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Serotonin modifies the spontaneous spiking activity of gracile nucleus neurons in rats: role of 5-HT_{1A} and 5-HT_2 receptors

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ABSTRACT

We tested the effects of microiontophoretic application of serotonin (5-HT) on the firing rate of neurons located in the gracile nucleus (GN) of rats. Application of 5-HT_{1A} and 5-HT_2 agonists and antagonists respectively mimicked/modulated and blocked the effects produced by the amine, respectively.

Among the tested neurons, 88.2% modified their background firing activity in the presence of 5-HT. Responsive neurons decreased their mean firing activity (MFA) in 56.7% of cases and increased it in the remaining 43.3%. To ascertain the specificity of the effects induced by 5-HT, we utilized 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and alpha-methyl-5-hydroxytryptamine (α-MET-5-HT), agonists for 5-HT_{1A} and 5-HT_2 receptors, respectively. The microiontophoresis of 8-OH-DPAT modified the background firing rate of all GN neurons (100% of tested neurons) mimicking the decrease of MFA evoked by 5-HT. The application of a-MET-5-HT modified the MFA in 76.9% of tested neurons, decreasing it in 61.5% of cases and increasing in the remaining 23.1%.

The decrease of MFA induced by 8-OH-DPAT was antagonized by application of the 5-HT_{1A} receptor antagonist N-[2-[-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635), while application of 5-HT_2 receptor antagonist ketanserine tartrate (KET) antagonized only the increase of MFA induced by a-MET-5-HT.

These results indicate that 5-HT is able to modulate the background firing activity of GN neurons by 5-HT_{1A} and 5-HT_2 receptors.

Key words

Gracile nucleus • Serotonin receptors • Microiontophoresis • Rat

Introduction

Neurons of the gracile nucleus (GN) receive somatotopically organized somatosensory information from the periphery and project toward to the ventroposterolateral nucleus of the contralateral thalamus (Nunez and Buno, 1999). Previous studies in rats, cats, raccoons, and monkeys demonstrated that GN neurons receive cutaneous input coming from hindlimbs and lower trunk (Strata et al., 2003; Qi and Kaas, 2006), which is more related to postural support than tactile exploration (Marino et al., 1999). Moreover, these neurons are involved in the transmission of nociceptive information to the central nervous system (CNS) (Miki et al., 2000; Porreca et al., 2002).

Gerhart and coll. (1981) showed an inhibitory pathway arising from the nucleus raphe magnus (NRM) and terminating in the GN; in fact the activation of GN neurons can be inhibited by electrical stimulation of raphe nuclei (RN). A dense network of serotonergic fibers coming from RN

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modulates the activity of dorsal column nuclei (DCN) (Willcockson et al., 1987). In addition, 5-HT-immunoreactive fibers have been observed in GN of cats (Pearson and Goldfinger, 1987), owl monkeys (Blomqvist and Broman, 1993), and anurans (Munoz et al., 1995). Other sources of serotoninergic afferent input to GN arise from the nucleus raphe obscurus (NRO) and the nucleus raphe pallidus (NRP) (Willcockson et al., 1987).

In the GN, 5-HT plays several roles influencing both the nociceptive input at the spinal cord level (Willcockson et al., 1987) and in the motor function (Blomqvist and Broman, 1993). The modulatory effect on the 5-HT spontaneous activity has been described in various central structures, such us the ventral-anterior (VA) and ventrolateral (VL) thalamic nuclei (Grasso et al., 2006), red nucleus (Licata et al., 1995), vestibular nuclei (Licata et al., 1990; Licata et al., 1993a; Licata et al., 1993b) and bulbar reticular neurons (Barresi et al., 2005).

Hybridization technique showed low levels of 5-HT_{1A} receptors in the GN (Pompeiano et al., 1992). This receptor subtype represents one of the principal targets of 5-HT and is involved in numerous physiological effects, including pain modulation (Millan et al., 1991).

Previous studies demonstrated the role of 5-HT_{2} receptors in nociceptive and motor functions. In particularly, in situ hybridization studies described the presence of detectable levels of mRNA for 5-HT_{2A} and 5-HT_{2C} subtype receptors in the GN (Fonseca et al., 2001).

The aim of this work was to study the effects of 5-HT on the MFA of single neurons of the GN and to provide a first analysis on serotoninergic receptors involved in these interactions. Our electrophysiological findings demonstrate the presence of 5-HT_{1A} and 5-HT_{2A} subtype receptors in the GN and their involvement in the 5-HT induced modulation of GN firing rate.

Methods

Experiments were performed on adult male Wistar rats, anesthetized with urethane (1.5 g/Kg i.p.).

All experiments complied with the guidelines for the acquisition and care of laboratory animals described in the European Communities Council Directive (86/609/EEC), in the National Institutes of Health Publication No. 80-23, revised 1996, and in the Italian laws on this matter. In addition, the experimental protocol was approved by the local chapter of the International Animal Care and Use Committee (IACUC) of the University of Catania.

