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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Effects of 5-HT3 Receptor Blockade on Visceral Nociceptive Neurons in the Ventrolateral Reticular Field of the Rat Medulla Oblongata

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Abstract—The caudal ventrolateral reticular formation of the medulla oblongata is the first layer of visceral nociceptive processing. In experiments on rats, neuronal responses in this zone to nociceptive stimulation of the large intestine were examined and the effects of selective blockade of 5-HT3 receptors on these responses were assessed. By the character of responses to nociceptive colorectal stimulation (CRS), the recorded medullary neurons were divided into three groups—excited, inhibited and indifferent. Intravenous injection of 5-HT3 antagonist granisetron (1 and 2 mg/kg) as well as local application of this agent on the surface of the medulla oblongata (1.25 and 2.5 nmole) suppressed the background and evoked firing of CRS-excited reticular neurons in a dose-dependent manner but did not exert as pronounced influence on the cells inhibited by visceral nociceptive stimulation. Spike activity in the group of CRS-indifferent neurons under similar conditions was 5-HT3-independent. The results obtained provide evidence that 5-HT3 receptors mediate the facilitating effect of serotonin on supraspinal transmission of the abdominal nociceptive stimulus which, at least in part, is realized via selective activation of visceral medullary nociceptive neurons. A shutdown of this mechanism may underlie the analgesic effect of 5-HT3 antagonists in abdominal pain syndromes.

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Key words: 5-HT3 receptors, granisetron, ventrolateral reticular field of the medulla, neurons, colorectal stimulation, abdominal pain.

INTRODUCTION

A major role in conducting nociceptive information from the organs of the abdominal cavity to the brain structures is played by fibers of the spinal ventrolateral system, which consists of the ventral spinothalamic, spinoreticular and spinomesencephalic tracts [1, 2]. The first supraspinal level responsible for processing nociceptive signals incoming via these tracts is represented by the medullary reticular formation [1, 3, 4]. The caudal ventrolateral medulla (CVLM), situated between the ventral pole of the spinal trigeminal nucleus and the lateral reticular nucleus [5], is commonly believed to be a center for integration of the cardiovascular and motor components of reflex behavioral responses (including those triggered by nociceptive stimuli) and considered as a crucial

link in the endogenous pain control system [4, 6].

It is exactly in this system where some authors revealed the neuronal populations specifically responding to painful stimulation of the organs in the abdominal cavity [7–10]. These cells were referred to visceral nociceptive neurons of the medulla oblongata, while changes in their functional activity may serve indicators of central conduction of the abdominal nociceptive signals [8–10].

Serotonin is known to be a major neurotransmitter of visceral nociception that realizes its effects via impacts on different classes of peripheral and central 5-HT receptors [11, 12]. It was established that 5-HT receptors play a key role in the serotoninergic mechanisms of abdominal pain arising in the organs of the gastrointestinal tract (GIT) [12, 13]. These receptors represent a cation-selective ion channel, activation of which promotes rapid depolarization of a cell and the release of some neurotransmitters, including glutamate, substance P, GABA, dopamine, acetylcholine and proper serotonin [14]. 5-HT3 type receptors are characterized by a predominantly presynaptic localization in the membrane of peripheral and central neurons. In the brainstem, they were found to be most abundant in the bulbar structures adjacent to the bottom of the fourth ventricle (calamus scriptorius area) and in the nucleus of the solitary tract [13].

The results of some studies indicate that blockade of 5-HT3 receptors promotes analgesia. It was thus shown that their antagonist, alosetron, reduces the intensity of painful sensations and resultant activity of the stem and limbic brain structures in patients with the irritable bowel syndrome [15, 16], as well as suppresses neuronal responses to nociceptive colorectal distension (CRD) in experimental animals [17, 18]. The analogous effect was demonstrated under experimental abdominal pain in dogs and rats by selective 5-HT3 blockers granisetron and ondansetron [19–22].

However, the neurophysiological mechanisms underlying the analgesic effect of 5-HT3 antagonists as well as the extent of their involvement in its realization in the central (specifically bulbar) structures remain largely unclear. Meanwhile, these data might promote deeper insight into the supraspinal serotonin-dependent processes, which control abdominal nociception, and thereby stir up the elaboration of more effective methods to manage visceral pain syndromes.

That is why the principal aim of our neurophysiological experiments was to study the effects of 5-HT3 receptor blockade by intravenous injection or local application of their selective antagonist granisetron on the background spike activity of neurons in the CVLM and their responses to nociceptive CRD.

MATERIALS AND METHODS

The experiments were conducted on 75 Wistar male rats with a body weight of 280–340 g in compliance with the requirements of the Committee for control on the maintenance and use of experimental animals at Pavlov Institute of Physiology, Russian Academy of Sciences. The main methods used in the study were as described previously [23]. Rats were anesthetized by intraperitoneal injection of urethane (ICN, USA; 1.5 g/kg). Following catheterization of the femoral vessels and tracheostomy, animals were placed into a stereotaxic apparatus (Medicor, Hungary). To facilitate access to the surface of the medulla oblongata, the muscles attached to the occipital bone were partially removed. The soft atlanto-occipital membrane was then cut with the occipital bone and dura mater removed thereafter.

Depth of anesthesia was assessed during experiments by the level of systemic arterial pressure, which was monitored through a cannula introduced into the femoral artery and connected to a semiconductor sensor (PDP-300, Russia). In the event of destabilization of this parameter or its divergence beyond the range of 70–100 mm Hg, additional urethane was injected. The body temperature was controlled intrarectally and maintained within 37–38°C using a heating plate and water thermostat (U-10, Germany).

