Inversion of the Response to Serotonin in Rats with Traumatic Shock

L. M. Kozhevnikova*, P. P. Avdonin, I. F. Sukhanova**, and P. V. Avdonin*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 145, No. 3, pp. 266-269, March, 2008
Original article submitted June 29, 2007

Normally serotonin reduced blood pressure. It was shown that in rats with traumatic shock its hypotensive effect was transformed into hypertensive one. *In vitro* serotonin exhibited a slight vasoconstrictor effect on isolated rat aorta, while 24 h after injury, the strength of aortic contractions in response to serotonin increased 2.2 times. Desensitization of glucocorticoid receptors caused by injection of high doses of dexamethasone (3 mg/kg) to rats for 5 days led to similar changes in serotonin effect. We hypothesized that inversion of the response to serotonin in shock was caused by increased activity and/or expression of vasoconstrictor serotonin receptors in blood vessels.

Key Words: *traumatic shock; vascular contractility; serotonin; serotonin receptors*

Serotonin (5-hydroxytryptamine; 5-HT) is a local hormone and neurotransmitter. Numerous 5-HT receptors are present in the CNS and peripheral tissues. Seven types of 5-HT receptors are described [11,12]. Except 5-HT3 receptors, 5-HT receptors of all types contain 7 transmembrane α-helix domains and are coupled to G proteins. 5-HT3 receptors form cationic channels. Interactions of 5-HT with 5-HT1 and 5-HT5 receptors leads to adenylate cyclase inhibition, while interaction of 5-HT with 5-HT4, 5-HT6, and 5-HT7 receptors results in its activation. The mechanism underlying the effect of 5-HT2 receptors is associated with activation of phospholipase C. Mammalian blood vessels contain 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2B, 5-HT4, and 5-HT7 receptors [7,11,15]. However, the role of these receptors in the regulation of vascular tone in health and their functioning in hypo- and hypertension are little studied. It was previously shown that one of the causes of hypotension in traumatic shock is sharp attenuation of the vascular contractile reaction to angiotensin II, endothelin 1, vasopressin, and norepinephrine [4,5].

We studied vascular reactions to 5-HT during the development of traumatic shock.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (200-250 g). Cannon model of traumatic shock was used. The reaction to 5-HT *in vivo* was evaluated by changes in the mean blood pressure (BPm) in rat femoral artery 3 and 24 h after injury in comparison with the initial status. Serotonin (Sigma-Aldrich) was injected intravenously in doses of 20, 60, and 100 nmol/kg with 30-min intervals 30 min before and 3 and 24 h after the injury. The force of contractions of thoracic aortic rings, isolated from control rats and traumatized rats 24 h after the injury was measured in an isometric mode in a thermostat at 37°C in Krebs—Henseleit solution aerated by carbogen (95/5 O_2/CO_2 mixture) [6]. Desensitization of glucocorticoid receptors was induced by treatment with dexamethasone in high doses (3 mg/kg intraperitoneally for 5 days). The

*Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences; **N. K. Koltsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow. ***Address for correspondence:*** pvavdonin@yandex.ru. P. V. Avdonin

0007-4888/08/1453-0298

reaction of isolated vessels to 5-HT, norepinephrine, and carbachol was studied.

The data were statistically processed using Student's *t* test. The differences between the values were considered significant at $p<0.05$.

RESULTS

Intravenous injection of 5-HT to intact animals (before injury) caused a short-term dose-dependent decrease in BPm (Fig. 1, *a*). The effect of the hormone in these doses was short-term: BPm returned to the initial level (138±5 mm Hg) 1 min after injection of 5-HT. Thirty minutes after injection of the maximum dose (100 nmol/kg), the injury was inflicted to animals, as a result of which BPm dropped to 40±1 mm Hg. By the 3rd hour, parameters of systemic hemodynamics in rats were more or less stabilized, which was seen from BPm elevation to 88±6 mm Hg. Injection of 5-HT 3 h after injury was associated with a pronounced dose-dependent elevation of BPm, in contrast to its reduction observed in animals before injury (Fig. 1, *a*). It is noteworthy that in about 30% animals, the effect of 5-HT in doses of 60 and 100 nmol/kg developed in 2 phases. The initial 5-HT-induced elevation of BPm and its normalization were followed by a short-term BPm drop. However, the hypotensive effect of 5-HT in animals of this group was less pronounced than in intact animals (DBPm was -16.0±1.7 *vs.* -37.3±3.1 mm Hg after injection of 60 nmol/kg 5-HT and -18.7±1.4 *vs.* -46.2±5.0 mm Hg after injection of 100 nmol/kg 5-HT).

