PHARMACOLOGY

A Different Mechanism of Inhibition by Buspirone and Its Camphorimide Analogs of Serotonin 5-HT_3 Effects

N. A. Kharin, I. I. Abramets, I. V. Komissarov, and I. M. Samoilovich

UDC 615.357:577.175.823].015.4.07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 167-169, August, 1993
Original article submitted March 18, 1993

Key Words: *serotonin receptors; buspirone*

In contrast to classical, e.g., benzodiazepine, tranquilizers, buspirone is effective in not only neurotic [8] but also psychotic anxieties [7]. This feature and the apomorphine antagonism of buspirone detected in various tests [4], as well as the capacity of this drug to accelerate the metabolic turnover of dopamine in the brain [6,12], make it possible to regard buspirone as an atypical neuroleptic. On the molecular level its action is thought to be caused not so much by serotonin-1A (5-HT_{1A}) receptor activation as much as by blocking of the dopamine D_2 receptors, to which buspirone displays an affinity in nanomolar concentration (IC_{50}=235 nM) [12].

Recently reports appeared about a relationship between the antipsychotic effect of some agents and serotonin-3 (5-HT_3) receptor blocking. The antipsychotic action of ondansetron (GR 38032), tropisetron (ICS 205-930), granisetron (BRL 43694 A), blocking 5-HT_3 receptors [10,11], and the appurtenance to the 5-HT_3-receptor blockers of some known atypical neuroleptics, e.g., clozapine [9], support these data.

The present research demonstrates that buspirone and its structural analogs (ipsapirone, campirone, piricapirone) suppress 5-HT_3 serotonin effects without necessarily being nerve cell 5-HT_3-receptor blockers.

MATERIALS AND METHODS

Experiments were carried out on isolated dorsal (sensory) radicular ganglia of adult white rats weighing 200±20 g. The membrane potential and individual neuronal membrane resistance were recorded by the standard microelectrode technique, as described previously [1]. Intracellular leading off was performed with glass microelectrodes filled with a solution containing 2.5 M KCl and 0.5 M CsCl. Serotonin-creatinine sulfate (Reanal, Hungary) in a concentration of 10 μmol was delivered to neurons with a micropipette under pressure in a dose of 0.025 ml, the micropipette tip being placed near the recording electrode.

Rat sensory ganglion neurons usually respond to serotonin application by hyperpolarization or by depolarization associated with increased input membrane resistance, caused by activation of, respectively, 5-HT_{1A} and 5-HT_2 receptors and by s change in the K^+ conductivity of membranes [2]. To suppress these K^+-dependent responses Cs^+ (1-2 nA; 1.5-5 min) was intracellularly injected. Suppression of delayed rectification during the passage of output current rectangular pulses through the

Department of General and Clinical Pharmacology, Donetsk Medical Institute. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0008-0963$12.50

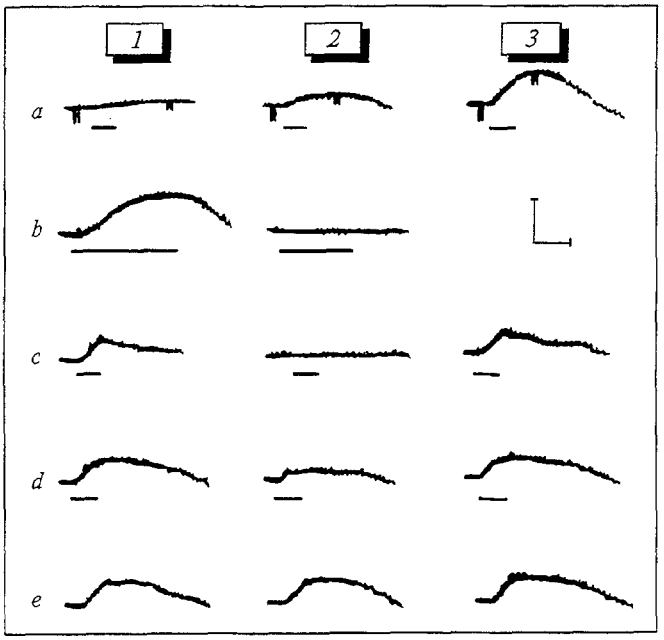

Fig. 1. Serotonin-induced responses of rat spinal ganglion neurons and effects of serotonin receptor blockers on these responses. *a*) reactions induced by serotonin in a concentration of 10 nM (*1*), 100 nM (*2*), and 1 μM (*3*); *b*) response to prolonged serotonin exposure (*1*) and 5-methoxytryptamine in a concentration of 10 μM (*2*); (*c–e*) changes in neuronal responses induced by serotonin in the presence of 50 nM tropisetron (*c*), 10 μM methoclopramide (*d*), and 1 μM methysergide (*e*). *1*) initial responses; *2*) the same at the 10th min of exposure to blockers; *3*) 40 min after start of washing. Calibration: 5 mV; 1 min.

membrane and of serotonin-induced hyperpolarizational and depolarizational (with increased input resistance) neuronal responses served as the criteria of complete blocking of neuronal membrane potassium conductivity by cesium ions.