Nociceptive colorectal stimulation (=distension, CRD) was performed by a thin-walled rubber balloon, 8 cm long, introduced via the anal sphincter and inflated with air up to 80 mm Hg using a syringe pump (DSh-09, Visma-Planar, Belorussia). Neurons responding to CRD were searched for in the caudal part of the medulla at a distance of 0.5 to 1.5 mm from the area postrema towards the rostrum and 1.8 to 2.3 mm laterally

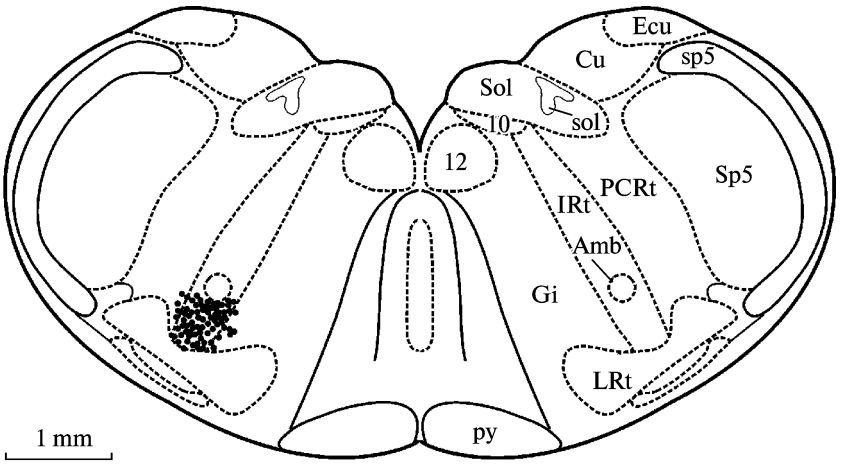

Fig. 1. Neuronal activity recording sites in the caudal ventrolateral reticular formation of the medulla oblongata at the level of 1.0 mm from the obex (area postrema) towards the rostrum. Adapted from the standard rat brain atlas [24]. Each point marks the localization of several neurons recorded. Designations: Amb—ambiguous nucleus, Cu—cuneate nucleus, Ecu—external cuneate nucleus, Gi—gigantocellular reticular nucleus, IRt—intermediate reticular nucleus, LRt—lateral reticular nucleus, PCRt—parvicellular reticular nucleus, py—pyramidal tract, Sol—solitary tract nucleus, sol—solitary tract, Sp5—spinal trigeminal nucleus, sp5—spinal trigeminal tract, 10—dorsal motor nucleus of vagus nerve, 12—hypoglossal nucleus.

to the midline, at a depth of 2.7 to 3.4 mm from the brain surface. Neuronal activity was recorded intracellularly using lacquer-coated tungsten microelectrodes with a tip diameter of 5 μm and resistance of 12 MΩ (Science Products GMBH, Germany).

Microelectrodes were moved vertically in the brain tissue with a pitch of 4 μm using an electron plunger (MP-2, Russia). Signal from the recording electrode following a requisite amplification (amplifier DAM 80, World Precision Instruments, USA) was fed into the PC sound card for digitalization at 44 kHz. Neuronal spike activity was visualized on the monitor screen, and the data were stored in the PC memory in a real time mode using an Audition 3 software program (Adobe Corp, USA). Recording was implemented within a 3-min interval: 1 min before, 1 min during and 1 min after CRD.

Experiments were conducted on two groups of rats. Animals in the first group were injected intravenously with granisetron (Sigma, USA), a selective blocker of 5-HT3 receptors, dissolved in 0.3 ml of physiological solution at doses of 1 mg/kg (n = 18) and 2 mg/kg (n = 11); the equivalent volume of physiological solution served as a control (n = 13). In the second group of rats, 0.3 μl of solution, containing 1.25 (n = 15) and 2.5 (n = 12) nmole of granisetron or the same volume of physiological solution (n = 8), were applied onto the dorsal surface of the medulla oblongata near the bottom of the fourth ventricle using a Hamilton syringe.

The background and CRD-evoked neuronal activities were recorded before the systemic injection or local application of the tested solutions, 5 min thereafter, and every 15 min (1st group) or 5 min (2nd group) later on during 60–90 min. After the cessation of each experiment, animals were euthanized by intravenous injection of a 3-fold dose of urethane with an electrolytic destruction of the brain via the recording electrode. The recording sites were localized on brain sections (40 μm thick) stained with toluidine blue using the rat brain atlas [24].

Further analysis, providing differential selection of neuronal spikes by their shape with separate processing of spike trains, was fulfilled using a Spike 2 software program (CED, UK). The mean rate of CRD-evoked firing during consecutive 1-min intervals before, in the course and after CRD, as well as changes in the first two parameters against the background of granisetron action, were estimated. Neurons were considered responsive to nociceptive stimulation if their firing rates changed during stimulation versus the background value no less