Five-barrel glass microelectrodes positioned by a micromanipulator (LPC, France) at coordinates corresponding to the GN were used to record unitary neuronal firing and to eject drugs. A barrel, filled with 4% Pontamine Sky Blu (Sigma) in 3 M NaCl (resistance 7-12 MΩ) was used to record electrical activity and to mark the final point of each penetration (negative current pulse of 20 μA for 20 min) in the GN (Fig. 1A). At the end of each penetration, after removal the electrode, tracks and recording sites were identified in 60 μm coronal sections of the brainstem, stained with Neutral Red. Electrical unitary activity was discriminated, stored using an interface (CED1401 plus, CED, UK) and analyzed using a personal computer provided with software (Spike2, CED, UK). Spiking activity was processed if the signal to noise ratio was at least 3:1 and if the spike amplitude remained unmodified during the trials. Barrels used for microiontophoresis contained the followings drugs: 5-hydroxytryptaminecreatininesulphates(5-HT,Sigma Aldrich, Milan, Italy, 30 mM, pH=4.5); 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, Sigma Aldrich, Milan, Italy, 20 mM, pH=4.5-5.0); alpha-methyl-5-hydroxytryptamine (a-MET-5-HT, Tocris Bioscience, Milan, Italy, 20 mM, pH=4.5-5.0); N-[2-[-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635, Sigma Aldrich, Milan, Italy, 20 mM, pH=4.5-5.0); ketanserin tartrate (KET, Sigma Aldrich, Milan, Italy, 10 mM, pH=4.5). All drugs were dissolved in water except for 5-HT (165 mM NaCl). The microiontophoretic system (Neurophore BH-2, Harvard Apparatus, Holliston, MA, USA) balanced currents automatically through a barrel filled with 3 M NaCl, to neutralize any voltage shift due to the applied currents. All drugs (5-HT agonists and antagonists) were retained by negative currents (2-10 nA) delivered to the barrels. 5-HT and agonists (8-OH-DPAT and a-MET-5-HT) were always ejected by mean of brief (duration 30 s) positive current pulses (intensity up to 120 nA). Antagonists (WAY100635 and KET) were always applied with long-lasting currents (up to 5 nA for 7-15 min).

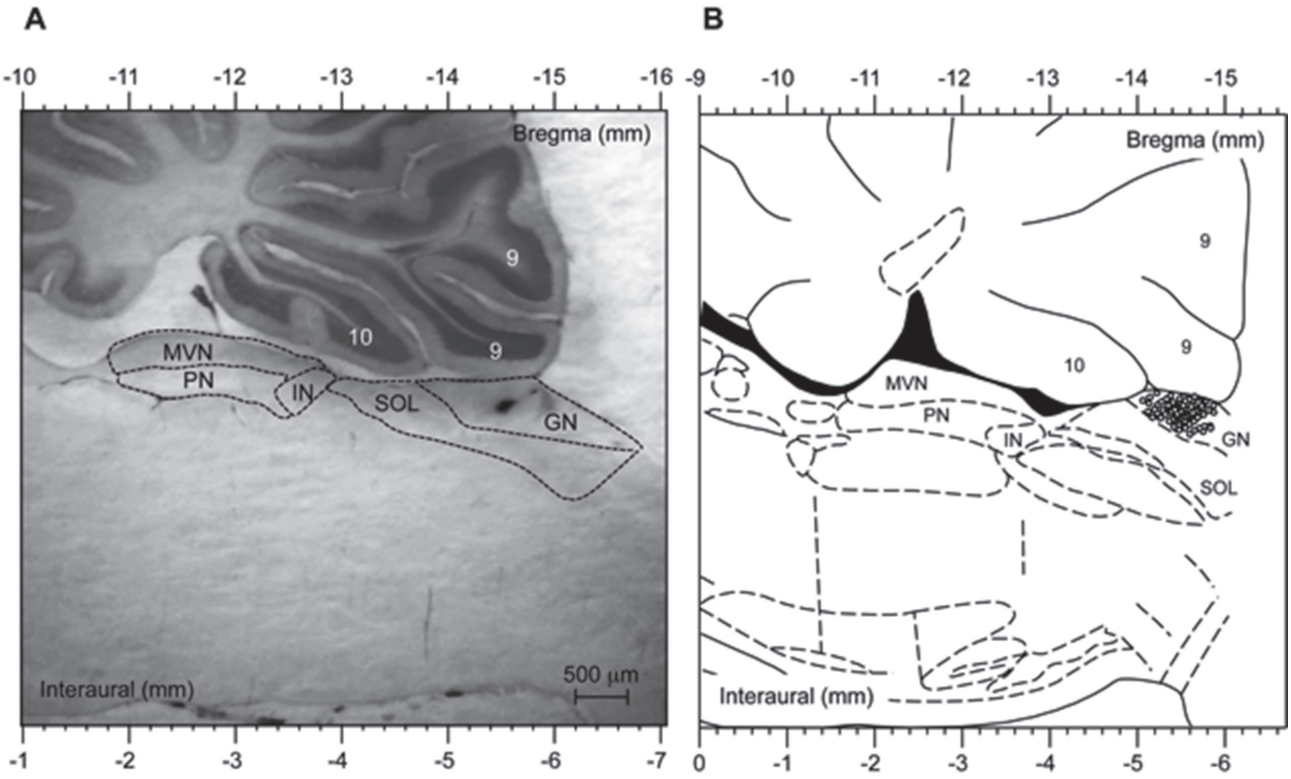

Fig. 1. - (A) Sagittal plane, showing the final point of the penetration, marked with Pontamine Sky Blu (40x magnification). (B) The locations of all 86 recorded neurons, reconstructed by histological examination of brain slices after each experiment, are projected onto a schematic drawing of a sagittal plane of the rat brain based on the Paxinos and Watson atlas (1998) for stereotaxic plane, L 0.4 (the units located outside of GN were omitted). Index of abbreviations: MVN: medial vestibular nucleus; PN: prepositus nucleus; IN: intercalated nucleus of the medulla; GN: gracile nucleus; SOL: nucleus of the solitary tract; IX: 9th cerebellar lobule; X: 10th cerebellar lobule.

