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COMPARATIVE AND ONTOGENIC BIOCHEMISTRY

Different Effects of 5-HT_1 and 5-HT_2 Receptor Agonists on Excitability Modulation of Motoneurons in Frog Spinal Cord

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Abstract—Effects of 5-HT_1 and 5-HT_2 receptor agonists and antagonists on membrane properties of motoneurons in the isolated lumbar segment of the frog spinal cord were investigated using intracellular recordings. Application of a 5-$HT_{2A,B,C}$ receptor agonist α-Me-5-HT evoked depolarization of the motoneuronal membrane. The co-application of α-Me-5-HT and a specific 5-HT_{2B} receptor antagonist SB 206553 did not result in depolarization. α-Me-5-HT reduced the amplitude of medium afterhyperpolarization and increased the number of antidromic action potentials (APs). The application of an antagonist SB 206553 abolished these effects. A 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT had a time-dependent effect on the number of antidromic APs, evoking an initial short-term excitation followed by an inhibition. The data obtained in our experiments indicate the presence of 5-$HT_{1A/7}$ and 5-$HT_{2B,C}$ receptors on the postsynaptic membrane of motoneurons. We suggest a possible co-modulation of the accommodative properties of motoneurons by the two types of serotonin receptors, 5-$HT_{2B,C}$ and 5-HT_{1A}.

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Keywords: spinal cord, motoneuron, 5-HT, frog.

Abbreviations: RMP—resting membrane potential; AP—action potential; 5-HT—serotonin; fAHP—fast afterhyperpolarization; mAHP—medium afterhyperpolarization; α-Me-5-HT (α-Methyl-5-hydroxytryptamine maleate)—specific agonist of 5-$HT_{2A,2B,2C}$-receptors; 8-OH-DPAT(±)-(8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide)—agonist of 5-$HT_{1A/7}$-receptors; sumatriptan (3-[2-(Dimethylamino) ethyl]-N-methyl-1H-indole-5-methanesulfonamide succinate)—agonist of 5-$HT_{1B/D}$-receptors; ketanserin (3-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-2,4[1H,3H]-quinazolinedione tartrate)—selective antagonist of 5-HT_{2A}-receptors; SB 206553 (3,5-Dihydro-5-methyl-N-3-pyridinylbenzo[1,2-b:4,5-b']dipyrrole-1(2H)-carboxamide hydrochloride—selective antagonist of 5-$HT_{2B, 2C}$-receptors.

INTRODUCTION

Serotonin (5-HT) is one of the basic neurotrans-

mitters and neuromodulators in the CNS of vertebrates and invertebrates [1]. Most serotonergic neurons reside in the raphe nuclei and the brainstem reticular formation. Descending projections of serotonergic neurons to the spinal cord provide modulation of locomotion, sexual function and urination [2, 3]. According to the hypothesis proposed by Jacobs and Fornal [4], 5-HT facilitates motor input and suppresses sensory input to spinal cord neurons. This hypothesis is supported by numerous experimental data [5–8], however neural and molecular mechanisms of action of 5-HT are only partially elucidated.

5-HT affects neuronal activity via 14 genetically, pharmacologically and functionally different 5-HT-receptors referring to 7 families, 5-HT$_1$–5-HT$_7$ [9, 10]. Except one type (5-HT$_3$), which is a ligand-gated ion channel, all other 5-HT-receptors are metabotropic G protein-coupled receptors (GPCR). In the membrane of mammalian spinal motoneurons, only a few types of 5-HT-receptors are expressed: 5-HT$_{1A,B,D}$, 5-HT$_{2A,B,C}$ and 5-HT$_{5A}$ [3]. Data on expression of 5-HT-receptors in the spinal cord in other classes of vertebrates are so far incomplete. Some pre- and postsynaptic mechanisms of 5-HT-mediated modulation of neuronal activity have been described in the spinal cord of lower vertebrates, specifically in cyclostomes [1] and amphibians [12–16]. Recently, we have shown that 5-HT decreases the frequency of glycinergic miniature inhibitory postsynaptic potentials (mIPSP) while exerting no apparent influence on the frequency of GABAergic mIPSP [17]; this effect is mediated by activation of presynaptic 5-HT$_{1B,D}$-receptors, leading to a partial motor output facilitation [18].

Postsynaptic mechanisms of action of 5-HT were previously studied on motoneurons of mammals [19, 20], cyclostomes (lamprey) [21, 22], and reptiles (turtles) [3]. As a rule, 5-HT reduces frequency accommodation of neurons due to decreased amplitude of medium afterhyperpolarization (mAHP), therefore a motoneuron discharges longer and at a higher frequency [23]. Using a selective 5-HT$_{1A/7}$-receptor agonist 8-OH-DPAT, it was shown that the implementation of this effect on spinal motoneurons of turtle [3, 24], rat [19] and lamprey [25] is provided via activation of 5-HT$_{1A}$-receptors. Also shown was the role of 5-HT$_2$-receptors in facilitating persistent inward currents (PIC) in rat spinal motoneurons [26, 27] and calcium currents that promote sustaining plateau potentials in turtle spinal motoneurons [28]. No studies of the kind have yet been conducted on amphibian motoneurons.