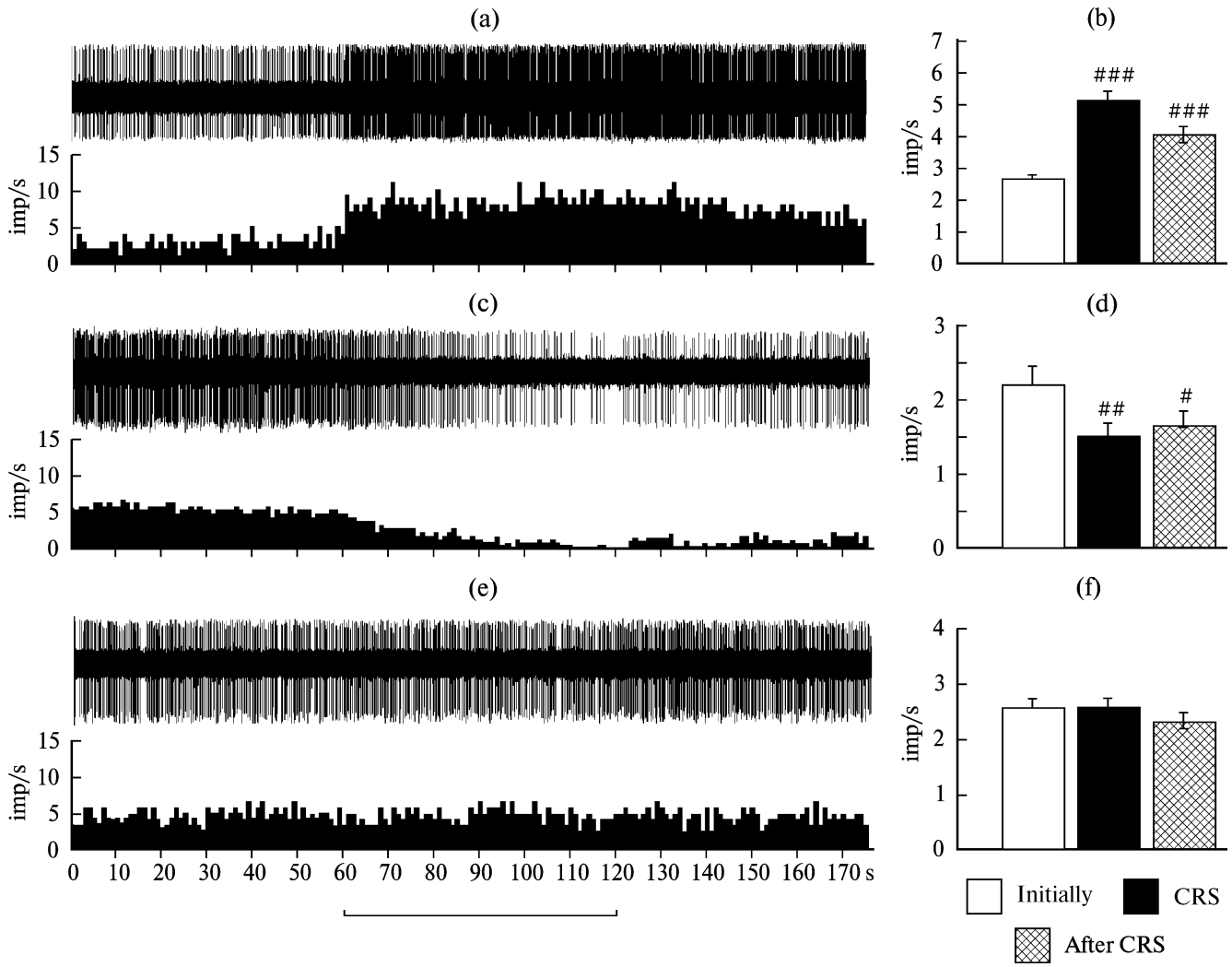

Fig. 2. Examples of recorded neuronal responses to nociceptive colorectal stimulation (*left part*), and diagrams showing respective changes in the mean rate of firing (*right part*) in neurons from the caudal ventrolateral medullary reticular formation attributed to the excited (a, b), inhibited (c, d) and indifferent (e, f) cell groups. In the upper part of each left fragment—native oscillograms, below—respective histograms. Histograms: *abscissa*: time, *ordinate*: rate of firing. *Long line*—colorectal stimulation (CRS). Diagrams: *ordinate*: mean rate of firing. Changes are significant versus respective initial values (#—$p < 0.05$, ##—$p < 0.01$ and ###—$p < 0.001$).

than by 20%. Graphic representation and statistical analysis of the results were carried out using Origin 7.5 (Original Corp, USA) and GraphPad InStat 3.02 (GraphPad Software Inc., USA) software programs using non-parametric methods for paired (Friedman and Wilcoxon tests) and unpaired (Kruskal–Wallis and Wilcoxon–Mann–Whitney tests) samples. The data are presented as mean ± SE.

RESULTS

During experiments, activity of 306 neurons located in the CVLM between the ambiguous and lateral reticular nuclei was detected (Fig. 1). All cells exhibited a stable background activity with a mean firing rate of 2.5 ± 0.1 imp/s (n = 306). By the character of the response to CRD, neurons were divided into three groups—excited, inhibited and indifferent. The mean rates of background neuronal firing in these groups did not differ significantly from each other (p = 0.12, KW = 4.29, Kruskal–Wallis test).

The cells of the first group made up 48% (n = 147) of the total number of the recorded neurons and were characterized by an increased firing ac-