One day after injury, BPm in survivors was 98±6 mm Hg. Intravenous injection of 5-HT in doses of 20, 60, and 100 nmol/kg increased BPm by 6.8±4.9, 20.2±6.3, and 37.2±5.4 mm Hg, respectively. The reaction to 5-HT was biphasic in 66% animals: the initial 5-HT-induced vasoconstrictor phase was followed by a hypotension phase. However, the second reduction of BPm after pressure increase was short, similarly as during the first hours of traumatic shock, and did not surpass 20-26% of BPm reduction in animals before the injury.

We hypothesized that inversion of the response to 5-HT in traumatic shock was caused by changes in vascular contractility. The pressor reaction of isolated vessels in response to 10^{-6} M 5-HT increased more than 2-fold in traumatic shock in comparison with the control (Fig. 1, *b*). Addition of 5-HT increased (by 184±29 mg) the strength of isometric contraction of the isolated aortic rings excised 24 h after injury, while in the control the strength of vascular contraction increased by only 83±14 mg ($p<0.001$). Changes in vascular contractility in response to 10^{-7} M norepinephrine were evaluated in the same experiments. The pressor reaction of vessels to norepinephrine decreased by 1.9 times (390±27 mg *vs.* 750±35 mg in the control; $p<0.01$), in contrast to reaction to 5-HT during shock. The endothelial function after stabilization of tonic contractions (when the curve representing tonic contractions in response to norepinephrine reached the plateau) was evaluated by adding carbachol (10^{-5} M), an acetylcholine analog not hydrolyzed by acetylcholine esterases, to vessel fragment. Carbachol-dependent relaxation of norepinephrine-precontracted aorta was 81% in the control and 79% in shock. After carbachol treatment, the duration of relaxation of vessels isolated from control and experimental rats was the same.

Intensification of aortic contractions in response to 5-HT after injury could be caused by elimination of its vasorelaxant effect. The presence of vasodilating 5-HT1A-like receptors in the endothelium was hypothesized [14]. Carbachol (10^{-5} M) caused rapid relaxation of control norepinephrine-precontracted (10^{-7} M) aortic rings (Fig. 2). By contrast, 10^{-5} 5-HT in the presence of norepinephrine not only failed to relax the vessels, but even caused additional contractions.

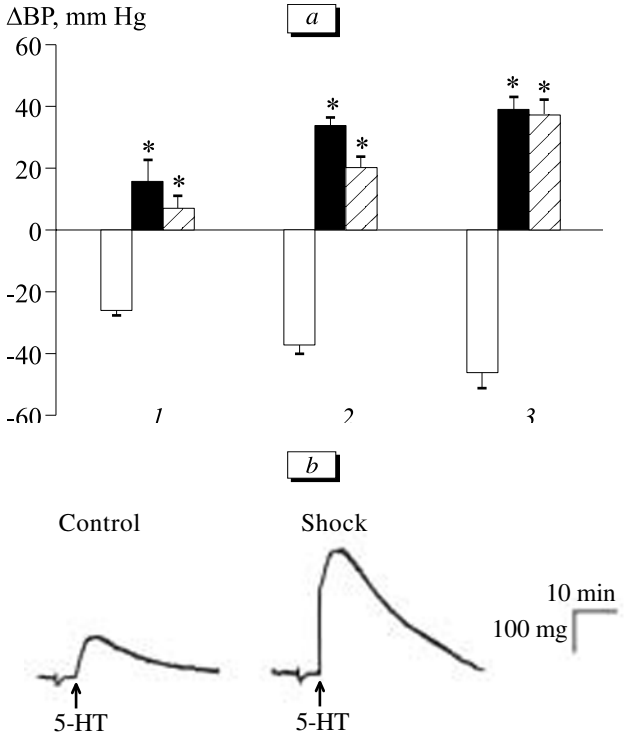

Fig. 1. Changes in the reactions of rat vessels to 5-HT in traumatic shock *in vivo* (*a*) and *in vitro* (*b*). *1*) 5-HT, 20 nmol/kg; *2*) 60 nmol/kg; *3*) 100 nmol/kg. Light bars: before injury; dark bars: 3 h after injury; cross-hatched bars: 24 h after injury. Arrow shows injection of 5-HT. **p*<0.001 compared to values before injury.

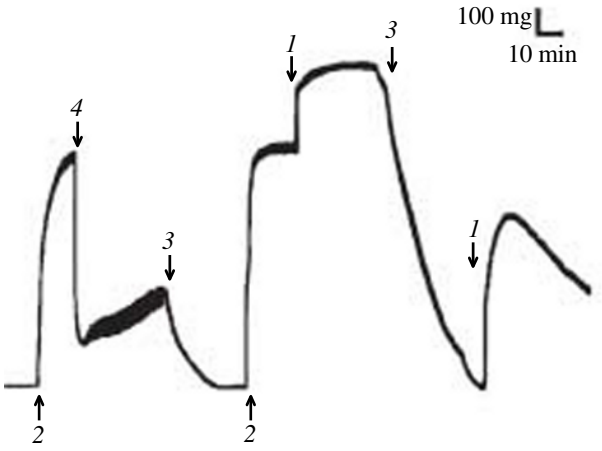

Fig. 2. Intensification of contraction of isolated aorta after addition of 10^{-5} M 5-HT (*1*) to the aorta, pre-contracted by 10^{-7} M norepinephrine (*2*) and repeatedly to relaxed aorta after its washing (*3*) from norepinephrine and 5-HT. *4*) 10^{-5} M carbachol.