During our research we adopted three different approaches in the use of drugs. In the first 7 experiments we used a three-barrel glass microelectrodes to elucidate the effects exerted by 5-HT application on GN neurons. In the following 8 experiments we used a five-barrel glass microelectrodes, in which 3 elements were filled with the 5-HT and its agonist for 5-HT_{1A} and 5-HT_{2} receptors. In the remaining experiments, we still used five-barrel glass microelectrodes, filled alternatively with 5-HT, agonists and antagonists for the 5-HT_{1A} and 5-HT_{2} receptors.

Whenever a single unit was isolated, application (30 s pulses) of 5-HT or an agonist (without a preferential order) at different intensities (20-120 nA) was routinely followed by applications of the same drug performed at the maximal intensity able to evoke reversible responses. In some cases the application of 5-HT and/or agonists were repeated during simultaneous application of an antagonist for a specific type of serotoninergic receptor.

The firing rate of each recorded unit was calculated and integrated over 1 s bins for analysis and 5 s bins for display. The mean value of the firing rate recorded over a sequence of 180 values (3 min) in the absence of any drug application was defined as the MFA. If the standard deviation (SD) exceeded 50% of the MFA, the unit was excluded from analysis.

A modification of the firing rate by at least 2 SD from the MFA during at least 20 s was defined as a response to a drug application. We used three parameters to quantify a response: the magnitude (M), the contrast (C) and the duration (D). M indicates the magnitude of the effect and was defined as the difference between the number of spikes recorded during the response and the number recorded during a period with the same duration immediately preceding the drug ejection. C (expressed as a percentage, %) is the ratio between these two values and indicated the signal-to-noise value. D estimates the duration of the effect in seconds (s).

Table I. - Effects of 5-HT, 8-OH-DPAT and α-MET-5-HT on GN neurons.

Gracile Nucleus	Decreases				Increases			
	M	C	D	n	M	C	D	n
5-HT	-113.5±9.4	-31.0±3.6	70.0±6.7	26	229.2±16.4	62.6±8.3	72.7±5.9	24
8-OH-DPAT	-194.3±19.4	-27.8±2.7	80.0±8.5	20	-	-	-	0
α-MET-5-HT	-60.3±4.7	-19.0±2.9	39.6±3.4	12	149.5±1.9	96.9±21.0	71.3±0.4	4

The table show the M (number of spikes); C, (%); D (s); n, number of units and ± S.E.

Statistical analyses of firing rate and discharge pattern modifications were performed with a statistical software package (GRAPH PAD Prism). Modification of MFA induced by 5-HT or its agonist was considered significant if at least two of the mean values of the parameters used (M, C or D) differed significantly from the mean values recorded during the preliminary control (two-tailed Student's t-test). Different sets of responses to amine and 5-HT_{1A} and 5-HT_2 agonists were compared with a two-tailed unpaired t-test. An antagonist action was considered effective if it reduced at least two of the three parameters of a response by 50-70% (partial antagonism) or more (blockade). In each neuron, different sets of single trials recorded under different conditions (e.g. 5-HT_{1A} and 5-HT_2 agonists responses before and during application of their respective antagonist) were compared with a two-tailed Student's t-test (Mann-Whitney U-test).

Results

Effects of 5-HT on background firing activity

The activity of 86 neurons was studied in the GN during short-lasting application of 5-HT, 8-OH-DPAT, a-MET-5-HT, WAY100635, and KET. The MFA ranged from 2 to 38 spikes/s (mean ± standard error = 9.3 ± 0.8 spikes/s; median = 6.7 spikes/s; mode = 5.9 spikes/s).

Administration of 5-HT (current intensity: 10-120 nA) was tested on 68 GN neurons, 88.2% of which responded to this application modifying their MFA. Two types of MFA modifications were observed: decrease in 56.7% (34 of 60), increase in 43.3% (26 of 60), whereas in the remaining 11.8% of cases (8 of 68) 5-HT was ineffective. Examples of dose-dependent decrease and increase of MFA changes induced by 5-HT are illustrated as histograms respectively in Fig. 2A and Fig. 2B. The mean value of parameters M, C and D for MFA changes, induced by 5-HT are shown in Table 1 and Fig. 2C. The selective 5-HT_{1A} receptor agonist 8-OH-DPAT induced a dose-dependent decrease of all firing parameters tested in 100% of the 20 GN neurons tested (Fig. 3A). The mean values for the changes induced by 8-OH-DPAT are shown in Table 1 and Fig. 2C. Application of WAY100635, a 5-HT_{1A} selective receptor antagonist, was tested in 4 neurons, where this treatment induced a partial antagonism of MFA decreases evoked by 8-OH-DPAT ($p<0.0426$) (Fig. 3C-D).

The changes induced by 5-HT and 8-OH-DPAT in the different parameters, shown in Table 1, were significantly different (M, $p=0.0002$; C, $p=0.0006$; D, $p<0.0001$).

In two cases, we recorded an 8-OH-DPAT induced decrease of MFA while 5-HT evoked increase of MFA. An example of these opposite effects was given in Fig. 3B.

Examples of dose-dependent response induced by 5-HT and 5-HT_{1A} agonist are shown in Fig. 3A.