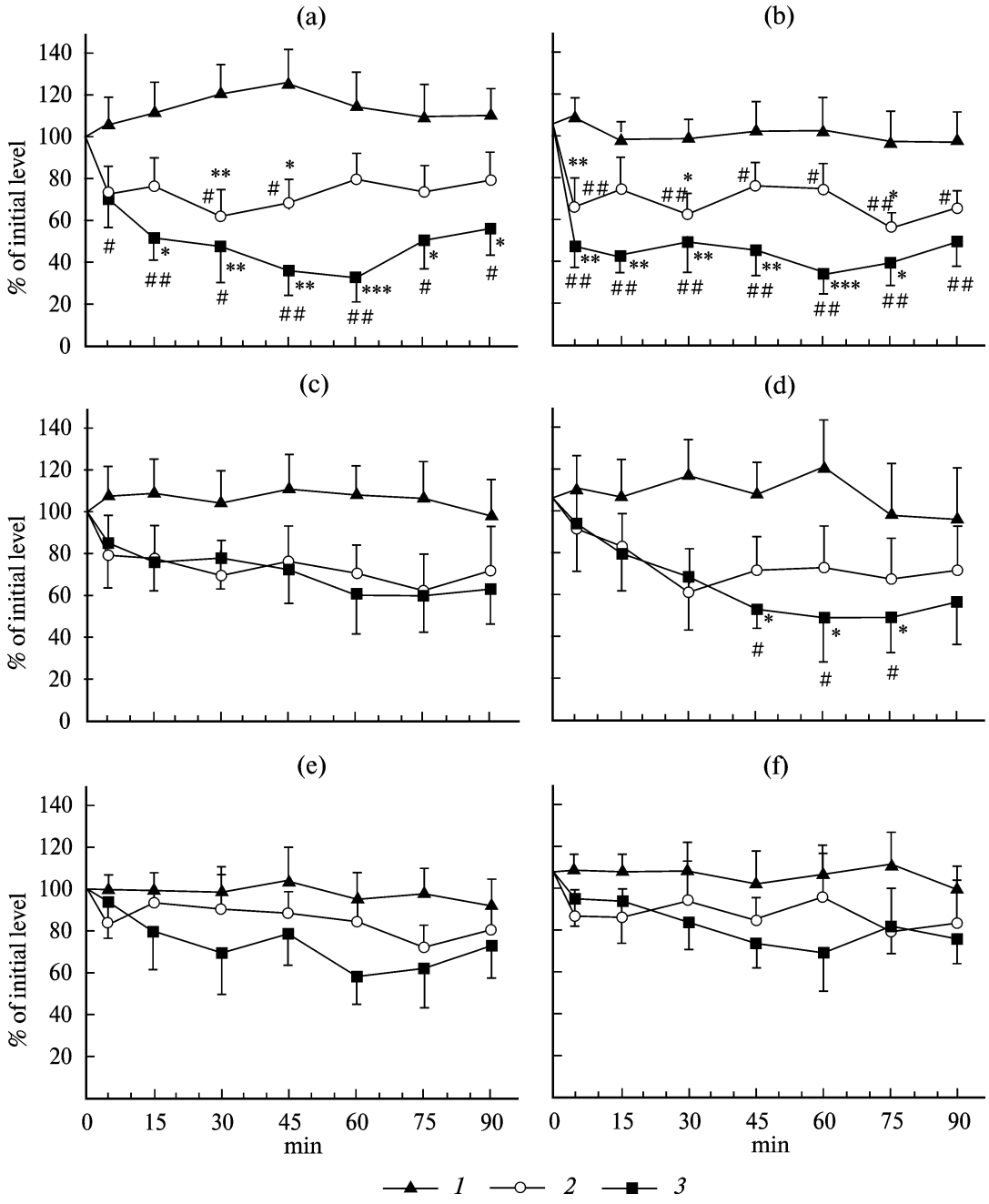

Fig. 3. Effect in intravenous injection of granisetron at doses of 1 and 2 mg/kg on background (*left column*) and colorectal distension evoked (*right column*) activity in neurons from the caudal ventrolateral medullary reticular formation attributed to the excited (a, b), inhibited (c, d) and indifferent (e, f) cell groups. *Abscissa*: time after granisetron injection, 0 min—initial values. *Ordinate*: mean rate of firing. Changes are significant versus respective initial values (#—$p < 0.05$, ##—$p < 0.01$, ###—$p < 0.001$) and control (*—$p < 0.05$, **—$p < 0.01$, ***—$p < 0.001$). *Vertical bars*—standard error of mean. For *left* and *right columns,* curves: (*1*) physiological solution, (*2*) granisetron, 1 mg/kg, (*3*) granisetron, 2 mg/kg.

tivity against the CRD background, which persisted for at least 1 min after the end of stimulation (Fig. 2). The mean firing rate of these neurons, being 2.6 ± 0.1 imp/s in the pre-stimulation period, increased during nociceptive CRD up to 5.1 ± 0.3 imp/s (221.6 ± 18.6% of the pre-stimulation value, $p < 0.0001$, Wilcoxon test, $n = 147$) and remained at the increased level of 4.0 ± 0.3 imp/s (153.6 ± 7.9 %, $p < 0.0001$, Fig. 2b) after the end of stimulation.