It was found that activity of cytosol glucocorticoid receptors decreased in traumatic and hemorrhagic shock [1-3]. Moreover, artificial desensitization of glucocorticoid receptors induced in rats by prolonged high-dose dexamethasone treatment led to reduction of vascular contractility under the effects of endogenous vasoconstrictors (angiotensin II, vasopressin, and endothelin 1), similarly as in traumatic shock [4,5]. These results suggested that disorders in the neuroendocrine regulation of vascular contractility in shock were realized through the glucocorticoid-dependent mechanism. Evaluation of the possible relationship between inversion of the response to 5-HT and function of cytosol glucocorticoid receptors in our study showed that the reaction to 5-HT in rats treated with dexamethasone (3 mg/kg intraperitoneally for 5 days) changed similarly as in shock. The mean blood pressure in response to 5-HT in a dose of 60 nmol/kg increased by 27±4 mm Hg, while in the control it decreased by 41.0±0.6 mm Hg (Fig. 3). The reaction to 5-HT was biphasic, like in animals with traumatic shock. The second serotonin-induced phase of BPm reduction was short and less pronounced in comparison with the control (-16.2±5.4 and -41.0±0.6 mm Hg, respectively).

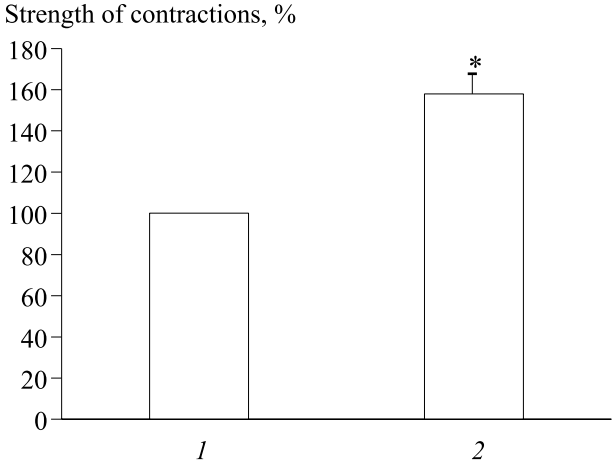

Fig. 3. Increase of the force of contractions of the isolated rat aortic rings *in vitro* in response to 5-HT in a dose of 60 nmol/kg after desensitization of glucocorticoid receptors. *1*) control; *2*) desensitization of glucocorticoid receptors. *$p<0.05$ compared to the control.

Addition of 10^{-6} M 5-HT *in vitro* led to intensification of isometric contractions of the isolated aorta, though not so strong as during shock (1.5 times in desensitization of glucocorticoid receptors and 2.2 times 24 h after injury; Fig. 3). *In vitro* studies showed that dexamethasone in a concentration of 10^{-5} M had no direct effect on contractility of control isolated rat aorta and did not modify the pressor reaction to 5-HT (10^{-6} and 10^{-5} M).

Importantly that the pressor reaction to norepinephrine did not change after desensitization of glucocorticoid receptors, in contrast to that in traumatic shock. Addition of 10^{-7} M norepinephrine to isolated aorta equally increased the strength of isometric contractions in the preparations from control (662±55 mg) and experimental (668±41 mg) rats. Long treatment with dexamethasone also did not lead to changes in the reactions of isolated vessels to carbachol. Addition of 10^{-5} carbachol caused a 75% relaxation of norepinephrine-precontracted (10^{-7} M) aorta.

Hence, the reaction to 5-HT is inverted in traumatic shock, this inversion being completely or largely due to changes in vascular contractility. Artificial desensitization of glucocorticoid receptors reproduced inversion of response to 5-HT *in vivo* and stimulated, though to a lesser degree than in shock, the contractile reaction of isolated aorta to 5-HT. The vasopressor reactions to angiotensin II, endothelin 1, vasopressin *in vivo* and vasoconstrictor reactions *in vitro* in traumatic shock and desensitization of the glucocorticoid receptors were significantly reduced.