The 5-HT_2 receptors agonist, a-MET-5-HT, was tested in 26 GN, 76.9% of which responded to this application, inducing decrease (Fig. 4A) of the MFA in 61.5% (16 of 26) and increase of it (Fig. 4B) in 15.4% (4 of 26) of cases tested, while in 23.1% of the remaining cases (6 of 26) 5-HT_2 agonist was ineffective. The mean values for parameters M, C, and D regarding decrease or increase of MFA, induced by a-MET-5-HT are shown in table 1 and Fig. 2C. In the 4 neurons whose firing rate was increased by a-MET-5-HT, this response was partially antagonized by the non-selective 5-HT_2 receptor antagonist KET ($p=0.0294$; Fig. 4C-D).

Discussion

Our findings demonstrate that 5-HT modulates the MFA of GN neurons in rats. The modulation was not

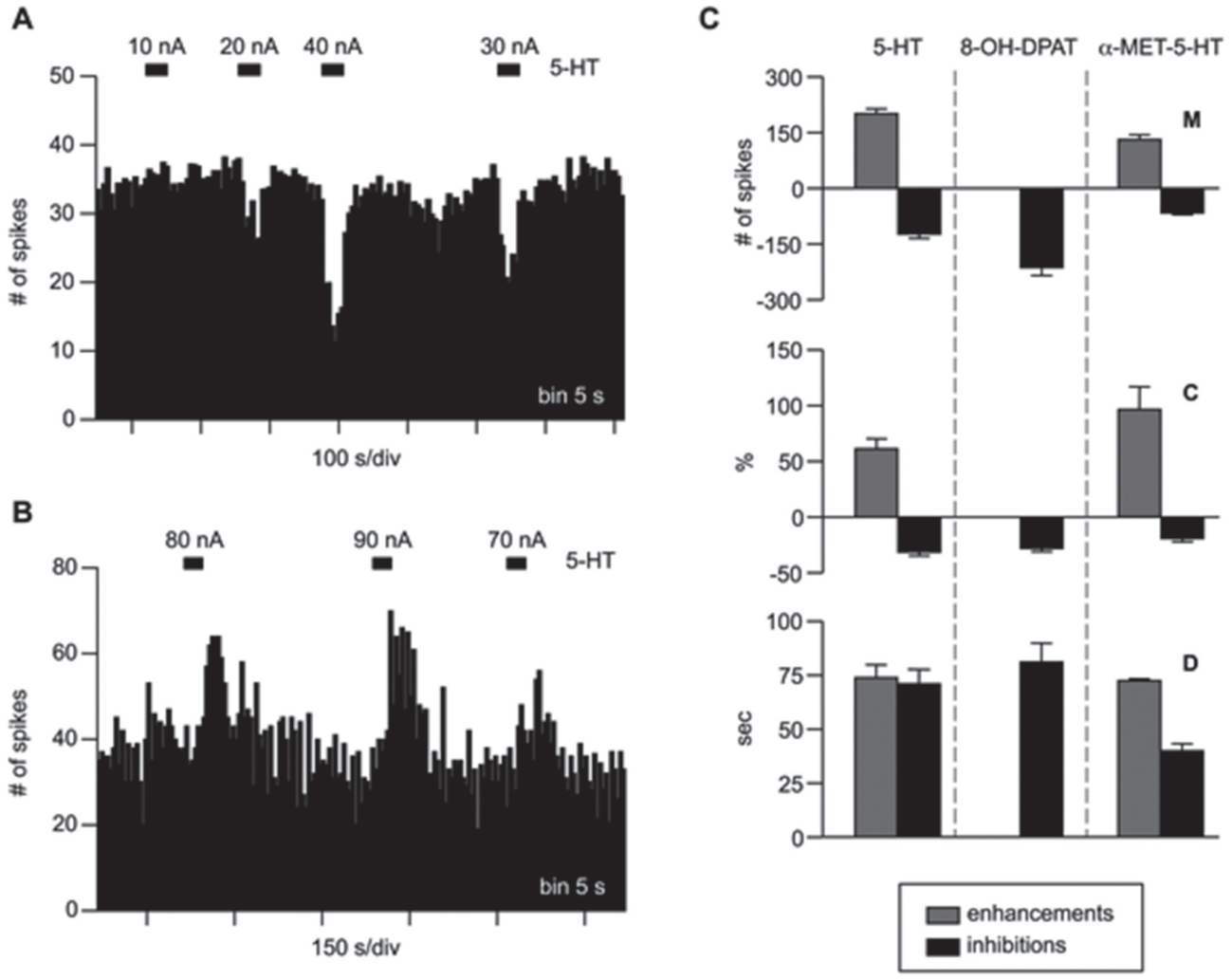

Fig. 2. - Rate histograms (5-s bins) showing the effects of 5-HTon MFA in two GN neurons. The horizontal bars above the histograms (5-s bins) indicate the duration of the ejection periods of 5-HT at the given current. (A) The application of 5-HT induced decrease of the MFA in one GN neuron. (B) The application of 5-HT induced increase of the MFA in one GN neuron. (C) Mean values of Magnitude (M), Contrast (C) and Duration (D) in presence of 5-HT, 8-OH-DPAT and α-MET-5-HT.

homogeneous; in fact, application of 5-HT induced decrease of MFA in the majority of cases and increase in the remaining ones. This indicates that 5-HT exerts its effect on at least two types of receptors: 5-HT_{1A} and 5-HT_{2}. Both types of response exhibited by GN neurons to microiontophoretic application of 5-HT were dose-dependent, indicating that increase and decrease of MFA were related to 5-HT receptor binding.