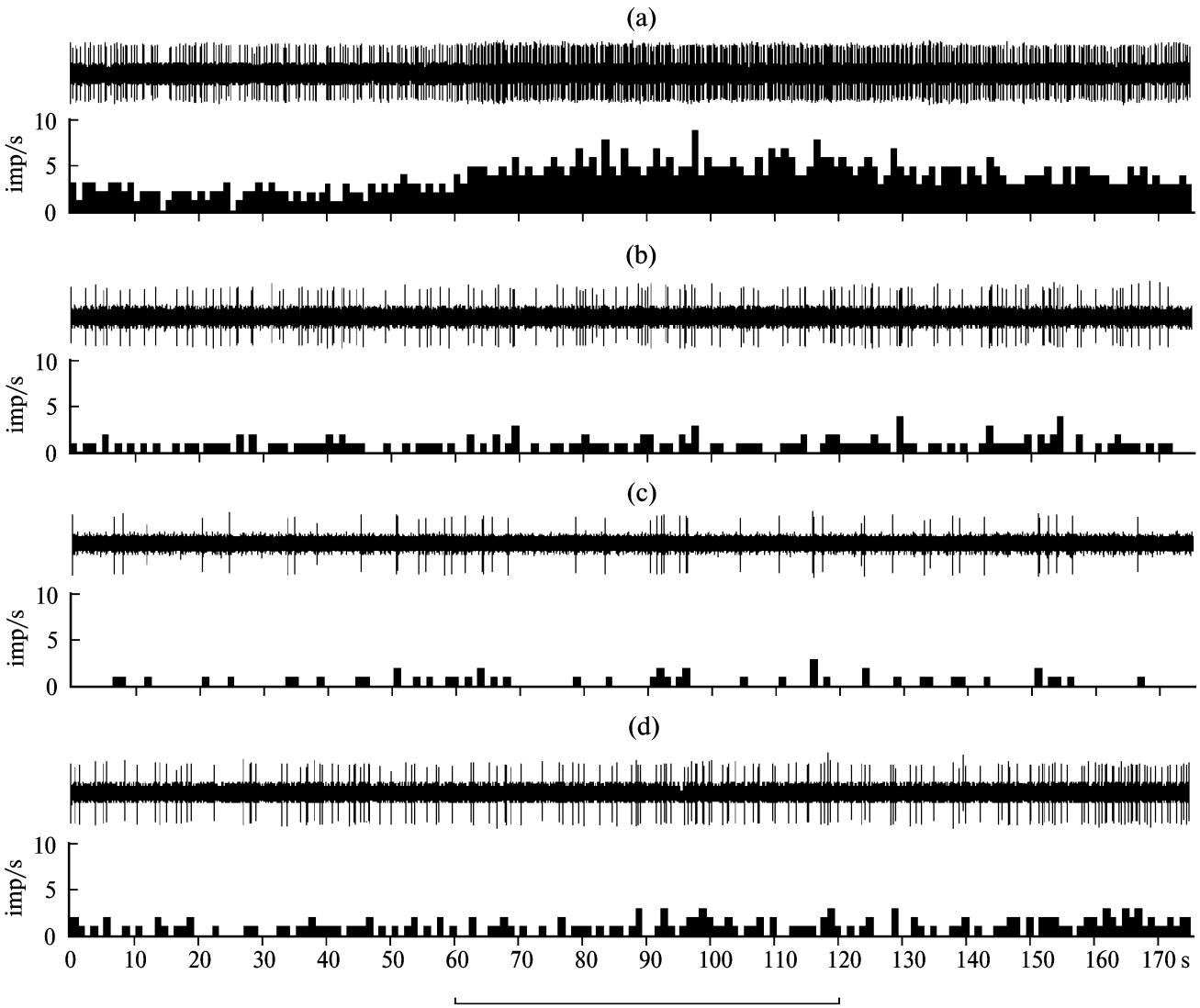

Fig. 4. Impulse activity of the excitable neuron in the caudal ventrolateral medullary reticular formation in response to colorectal stimulation before (a) and 15 (b), 60 (c) and 90 (d) min after intravenous injection of granisetron at a dose of 2 mg/kg. In the upper part of each fragment—native oscillograms, below—respective histograms. *Abscissa*: time, *ordinate*: rate of firing. *Long line*—colorectal stimulation.

The second group of cells, making up 20% (n = 61) of the total number of neurons, demonstrated in response to CRD a decrease in the mean firing rate from 2.2 ± 0.2 imp/s to 1.5 ± 0.2 imp/s (68.3 ± 2.2% of the appropriate level before stimulation, p = 0.005, Wilcoxon test, n = 61), and continued to generate spikes at a reduced rate of 1.6 ± 0.2 imp/s (76.1 ± 4.1%, p = 0.02) even after the end of nociceptive CRD (Fig. 2d).

The group of indifferent neurons included 98 cells (32% of the total number of the recorded neurons) with activity that did not change significantly during and after CRD (Fig. 2f).

Effects of intravenous injection of granisetron. Intravevous inject of physiological solution did not cause significant changes in the rate of background and CRD-evoked firing in excited (n = 28), inhibited (n = 13) and indifferent (n = 29) neurons (Fig. 3). In turn, granisetron injection had a dose-dependent inhibitory effect on firing activity of the cells that demonstrated excitation in response to CRD.

At the same time, the inhibitory influence of granisetron at a dose of 1 mg/kg on background (p = 0.04, Fr = 14.73, n = 29, Friedman test) and CRD-evoked (p = 0.002, Fr = 22.44) firing in

this group was significant, although unstable. A significant decrease in the rate of background firing was detected only at the 30th and 45th min of the experiment, when its mean values were 62.2 ± 12.8 ($p = 0.013$, $n = 29$, Wilcoxon test) and 68.9 ± 10.7% ($p = 0.017$) of the initial level, respectively (Fig. 3a). At these time points, the effect of granisetron injection was also significant versus control ($p < 0.05$, Wilcoxon–Mann–Whitney test). At each time point, changes in CRD-evoked neuronal activity became significant relative to the initial values ($p < 0.05$, Wilcoxon test) as soon as 5 min after granisetron injection until the end of the experiment. However, a significant versus control decrease in the firing rate was observed only at the 5th, 30th and 75th min, when it dropped to 62.5 ± 12.4 ($p = 0.004$, U = 113, Wilcoxon–Mann–Whitney test), 59.5 ± 8.5 ($p = 0.013$, U = 129) and 52.8 ± 7.2 % ($p = 0.042$, U = 129), respectively (Fig. 3b).

An increased dose of granisetron (up to 2 mg/kg) led to a more pronounced and stable suppression of neuronal firing. As soon as 15 min after intravenous injection, the mean rates of background and CRD-evoked firing were reduced versus the initial values to 51.8 ± 10.6 ($p = 0.008$, $n = 18$, Wilcoxon test) and 40.3 ± 7.1% ($p = 0.002$), respectively (Figs. 3a, 3b, 4). By the end of the experiment (90th min), both parameters remained appreciably lower than their initial values, coming to 56.1 ± 12.8% ($p = 0.016$) and 46.9 + 11.4% ($p = 0.002$). A most clear-cut reduction versus control in the rate of background and CRD-evoked neuronal activity was detected 60 min after granisetron injection, when the appropriate values decreased to 32.8 ± 11.1 ($p = 0.0008$, U = 18, Wilcoxon–Mann–Whitney test) and 32.5 ± 9.4% ($p = 0.0002$, U = 25), respectively (Figs. 3a, 3b, 4). As shown by an intergroup comparison, at the 45th and 60th min of the experiment granisetron at a dose of 2 mg/kg was much more effective than at a dose of 1 mg/kg ($p < 0.05$, Wilcoxon–Mann–Whitney test).

