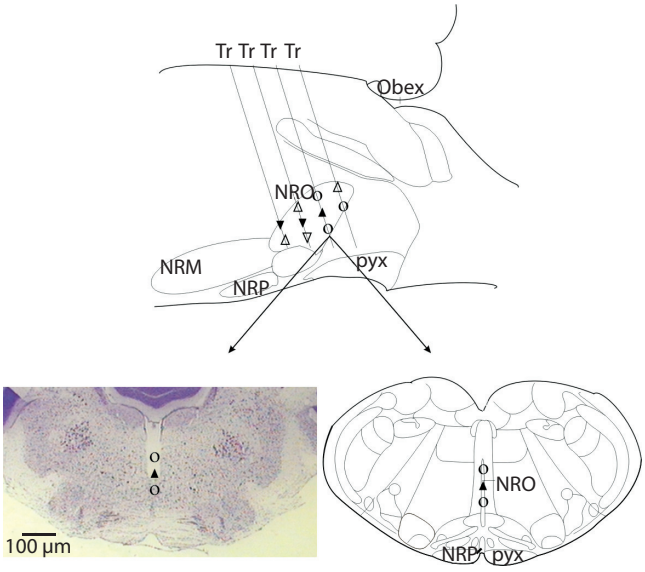
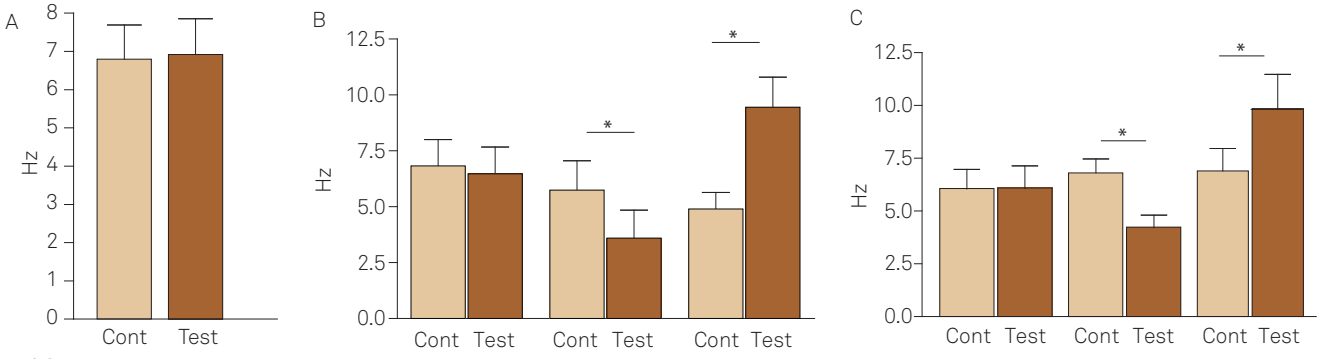


Fig 2. Representative sagittal, coronal sections and microphotography of the rat brainstem, displaying electrode track (Tr) and recording sites plotted from four animals. ▲: increase in unitary discharge rate due to warm stimulation; ▼: decrease in unitary discharge rate due to warm stimulation; O: no response; Δ: increase in unitary discharge rate due to cold stimulation; ∇: decrease in unitary discharge rate due to cold stimulation; NRM: nucleus raphe magnus; NRO: nucleus raphe obscures; NRP: nucleus raphe pallidus; pyx: pyramidal tract. Representative sections modified from Paxinos and Watson[24].



*p<0.01.

Fig 1. Firing rate responses of nucleus raphe obscurus neurons to thermal stimulation in the rat tail. (A) Time-matched control group; (B) effect of cold stimulation in the rat tail; (C) effect of warm stimulation in the rat tail. In (B and C), cells were separated according to their response profiles to thermal stimulation; i.e.,no response (left double columns), reduction (middle double columns) or increase in firing rate (right double columns). Cont = unitary basal firing rate. Test = unitary firing rate after thermal challenge.