In the GN, 5-HT plays a key role in the modulation of somatosensory, nociceptive input (Willcockson et al., 1987; Gosselin et al., 2010) and in motor functions (Blomqvist and Broman, 1993).

It is known that stimulation of various cortical regions modifies nociception both in humans and rodents. These modifications depend upon a pathway that arise from the cortex and reach the DCN (Millan, 2002). In particular, stimulation of the RN and periacqueductal gray (PAG) inhibits neural transmission in the GN (Jundi et al., 1982). The inhibitory activity of the RN on GN neurons may involve the release of 5-HT by neurons belonging to the NRM that is an important inhibitory pathway of the GN in cats (Gerhart et al., 1981). This seems to be the case despite of the fact that only anatomical studies suggest a connection between the NRM and GN in rat (Willcockson et al., 1985).

With regard to 5-HT-evoked responses, our findings are in line with those obtained in the rat VA and VL

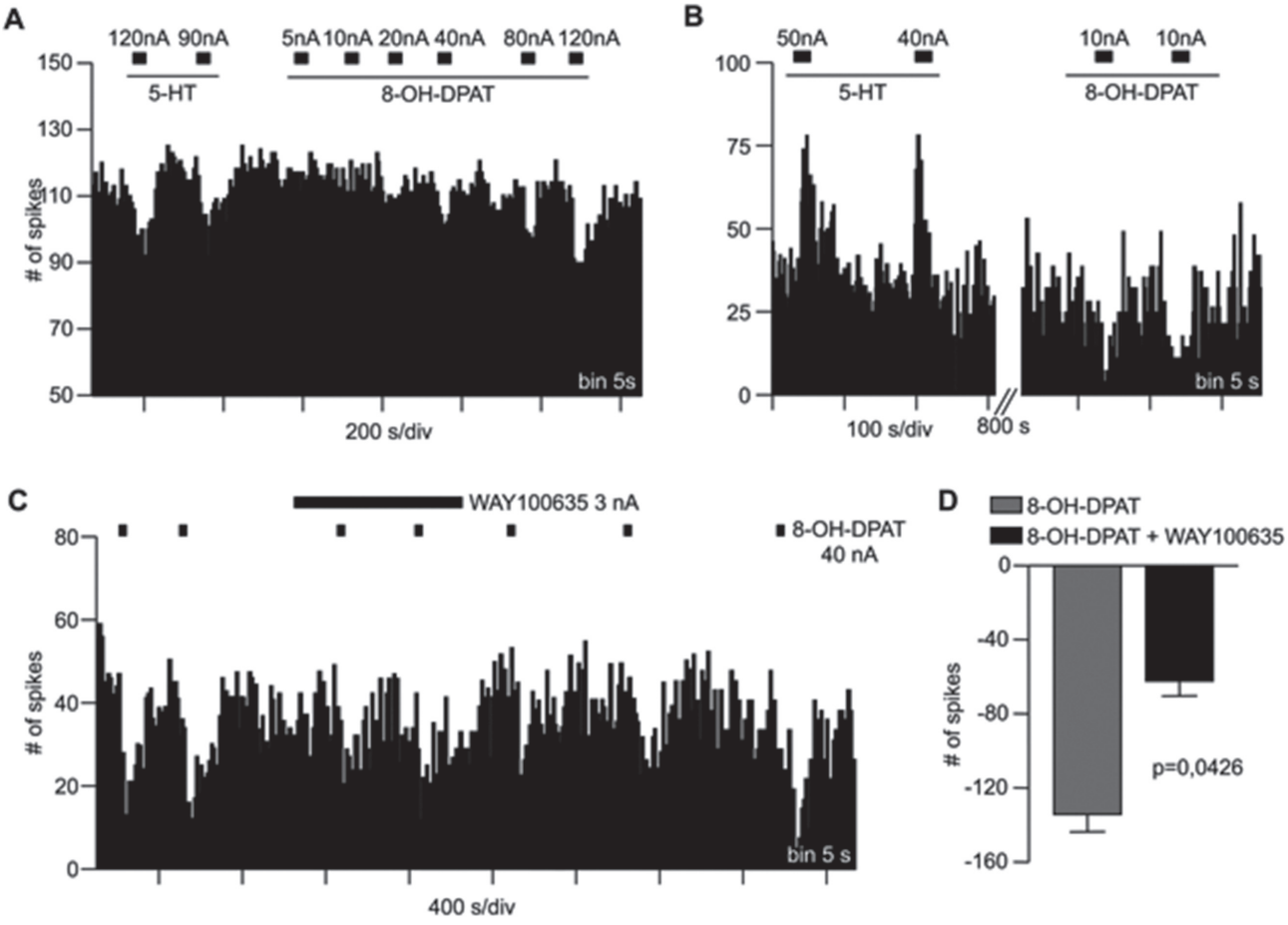

Fig. 3. - Rate histograms (5-s bins) showing the effects of the 5-HT, 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin(8-OH-DPAT) and the antagonist N-[2-[-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635) on two GN neurons. The horizontal bars above the histograms (5s bins) indicate the duration of the ejection periods of 5-HT, 8-OH-DPAT and WAY100635 at the given current. (A) The application of 8-OH-DPAT induced decrease of the MFA that mimicked the 5-HT evoked response. (B) Opposite effects induced by 5-HT and 8-OH-DPAT in the same unit. (C) The histogram illustrates the 8-OH-DPAT-evoked decrease antagonized by the application of WAY100635. (D) Mean values of magnitude modification in the presence of 5-HT_{1A} agonists, 8-OH-DPAT (grey column) and during coiontophoresis of 8-OH-DPAT and 5-HT_{1A} antagonists WAY100635 (black column).