In the group of neurons that responded to CRD by an inhibition of firing activity, intravenous injection of granisetron at a dose of 1 mg/kg exerted no significant influence on the rates of background ($p = 0.28$, Fr = 8.66, $n = 9$, Friedman test) and CRD-evoked firing ($p = 0.26$, Fr = 8.86), although there was a tendency towards reduction in these parameters (Figs. 3c, 3d). The lack of an appreciable effect on impulse firing in these cells was also observed after granisetron injection at a dose of 2 mg/kg ($p = 0.23$, Fr = 9.35, $n = 7$). However, the latter dose promoted a significant enhancement of the inhibitory neuronal response to nociceptive stimulation, which manifested itself 45 min after intravenous injection (Fig. 3d). Since this moment and until the 75th min of the experiment, the rate of CRD-evoked firing at each time point was significantly reduced versus the appropriate level before granisetron injection ($p < 0.05$, Wilcoxon test). Relative to control, this change was significant at the 45th, 60th and 75th min, when the indicated parameter was 50.1 ± 7.7 ($p = 0.019$, U = 11, Wilcoxon–Mann–Whitney test), 46.2 ± 18.9% ($p = 0.014$, U = 10) and 46.5 ± 15.5% ($p = 0.021$, U = 12) of its initial values.

Neurons that expressed no distinct responses to nociceptive CRD were indifferent to intravenous granisetron injection. Even following its injection at a dose of 2 mg/kg, despite some reduction in the firing rate, there were detected no significant changes versus the initial level neither in background ($p = 0.12$, Fr = 11.57, $n = 12$, Friedman test), nor CRD-evoked neuronal activity ($p = 0.07$, Fr = 12.93; Figs. 3e, 3f).

Effects of granisetron application onto the bottom of the fourth cerebral ventricle. The differential effect of granisetron on functionally dissimilar groups of bulbar neurons was also expressed upon its direct application onto the dorsal surface of the medulla oblongata. As in the experiments with intravenous injection of granisetron, the most pronounced inhibitory effect of its local application was revealed in the group of cells that were excited in response to nociceptive CRD. In doing this, the effect of granisetron retained its dose-dependent character.

For example, application of 1.25 nmole of granisetron onto the bottom of the fourth ventricle promoted some decrease in the rate of background ($p = 0.03$, Fr = 16.0, $n = 29$, Friedman test; Fig. 5a) and CRD-evoked firing ($p = 0.0018$, Fr = 26.35, Fig. 5b). However, these changes were insignificant versus control ($p > 0.05$, Wilcoxon–Mann–Whitney test). Nonetheless, 5 min after local application of granisetron at a dose of 2.5 nmole there was detected a significant, versus the initial