Under such conditions the effects of buspirone, ipsapirone (Troponwerke, Germany), and their camphorimide analogs campirone and piricapirone on serotonin-induced reactions caused by 5-HT_3-receptor activation were studied. Each drug was tested in three concentrations in the range from 10^{-7} to 10^{-5} M. Serotonin-induced 5-HT_3 effects before and after 15 min superfusion of the ganglion with a salt solution containing the test agent were compared and its minimal (IC_{16}) and mean (IC_{50}) effective concentrations were graphed. For analysis of serotonin-induced 5-HT_3 neuronal responses and the effects of the test agents the following were used: 5-methoxytryptamine (methoxamine, Research Chemical and Pharmaceutical Institute), ICS 205-930 5-HT_3-receptor blockers (Research Biochemicals, USA), and methoclopramide (cerucal, Germany) in respective concentrations of 50 nM and 10 μM, methysergide, a 5-HT_2-receptor blocker (Sandoz, Switzerland) in a dose of 1 μM, and butamide (a proteinkinase A inhibitor of Russian manufacture) in a concentration of 100 μM.

RESULTS

After addition to the cells of cesium ions, suppressing K^+-dependent responses mediated by 5-HT_{1A} and 5-HT_2 receptors, about one half of the sensory ganglia neurons react to serotonin microapplication by depolarization, accompanied by a drop of membrane resistance (Fig. 1, *a*, *1-3*). If serotonin microapplications are repeated at 3-5-min intervals or are prolonged, the amplitude of the depolarization potentials evoked by them remains constant (Fig. 1, *b*, *1*), this pointing to the absence of receptor desensitization. Neurons responding by depolarization to serotonin microapplication do not react to 5-methoxytryptamine, a 5-HT_4-receptor activator, in a dose of 10 μM (Fig. 1, *b*, *2*). Serotonin-evoked depolarization responses of neurons are not changed by 15 min superfusion of the ganglion with a salt solution containing methysergide, a 5-HT_2-receptor blocker (Fig. 1, *e*, *2*), but the amplitude of these responses noticeably decreases in the presence of 10 μM methoclopramide (Fig. 1, *d*, *2*), whereas tropisetron, a more potent 5-HT_3-receptor blocker, in a dose of 0.05 μM prevents the depolarization effect of serotonin (Fig. 1, *c*, *2*). These data indicate that serotonin-induced depolarization under conditions of suppressed K^+ conductivity of sensory ganglia neuronal membranes, which goes along with a drop of membrane resistance, is caused by 5-HT_3-receptor activation.

Serotonin-induced 5-HT_3-receptor effects are either reduced or not reproduced at all if the gan-

TABLE 1. Concentrations of Buspirone and Its Structural Analogs Suppressing 5-HT_3 Responses of Sensory Ganglion Neurons ($M \pm m$)

Agent	Number of neurons	Concentration (in μM) reducing serotonin 5-HT_3 effects	
		IC_{16}	IC_{50}
Buspirone	10	5	6±1.0
Ipsapirone	7	6	8±0.9
Campirone	8	1	5±0.6
Piricapirone	14	0.1	2±0.2