thalamic nuclei (Grasso et al., 2006), red nucleus (Licata et al., 1995), lateral vestibular nucleus (LVN) (Licata et al., 1990), medial vestibular nucleus (MVN) (Licata et al., 1993a), superior vestibular nucleus (SVN) (Licata et al., 1993b), bulbar reticular neurons (Barresi et al., 2005), subthalamic nucleus (Stanford et al., 2005), dorsal raphe nucleus (Gobert et al., 1995; Martin et al., 1999; Wang et al., 2009b), and prefrontal cortex (Wang et al., 2009a). In these regions the 5-HT-evoked decreases of the MFA in GN neurons were mediated by 5-HT_{1A} subtype receptors. In fact, the selective agonist, 8-OH-DPAT, mimicked 5-HT effects by decreasing the spontaneous firing rate in all tested neurons. The 5-HT_{1A} selective receptor antagonist, WAY100635, induced a partial antagonism of the decrease evoked by 8-OH-DPAT on MFA.

The 5-HT_{1A} receptor is the most abundant 5-HT subtype expressed in the brain and seems to play a predominant role in the modulation of pain (Millan, 2002) through peripheral and central mechanisms (Millan, 1995). In fact, previous studies have shown antinociceptive effects following administration of 8-OH-DPAT (Colpaert, 2006; Jiang et al., 2014) and, in humans, chronic pain was associated with low expression of 5-HT_{1A} receptors (Lindstedt et al., 2011).

Regarding the 5-HT_2 receptors, our data show that in the GN, a-MET-5-HT is able to mimick both type of 5-HT effects, inducing a decrease of the firing rate