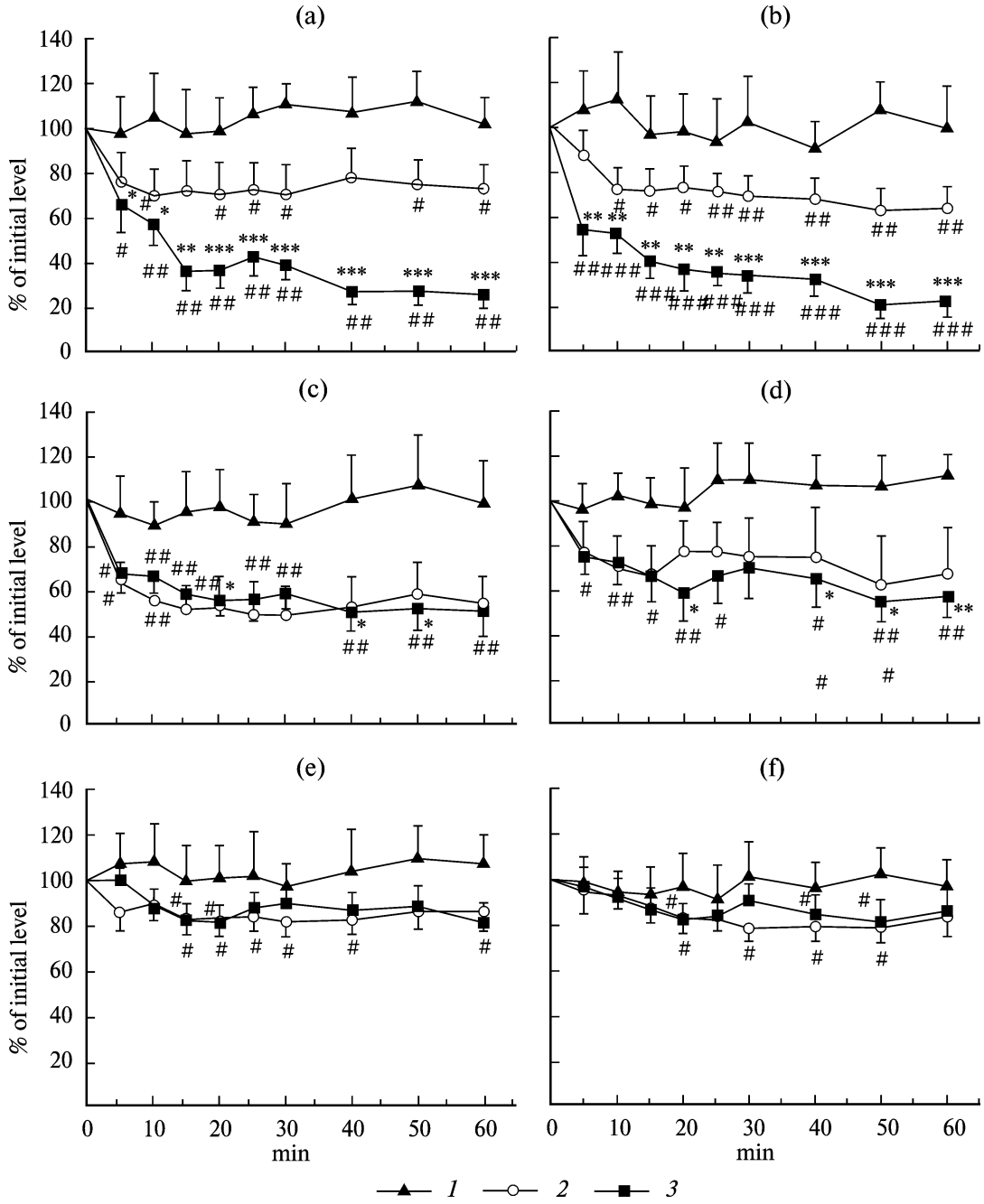

Fig. 5. Effect of granisetron application onto the bottom of the fourth ventricle at doses 1.25 and 2.5 nmole on background (*left column*) and colorectal distension evoked (*right column*) activity in neurons from the caudal ventrolateral medullary reticular formation attributed to the excited (a, b), inhibited (c, d) and indifferent (e, f) cell groups. *Abscissa*: time after application of granisetron, 0 min—initial values. *Ordinate*: mean rate of firing. For *left* and *right columns,* curves: (*1*) physiological solution, (*2*) granisetron, 1.25 nmole, (*3*) granisetron, 2.5 nmole. Other symbols as in Fig. 3.

level, decrease in the rates of background ($p = 0.02$, $n = 27$, Wilcoxon test) and CRD-evoked firing ($p = 0.002$), respectively, to 65.7 ± 12.6% and 54.4 + 11.5 % of the values recorded before the impact of 5-HT3 antagonist. The inhibitory effect increased gradually, attaining its maximum by the end of the experiment (60th min), when the analyzed parameters were on average 25.5 ± 5.9% ($p < 0.0001$) and 22.4 + 6.4% ($p < 0.0001$) of their initial values. From the 5th to 60th min

after granisetron application, changes it induced in background and CRD-evoked neuronal activities at each time point were significant versus control ($p < 0.05$, Wilcoxon–Mann–Whitney test; Figs. 5a, 5b).

The group of neurons, that responded to CRD by inhibition of the current impulse activity, after application onto the bottom of the fourth ventricle of granisetron at doses of 1.25 ($n = 12$) and 2.5 ($n = 11$) nmole also demonstrated a reduction in the rate of background and CRD-evoked firing ($p < 0.05$, Friedman test; Figs. 5c, 5d). However, this effect was dose-independent, and even after its application at a dose of 2.5 nmole it was less pronounced than in the group of cells that were excited upon CRD. At the same time, an appreciable (versus control) reduction in the rate of background firing was detected only at the 20th, 40th and 50th min of the experiment, when it decreased, respectively, to 54.4 ± 5.8 ($p = 0.04$, U = 8, Wilcoxon–Mann–Whitney test), 49.6 ± 8.1 ($p = 0.042$, U = 9) and 50.5 ± 10.9% ($p = 0.04$, U = 8) of its values before granisetron application (Fig. 5c). At the same time points, as well as at the 60th min, there was detected a significant (versus control) decrease in the rate of CRD-evoked firing, respectively, to 57.4 ± 10.9 ($p = 0.013$, U = 5, Wilcoxon–Mann–Whitney test), 64.3 ± 12.3 ($p = 0.04$, U = 8), 54.5 ± 9.5 ($p = 0.028$, U = 7) and 56.4 ± 9.7% ($p = 0.008$, U = 4) of the initial level (Fig. 5d).

In the group of CRD-indifferent bulbar neurons, local application of granisetron at doses of 1.25 ($n = 18$) and 2.5 ($n = 24$) nmole induced a little but statistically significant decrease in the rate of background and CRD-evoked firing ($p < 0.05$, Friedman test, Figs. 5e, 5f). However, these changes were dose-independent, being significant at none of the time points versus control ($p > 0.05$, Wilcoxon–Mann–Whitney test).

DISCUSSION

The data obtained in this study indicate that blockade of 5-HT3 receptors by granisetron exerts a differential effect on neurons in the CVLM, which differ in their responses to nociceptive CRD.

According to the character of the response to painful colorectal stimulation, we identified three types of such neurons—excited, inhibited and indifferent. This is in line with the data of other studies of the CVLM that demonstrated similar neuronal populations in this brain region [7, 10]. The groups of cells, that became excited or inhibited upon CRD, were found in the lumbosacral and thoracolumbar segments of the spinal cord [25], nucleus raphe magnus [26] and ventral dorsolateral nuceus of the thalamus [27].

Differences in responses to painful visceral stimulation allow suggesting that the detected neuronal groups refer to different functional systems. The CRD-excited cells in the CVLM appear to be directly related to the ascending pathways of visceral nociceptive signal conduction, representing the above-mentioned specific visceral nociceptive neurons. An indirect support for this assumption comes from the results of other studies, in which the functionally analogous neuronal populations in the medulla oblongata and medulla spinalis were found to exhibit an enhancement of impulse activity under colorectal inflammation, being also major recipients of influences from the endogenous inhibitory control structures as well as targets of the analgesic effect of opioids [8, 10, 25]. Some authors refer these neurons in the CVLM to on-cells in the rostral ventromedial medulla representing main components of the excitatory pronociceptive system [4, 10].

Neurons responding to visceral nociceptive stimulation with an inhibition of current impulse activity, probably, relate to the mechanisms of antinociception. Previously, it was established that the number of such cells at bulbar and spinal levels, as well as their response to colorectal stimulation, decrease considerably under experimental chronic pain characterized by an attenuation of the endogenous inhibitory control [10, 25]. This neuronal population is suggested to be functionally similar to inhibitory off-cells in the bulbar antinociceptive system [4, 10].

It its turn, the group of neurons in the CVLM, activity of which remains basically unchanged under nociceptive colorectal stimulation, is likely to provide regulation of visceral and motor functions that are irrelevant to colorectal nociception.

Our study has shown that functional activity of the first, excited, group of bulbar cells is largely

5-HT3-dependent. Intravenous injection of the selective agonist of 5-HT3 receptors, granisetron, exerts a dose-dependent inhibitory effect both on background and CRD-evoked firing of these cells, indicative of a general decline in neuronal excitability and/or reduced inflow of visceral nociceptive information.