References

1. Morrison SF. 2010 Carl Ludwig Distinguished Lectureship of the APS Neural Control and Autonomic Regulation Section: central neural pathways for thermoregulatory cold defense. J Appl Physiol 2011;110:1137-1149.
2. Gordon CJ. Thermal biology of the laboratory rat. Physiol Behav 1990;47:963-991.
3. Carlisle HJ, Laudenslager ML. Observations on the thermoregulatory effects of preoptic warming in rats. Physiol Behav 1979;23:723-732.
4. Kanosue K, Yanase-Fujiwara M, Honoso T. Hypothalamic network for thermoregulatory vasomotor control. Am J Physiol 1994;267(1 Pt 2):R283-288.
5. O'Leary DS, Johnson JM, Taylor WF. Mode of neural control mediating rat tail vasodilatation during heating. J Appl Physiol 1985;59:1533-1538.
6. Rathner JA, McAllen RM. Differential control of sympathetic drive to the rat tail artery and kidney by medullary premotor cell groups. Brain Res 1999;834:196-199.
7. Smith JE, Jansen AS, Gilbey MP, Loewy AD. CNS cell groups projecting to sympathetic outflow of tail artery: neural circuits involved in heat loss in the rat. Brain Res 1998;786:153-164.
8. Coote JH, Futuro Neto HA, Logan SD. The involvement of serotonin neurones in the inhibition of renal nerve activity during desynchronized sleep. Brain Res 1985;340:277-284.
9. Taber E, Brodal A, Walberg F. The raphe nuclei of the brain stem in the cat. I. Normal topography and cytoarchitecture and general discussion. J Comp Neurol 1960;114:161-187.
10. Bowker RM, Abbott LC. Quantitative re-evaluation of descending serotonergic and non-serotonergic projections from the medulla of the rodent: evidence for extensive co-existence of serotonin and peptides in the same spinally projecting neurons, but not from the nucleus raphe magnus. Brain Res 1990;512:15-25.
11. Ray RS, Corcoran AE, Brust RD, et al. Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition. Science 2011;333:637-642.
12. Berthoud HR, Patterson LM, Sutton GM, Morrison C, Zheng H. Inputs to caudal raphé neurons involved in thermal, cardiovascular, and gastrointestinal regulation. Histochem Cell Biol 2005;123:147-156.
13. Morrison SF, Nakamura K. Central neural pathways for thermoregulation. Front Biosci 2011;16:74-104.
14. Nakamura K, Morrison SF. Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol 2007;292:R127-136.
15. Smith JE, Jansen AS, Gilbey MP, Loewy AD. CNS cell groups projecting to sympathetic outflow of tail artery: neural circuits involved in heat loss in the rat. Brain Res 1998;786:153-164.
16. Tanaka M, Nagashima K, McAllen RM, Kanosue K. Role of the medullary raphé in thermoregulatory vasomotor control in rats. J Physiol 2002;540:657-664.
17. Tanaka M, Ootsuka Y, McKinley MJ, McAllen RM. Independent vasomotor control of rat tail and proximal hairy skin. J Physiol 2007;582:421-433.
18. Nakamura K, Matsumura K, Hübschle T, et al. Identification of sympathetic premotor neurons in medullary raphe regions mediating fever and other thermoregulatory functions. J Neurosci 2004;24:5370-5380.
19. Ootsuka Y, Blessing WW, McAllen RM. Inhibition of rostral medullary raphé neurons prevents cold-induced activity in sympathetic nerves to rat tail and rabbit ear arteries. Neurosci Lett 2004;357:58-62.
20. Ootsuka Y, Blessing WW. Inhibition of medullary raphé/parapyramidal neurons prevents cutaneous vasoconstriction elicited by alerting stimuli and by cold exposure in conscious rabbits. Brain Res 2005;1051:189-193.
21. Ootsuka Y, McAllen RM. Interactive drives from two brain stem premotor nuclei are essential to support rat tail sympathetic activity. Am J Physiol Regul Integr Comp Physiol 2005;289:R1107-1115.
22. Futuro-Neto HA, Coote JH. Changes in sympathetic activity to heart and blood vessels during desynchronized sleep. Brain Res 1982;252:259-268.
23. Dantas MA, Có W, Futuro-Neto HA. Responses of neurons of the nucleus raphe obscurus to noxious stimuli. Braz J Med Biol Res 1990;23:923-926.
24. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. San Diego: Elsevier, 2007. p. 1-166.
25. Futuro Neto HA, Dantas MA, Gilbey MP. A comparison of the effects of eserine sulfate on the activity of medullary raphe neurons in the anesthetized rabbit and rat. Braz J Med Biol Res 1994;27:1445-1454.
26. Jacobs BL, Azmitia EC. Structure and function of the brain serotonin system. Physiol Rev 1992;72:165-229.
27. Chéry-Croze S. Painful sensation induced by a thermal cutaneous stimulus. Pain 1983;17:109-137.
28. Sternbach H. The serotonin syndrome. Am J Psychiatry 1991;148:705-713.