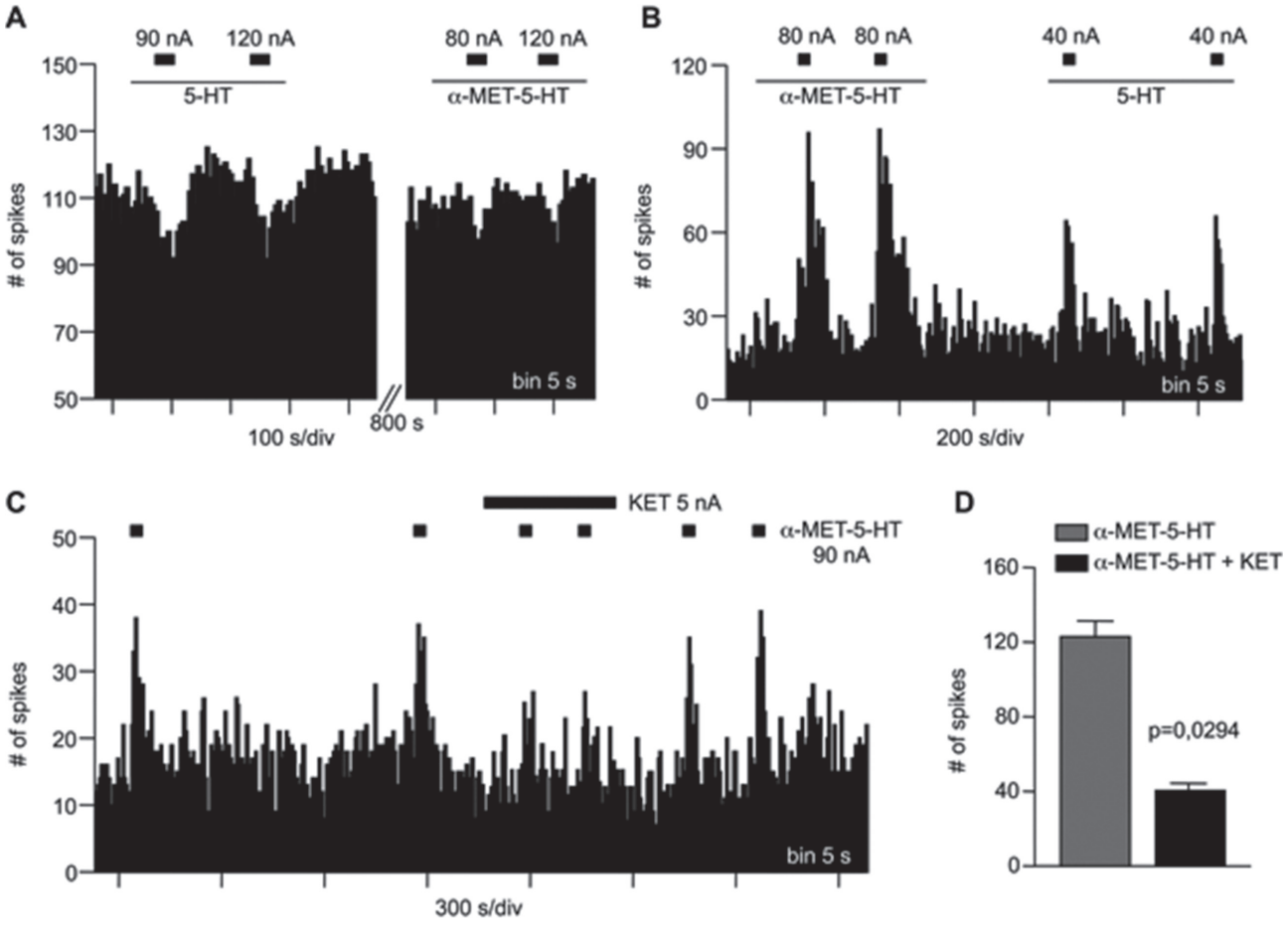

Fig. 4. - Rate histograms (5-s bins) showing the effects of 5-HT, 5-HT_2 agonists alpha-methyl-5-hydroxytryptamine (a-MET-5-HT) and antagonist ketanserin tartrate (KET) on three GN neurons. The horizontal bars above the histograms (5-s bins) indicate the duration of the ejection periods of 5-HT, a-MET-5-HT and KET at the given current. (A) The application of a-MET-5-HT induced decrease of the MFA that mimicked the 5-HT evoked response. (B) The application of a-MET-5-HT induced increase of the MFA that mimicked the response evoked by 5-HT. (C) The histogram illustrates a-MET-5-HT-evoked increase of MFA antagonized by the application of KET. (D) Mean values of magnitude modification in the presence of 5-HT_2 agonists, a-MET-5-HT (grey column) and during coiontophoresis of a-MET-5-HT and 5-HT_2 antagonists KET (black column).

in the majority of cases and an increase in a minor number of cases. Similar results have been obtained in our previous studies in VA, VL thalamic nuclei (Grasso et al., 2006) and in the bulbar reticular formation (Barresi et al., 2005).

An interesting issue could be address, concerning the decrease of MFA induced by a-MET-5-HT. In fact, in vitro studies, shown that a-MET-5-HT has moderate affinity for 5-HT_{1A-B} receptors (Ismaiel et al., 1990) and could be able to displace 8-OH-DPAT from its binding to 5-HT_{1A} receptor with an IC50 of 19.6 nM (May et al., 2003). Thus, the MFA decrease induced by a-MET-5-HT could be due to a "nonspecific" activation of 5-HT_{1A} receptors. Nonetheless, this occurs only if the a-MET-5-HT concentration is higher than the concentration above mentioned. Since in the present study we never reached the critical value of 19.6 nM with the a-MET-5-HT, both increase and decrease of MFA were induced by 5-HT_2 receptors. As shown in Fig. 5, the effect of 5-HT was mimicked by the 5-HT_{1A} agonist (8-OH-DPAT). Further microiontophoretic application of a-MET-5-HT had no effect on MFA. Our conclusion is that the 5-HT_2 agonist, at the concentration used in our experiments, acts only through 5-HT_2 receptors.

The opposite effects induced by a-MET-5-HT could be also accounted for by the different affinity of this agonist for 5-HT receptors, classified as 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} (Hoyer et al., 1994; Barnes and