Under systemic injection of granisetron, its antinociceptive effect may be equally mediated by peripheral and central mechanisms. Specifically, some contribution can be made by granisetron-induced blockade of 5-HT3 receptors located in pelvic and splanchnic colonic afferents, what has been established to reduce the sensitivity of these nerves to nociceptive stimuli and bring down their activating effect on appropriate spinal structures [28, 29]. However, there is evidence that the response of colonic afferents to mechanical stimulation, used in our study, is 5-HT3-independent [29], suggesting that central mechanisms play a leading role in the realization of the above-demonstrated effect of granisetron.

Particularly, the decrease in background and CRD-evoked activity of bulbar reticular neurons, observed in our experiments after intravenous granisetron injection, may result from the inhibitory effect of the agent on 5-HT3-dependent activity of central terminals of primary spinal afferents, which conduct sensory information from the colon (large intestine), as well as on associated excitatory inter- and projection neurons in the dorsal horn [30, 31]. This effect can be expected to reduce visceral nociceptive flow ascending to the medulla oblongata. In fact, the antinociceptive effect of granisetron at the spinal level is corroborated by the data on the suppression of behavioral response to colorectal stimulation after intrathecal injection of the agent to the thoracolumbar segment in rats [20]. Indirectly, it is also supported by the findings on the inhibitory influence of systemic blockade of 5-HT3 receptors on functional activity of neurons in the lumbosacral segment of the spinal cord under mechanical nociceptive stimulation of the colon [17].

Besides, the inhibitory effect of granisetron on neurons of the CVLM may result from immediate blockade of 5-HT3 receptors found in numbers in this medullary zone [32]. There is no evidence of local functions of these receptors, however, their general excitatory role [14] suggests that 5-HT3 antagonism leads to a reduced functional activity, at least, of some bulbar reticular neurons and dampening their responses evoked by visceral nociception.

A substantial contribution to this process can also be made by granisetron-induced blockade of 5-HT3 receptors in the nucleus of the solitary tract, shown more than once to be implicated in processing nociceptive information flowing from the colon along the vagus nerve fibers and spinal tracts [22, 23, 33]. The nucleus of the solitary tract is known to exert a 5-HT3-dependent excitatory effect on neurons in the CVLM [34], while the own responsiveness of this nucleus to visceral nociceptive stimulation is also provided by 5-HT3 receptors [21, 22]. That is why it can be expected that the final result of the effect of granisetron on this formation is a reduced excitation of cells in the CVLM under colorectal stimulation. The possibility of direct involvement of the above-considered local bulbar mechanisms in the realization of the effects of granisetron is supported by our experiments with application of the agent onto the dorsal surface of the medulla oblongata, which showed a more pronounced and stable (than after intravenous injection) suppression of background and CRD-evoked activity of reticular neurons.

We also cannot rule out the involvement in the sphere of granisetron activity of the upstream medullary structures, for example, the nuclei in the rostral ventromedial medulla expressing 5-HT3 receptors [32]. Serotoninergic on-cells of the endogenous pronociceptive system, located in these formations, may be a target for the inhibitory effect of the 5-HT3 antagonist. This would attenuate their activating influence on downstream visceral nociceptive neurons in the medulla spinalis and medulla oblongata [35], promoting thereby a decline in the excitability level of the latter.

Our study has shown that functional activity of the second, CRD-inhibited, group of neurons in the CVLM is to a great extent 5-HT3-independent. Intravenous injection of granisetron induced no considerable changes versus control in the rate of background firing of these cells, although a tendency towards its reduction was observed. This tendency showed up as statistically significant in experiments with application of the tested 5-HT3

antagonist onto the medullary surface, although in this case it was small and independent of a granisetron dosage. Overall this suggests that the reduction in impulse activity of this neuronal group is a secondary, nonspecific manifestation of the inhibitory effect of granisetron on other, positively interacting with the cells under consideration, populations of bulbar neurons, for example, those located in the nucleus of the solitary tract [34].

In turn, after intravenous injection of 2 mg/kg granisetron, the inhibitory response of neurons in the CVLM to visceral nociceptive stimulation gradually increased in our experiments, becoming statistically significant versus control by the 45th min. Apparently, the development of this process did not involve the bulbar 5-HT3-dependent mechanisms, since after local granisetron application onto the medullary surface no temporally similar changes in the inhibitory neuronal responses were detected. The effect may result from a progressive enhancement of the neuronal impact on this group of reticular cells from the upstream structures of the endogenous inhibitory control system, for example, the hypothalamic paraventricular nucleus [10] developing against the background of granisetron-reduced inflow of 5-HT3-dependent excitatory influences.

Finally, the group of CRD-indifferent neurons in the CVLM expressed no significant responses versus control to intravenous injection of granisetron, being actually nonresponsive to application of the agent onto the bottom of the fourth ventricle. In general, this may indicate a 5-HT3-independent character of impulse activity regulation in these cells.

CONCLUSION

The CVLM contains two functionally dissimilar neuronal populations, which exhibit, respectively, enhancement or inhibition of their impulse activity in response to colorectal nociceptive stimulation. The first group of cells may be involved directly in ascending visceral nociceptive signaling, whereas the second group is supposed to provide the endogenous antinociceptive mechanisms. Granisetron-induced selective blockade of 5-HT3 receptors, which acts, at least partially, via the local bulbar mechanisms, inhibits CDR-excited neurons but has no immediate effect on cells inhibited by nociceptive stimulation. Overall this indicates a specific activating role of 5-HT3 receptors within the bulbar visceral nociceptive systems. A shutdown of this mechanism may represent one of the ways to actualize the analgesic effect of 5-HT3 antagonists under abdominal pain. To elucidate specific neurophysiological and neurochemical processes that provide this function of 5-HT3 receptors, special studies needed.

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