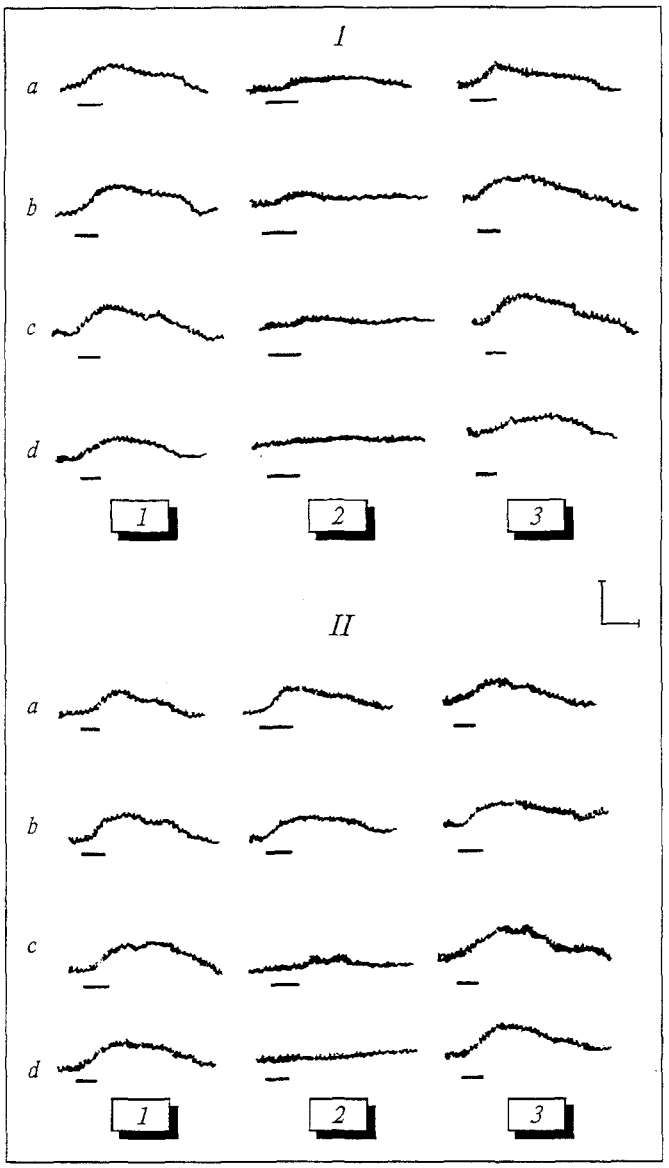

Fig. 2. Effects of buspirone and its structural analogs on serotonin-induced neuronal responses (*I*) and changes in these effects after 10-min exposure to 100 μM butamide (*II*). *1*) neuronal responses to serotonin microapplication; *2*) the same after 15 min superfusion of the ganglion with salt solution containing 10^{-5} buspirone (*a*), ipsapirone (*b*), campirone (*c*), and piricapirone (*d*); *3*) responses to serotonin microapplication after 40 min washing. Calibration : 5 mV; 1 min.

glion is pre-superfused for 15 min with a salt solution containing buspirone or its structural analogs (Fig. 2). This antiserotonin effect of the agent is detected in micromolar concentrations; piricapirone is the most active analog (Table 1).

The capacity of buspirone and its analogs to resist serotonin 5-HT_3 effects may result from direct blocking of the 5-HT_3 receptors or be indirectly realized via activation of the 5-HT_{1A} receptors with the named agents. Buspirone activation of these receptors is known to reduce adenylate cyclase activity and the cAMP concentration in brain tissue [5]. Serotonin or ipsapirone (campirone) activation of the 5-HT_{1A} receptors of sensory ganglia neurons leads to the development of their hyperpolarization, which is also realized via a reduction of the cAMP intracellular level and cAMP-dependent proteinkinase A activity [2]. The role of this enzyme in the metabolic regulation of membrane receptor affinity is well known [3].

To elucidate the possibility of indirect buspirone reduction of 5-HT_3-receptor sensitivity, we carried out experiments in which proteinkinase A activity in sensory neurons was suppressed by preliminary superfusion of the ganglion with a solution containing 100 μM butamide. Butamide inhibition of proteinkinase A was found to rule out the possibility of buspirone and ipsapirone suppression of serotonin-induced neuronal 5-HT_3 reactions (Fig. 2, *II*). However, serotonin 5-HT_3 effect inhibition with campirone and piricapirone is reproducible during butamide suppression of proteinkinase A activity as well.

Hence, buspirone and its structural analogs inhibit serotonin-induced 5-HT_3 responses of sensory spinal neurons. Buspirone camphorimide analogs campirone and piricapirone suppress serotonin 5-HT_3 effects as a result of 5-HT_3-receptor blocking, but buspirone and ipsapirone modulate the affinity of these receptors to serotonin by activation of 5-HT_{1A} receptors, reduction of the intraneuronal cAMP concentration, and lowering of the cAMP-dependent proteinkinase A activity. The process of proteinkinase A activity reduction appears to be attended by decrease of the degree of 5-HT_3-receptor phosphorylation and by desensitization of the receptors. The ability of piricapirone and campirone to block the 5-HT_3 receptors is most likely due to the steric similarity of the camphorimide bicycle to the bicyclic structure of tropin, whose derivatives are such 5-HT_3-receptor blockers as MDL 72222 and ICS 205-930, as well as cocaine, long known to be a blocker of 5-HT_3 receptors previously classified as type M serotonin receptors.