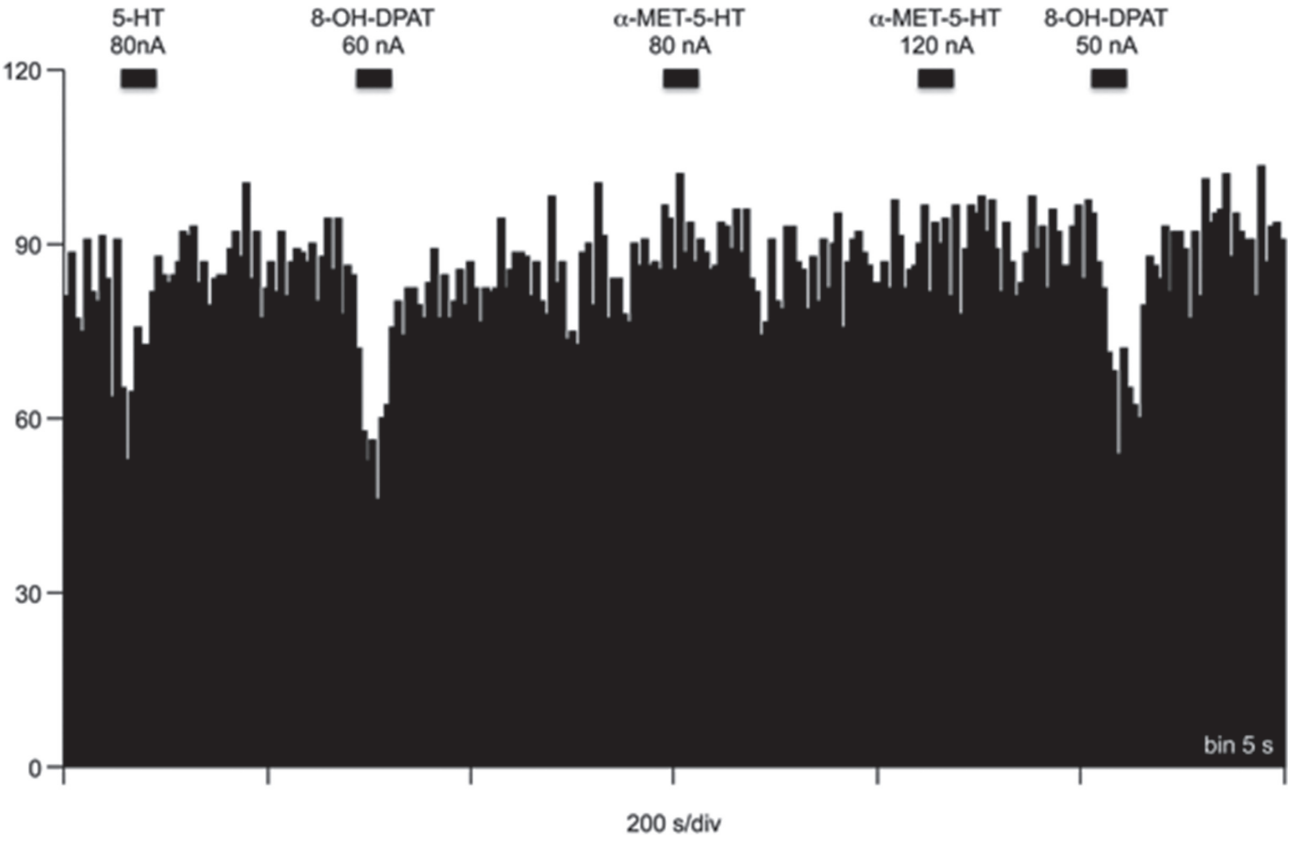

Fig. 5. - Rate histograms (5-s bins) showing the effects of 5-HT, 5-HT_{1A} agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 5-HT_2 agonists alpha-methyl-5-hydroxytryptamine (a-MET-5-HT) on one GN neuron. The horizontal bars above the histograms (5-s bins) indicate the duration of the ejection periods of 5-HT, 8-OH-DPAT and a-MET-5-HT at the given current. The decrease induced by the application of 5-HT was mimicked by 8-OH-DPAT applications. In the same neuron the application of a-MET-5-HT had no effects on MFA.

Sharp, 1999). It was observed that 5-HT_{2A} is the main receptor mediating excitatory effects among the 5-HT receptors' family (Aghajanian and Marek, 1999), even though it may also mediate inhibitory effects (Martin et al., 1998), while 5-HT_{2C} receptors mediate the inhibitory effects exerted by 5-HT (Liu et al., 2000; Jakus et al., 2003; Boothman et al., 2006). Both 5-HT_{2A} and 5-HT_{2C} are expressed at relatively higher levels in the CNS (Barnes and Sharp, 1999) while 5-HT_{2B} seems to be sligthtly expressed in few structures of CNS like the cerebellum, the hypothalamus, the lateral septum and the amygdala (Duxon et al., 1997). There is no evidence about the presence of 5-HT_{2B} receptors in the GN, therefore the greater affinity for the a-methyl-5-HT_{2B} subtype compared to 5-$HT_{2A/C}$ has no relevance in our studies (Barnes and Sharp, 1999).

The 5-HT_2 receptor antagonist KET was able to partially antagonize only the increase of the MFA induced by 5-HT_2 agonist. KET is a general antagonist with high affinity for 5-HT_{2A} receptors, moderate affinity for 5-HT_{2C} receptors and very low affinity for 5-HT_{2B} in rats (Kristiansen and Dahl, 1996). These findings suggest the involvement of 5-HT_{2A} subtype receptors in the increase of MFA in GN neurons, which is in agreement with previous research in LVN neurons, where KET reduced only the increase component of the biphasic effect induced by 5-HT on MFA (Licata et al., 1990). In SVN and MVN, KET was effective in blocking 5-HT-induced increase of MFA, but its influence on the MFA decrease was inconsistent (Licata et al., 1993a; Licata et al., 1993b). Previous studies have shown the important role played by the 5-HT_{2A} receptor in pain perception and modulation; in fact, experiments in knockout mice for this receptor have highlighted a lower nociceptive response to noxious stimuli (Kayser et al., 2007). The modulation of nociception of 5-HT_{2A} receptors may involve both the peripheral and central nervous systems. In the peripheral nervous system, this receptor is expressed in dorsal root ganglion cells and the

application of 5-HT_{2A} receptors antagonist, reduces the nociceptive response in pain models (Sasaki et al., 2006). In the central nervous system, a positron emission tomography study conducted in humans showed a relationship between 5-HT_{2A} binding in the brain and the response to noxious stimuli, in which the availability of the receptor co-varies along with the response to pain stimuli (Kupers et al., 2009). A similar effect has been observed in other central structures, such as the hippocampus (Soleimannejad et al., 2006) and the spinal cord (Kayser et al., 2007; Van Steenwinckel et al., 2008; Van Steenwinckel et al., 2009).

Furthermore, studies on the spinal cord and the GN demonstrate the role of cyclo-oxygenase-1 and 2 (COX-1, COX-2) in inflammatory pain (Yamamoto and Nozaki-Taguchi, 1997; Zhu et al., 2003), and show that activation of 5-HT_{2A} receptor in the parietal cortex is responsible for the increased level of COX-2 during pain (Mackowiak et al., 2002). All these pieces of evidence support the hypothesis that 5-HT_{2A} receptors may be involved in pain modulation in the GN.

Previous studies in rats using the double labeling immunohistochemistry technique have evidenced that the GN receive serotoninergic projections by the NRO, NRP and NRM (Willcockson et al., 1987). Furthermore, 5-HT-immunoreactive fibers have been observed in GN of cats (Pearson and Goldfinger, 1987), owl monkeys (Blomqvist and Broman, 1993), and anurans (Munoz et al., 1995).

This finding supports the microiontophoretic data, which demonstrates the role of 5-HT in the modulation of the MFA of GN neurons through 5-HT_{1A} and 5-HT_2 subtype receptors.

Our results show that 5-HT is able to modulate the background MFA of GN neurons and this action is mediated by 5-HT_{1A} and 5-HT_2 receptors. They contribute to deeper understanding of the regulation of nociception mechanisms and may serve as preliminary investigation for drug-related experimental protocols.

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