Several types of vasoconstrictor and vasodilator 5-HT receptors are expressed in the blood vessels. Presumably, stimulation of vasoconstrictor reaction to 5-HT in traumatic shock is caused by activation of the working vasoconstrictor 5-HT receptors (5-HT2A, 5-HT1B/D) in the smooth muscle cells and by additional triggering of 5-HT receptors of another type (as a result of expression or transition from inert to active status). This phenomenon is observed in rats during hypertension, when pressure increase correlates with expression of 5-HT2B and 5-HT1B vasoconstrictor receptors in vascular

smooth muscle cells, in addition to 5-HT2A receptors [8,13]. Hyperexpression of 5-HT1B receptors in cerebral vessels leading to vasospasm takes place during ischemia in hemorrhagic stroke [10]. The increase in pressor effect of 5-HT in shock can be caused by reduced activity of relaxation receptors 5-HT1A or 5-HT2B receptors located in the endothelium, as a result of endothelial injury. However, we failed to obtain the data confirming this hypothesis. If vasodilator 5-HT receptors function in the endothelium, their effect is to manifest in a precontracted aorta similarly to the effect of carbachol through the muscarinic receptors. However, 5-HT caused no relaxation of norepinephrine-precontacted aorta in control preparations, but stimulated contractions. In addition, 24 h after injury, no endothelial damage was observed, which was seen from normal relaxation under the effect of carbachol (Fig. 2).

Previous data on reduced activity of glucocorticoid receptors in traumatic and hemorrhagic shock [1-3] suggest that disorders in the neuroendocrine regulation of vascular contractility in shock, specifically, inversion of the reaction to 5-HT *in vivo* and stimulation of its vasoconstrictor effect *in vitro*, are realized by the glucocorticoid-dependent mechanism. It is also possible, that activity of 5-HT receptors in normal functioning of glucocorticoid receptors is subjected to the negative control, while in shock this control is canceled. Since inversion of the response to 5-HT manifests from the first hours of shock, we hypothesized that this alteration could be also caused by the effects of glucocorticoid hormones realized by immediate nontranscription mechanisms. However, this hypothesis was not confirmed, because dexamethasone did not modify contractility of isolated rat aorta and vascular reaction to 5-HT. The nontranscription mechanism of vasopressor effect of 5-HT during the first hours of shock can consist in activation of silent 5-HT1-like receptors [9].

The study was supported by the Russian Foundation for Basic Research (grants No. 07-04-01443 and No. 05-04-49166) and a grant from Swiss National Research Foundation (SCOPES, IB 74AO-110940).

REFERENCES

1. P. P. Golikov, L. M. Kozhevnikova, Yu. V. Arkhipenko, and N. I. Nikolaeva, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 12, 23-29 (2001).
2. P. P. Golikov, L. M. Kozhevnikova, N. I. Nikolaeva, and Yu. V. Arkhipenko, *Vopr. Med. Khim.*, **48**, No. 5, 503-512 (2002).
3. P. P. Golikov, L. M. Kozhevnikova, N. I. Nikolaeva, *et al.*, *Ibid.*, **43**, No. 3, 139-147 (1997).
4. L. M. Kozhevnikova and P. V. Avdonin, *Byull. Eksp. Biol. Med.*, **141**, No. 5, 574-577 (2006).
5. L. M. Kozhevnikova, P. P. Avdonin, I. F. Sukhanova, and P. V. Avdonin, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 6, 3-8 (2007).
6. I. F. Sukhanova, L. M. Kozhevnikova, E. G. Popov, *et al.*, *Dokl. Rossiisk. Akad. Nauk*, *Biol. Sciences*, **411**, No. 5, 1-5 (2006).
7. A. K. Banes and S. W. Watts, *Hypertension*, **38**, No. 4, 891-895 (2001).
8. A. K. Banes and S. W. Watts, *Ibid.*, **39**, No. 2, Pt. 2, 394-398 (2002).
9. J. Chen, O. Yildiz, and R. E. Purdy, *J. Cardiovasc. Pharmacol.*, **35**, No. 6, 863-870 (2000).
10. J. Hansen-Schwartz, N. L. Hoel, C. B. Xu, *et al.*, *J. Neurosurg.*, **99**, No. 1, 115-120 (2003).
11. W. K. Kroeze, K. Kristiansen, and B. L. Roth, *Curr. Top. Med. Chem.*, **2**, No. 6, 507-528 (2002).
12. D. L. Nelson, *Curr. Drug Targets CNS Neurol. Disord.*, **3**, No. 1, 53-58 (2004).
13. A. Russel, A. Banes, H. Berlin, *et al.*, *J. Pharmacol. Exp. Ther.*, **303**, No. 1, 179-187 (2002).
14. P. R. Saxena and C. M. Villalon, *J. Cardiovasc. Pharmacol.*, **15**, Suppl. 7, S17-S34 (1990).
15. C. Ullmer, K. Schmuck, H. O. Kalkman, and H. Lubbert, *FEBS Lett.*, **370**, No. 3, 215-221 (1995).