REFERENCES

1. I. I. Abramets, I. V. komissarov, and I. M. Samoilovich, *Byull. Eksp. Biol. Med.*, **108**, № 8, 172-174 (1989).
2. I. I. Abramets, I. V. Komissarov, and I. M. Samoilovich, *Neirofiziologiya*, **20**, № 1, 86-93 (1989).
3. A. R. Akopyan, B. N. Veprintsev, and N. K. Chemeris, *Dokl. Akad. Nauk SSSR*, **259**, № 3, 753-756 (1981).
4. L. E. Allen, H. Fergusson, and R. H. Cox, *Arzneimittel-Forsch*, **24**, № 1, 917-922 (1974).
5. M. De Vivo and S. Maayani, *J. Pharmacol. Exp. Ther.*, **238**, № 1, 248-253 (1986).
6. R. W. Fuller and K. W. Perry, *Ibid.*, **248**, № 1, 50-56 (1989).

7. M. J.-B. Napoliello, *Brit. J. Clin. Pract.*, **40**, № 1, 71-73 (1986).
8. K. Rickels, K. Weisman, N. Norstad, *et al.*, *J. Clin. Psychiat.*, **43**, Sect. 2, 81-86 (1982).
9. A. W. Schmidt and S. J. Peroutka, *Molec. Pharmacol.*, **36**, № 4, 505-511 (1989).
10. M. D. Triclebank, *Trends Pharmacol. Sci.*, **10**, № 4, 127-129 (1989).
11. M. B. Tyers, *Drug. Des. Deliv.*, **7**, № 3, 175-181 (1991).
12. P. L. Wood, N. P. Nair, S. Lal, *et al.*, *Life Sci.*, **33**, № 3, 269-273 (1983).

Mechanism of the Antiarrhythmic Effect of Agonists and Antagonists of Opioid Receptors

L. N. Maslov, Yu. B. Lishmanov, and I. I. Shekeli

UDC 615.22+612.178.2+612.173

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 169-171, August, 1993
Original article submitted March 16, 1993

Key Words: *arrhythmia; opioid receptor ligands*

Infranodal arrhythmia is one of the most crucial problems in modern cardiology. Recent investigations have demonstrated the antiarrhythmic activity of enkephalins [4,6], but only one synthetic analog of enkephalins - dalargin - was used in these studies. We have not found any published data on the antiarrhythmic properties of other opioid peptides (OP). Moreover, there is a prevailing opinion that the endogenous OP are arrhythmogenic agents, because naloxone, an opioid receptor (OR) antagonist, has an antiarrhythmic activity [9,17].

It thus seemed interesting to study the antiarrhythmic effect of OR agonists and antagonists and to reveal its possible mechanism.

MATERIALS AND METHODS

Experiments were carried out on 508 male Wistar rats weighing 150-200 g under ether anesthesia.

Arrhythmia was simulated by i.v. epinephrine injection in a dose of 90 μg/kg [10]. The electrocardiogram was recorded in the standard lead II 5 min after injection.

OR ligands were administered 15 min before and 6 h after epinephrine injection. D-Ala2-Leu5-Arg6-enkephalin (dalargin, Research Institute of Experimental Cardiology of the Russian Academy of Medical Sciences), Des-leucyl5-Ala 2[β-4-nitrophenyl]-α-alaninamide-4 enkephalin (tetrapeptide, Research Institute of Experimental Cardiology), D-Ala2-D-Leu5-enkephalin (DADLE, Vector Scientific-Conglomerate), Leu-enkephalin (Serva, Germany), and D-Met2-Pro5-enkephalinamide (enkephalinamide, Institute for Drug Research, Hungary) were used. The peptides were dissolved in 0.9% NaCl ex tempore. We demonstrated previously [4] that dalargin in a dose of 0.1 mg/kg expresses a high antiarrhythmic activity, and therefore the OP were injected in a dose of 0.1 mg/kg. An antiarrhythmic dose of morphine was 1.5 mg/kg [10]. Naloxone was injected in a dose of 0.5 mg/kg, which produced a blocade of the μ-OR [12], and in a dose of 2 mg/kg, enough to block all types of OR and to prevent arrhythmia [9,12,17]. Control animals were treated with epinephrine and NaCl instead of opioids. To obtain a complete inhibition of prostaglandin synthesis [8], the animals were treated with indomethacin together with

Laboratory of Radionuclide Experimental Methods, Research Institute of Cardiology, Tomsk Scientific Center; Institute of Drug Research, Budapest, Hungary. (Presented by R. S. Karpov, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0008-0966$12.50