The aim of this study was to analyze a modulatory effect of agonists and antagonists of type 1 and 2 serotonin receptors (5-HT$_{1,2}$) on membrane properties of frog spinal motoneurons.

MATERIALS AND METHODS

Experiments were carried out on a preparation of the isolated spinal cord of the frog *Rana ridibunda*. Dorsal laminectomy was executed under ether anesthesia. After the removal of meninges, the lumbar spinal cord segments IX and X were isolated together with their rootlets as frontal sections 2–3 mm thick. One of them was fixed in an experimental chamber with the rostral surface upwards, while the second was placed into physiological saline and left overnight at 4°C until use in the next-day experiment. The time lapsed between transection of descending serotonergic fibers and intracellular recording from motoneurons was 4–5 h on the first day and 26–30 h on the second one.

For superfusion, a solution of the following composition was used (mM): 100 NaCl, 2 KCl, 0.5 $MgCl_2$, 5.5 glucose, 1.5 $CaCl_2$, 9 $NaHCO_3$, 2 Tris, pH 7.4–7.6; it was aerated by a gas mixture (98% O_2 and 2% CO_2) and maintained at 16–18°C. The flow rate was 6 mL/min, the bath volume 0.5 mL. Motoneurons were identified by an antidromic action potential that arises upon stimulation of the ventral rootlet. The resting membrane potential (RMP) was controlled using a digital voltmeter.

Potentials were recorded intracellularly from the IX and X segmental motoneurons using sharp glass microelectrodes with a tip diameter of 1–1.5 μm filled with 3 M KCl and having a resistance of 5–10 MΩ. In part of experiment (when testing the effect of apamin, a blocker of small-conductance Ca^{2+}-activated potassium channels), microelectrodes were filled with a mixture of 3M KCl and 2M CsCl in equal proportions. Potentials were registered by a microelectrode

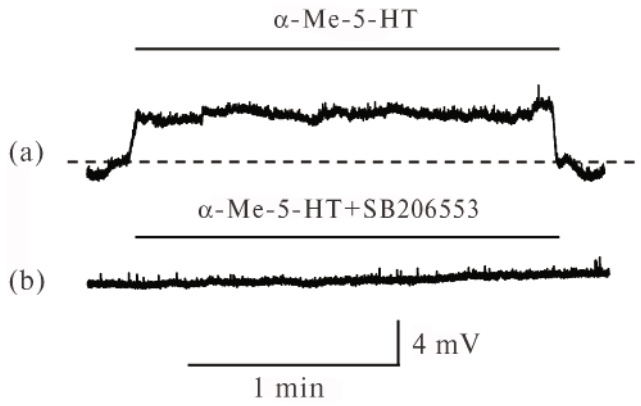

Fig. 1. Effect of 5-HT_2-receptor agonist α-Me-5-HT and antagonist SB 206553 on RMP. (a) α-Me-5-HT application (10 μM, 2 min) evokes depolarization of motoneuron (TTX block); (b) depolarization abolished by α-Me-5-HT and SB 206553 co-application (10 μM).

differential amplifier elaborated in our laboratory (B.T. Ryabov), digitalized at a frequency of 10–20 kHz using ADC USB-6211 (National Instruments, USA), and recorded on a computer using WinWCP (Strathclyde Electrophysiology Software, UK).

During experiments, we recorded the following parameters: RMP, input resistance of a neuron (as measured by a change of the membrane potential in response to an injected permanent current), AP amplitude and half-width, fast and medium afterhyperpolarizations of the antidromic AP.

A specific agonist of 5-$HT_{2A,2B,2C}$-receptors α-Me-5-HT (α-Methyl-5-hydroxytryptamine maleate) (10 μM), an agonist of 5-$HT_{1A/7}$-receptors 8-OH-DPAT (±)-8-Hydroxy-2-(di-n-propylamino)tetralin hydrobromide (10 μM), an agonist of 5-$HT_{1B/D}$-receptors sumatriptan (3-[2-(Dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulfonamide succinate) (10 μM), a selective antagonist of 5-HT_{2A}-receptors ketanserin (3-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl] ethyl]-2,4[1*H*,3*H*]-quinazolinedione tartrate) (10 μM), a selective antagonist of 5-$HT_{2B,2C}$-receptors SB 206553 (3,5-Dihydro-5-methyl-*N*-3-pyridinylbenzo[1,2-*b*:4,5-*b*']dipyrrole-1(2*H*)-carboxamide hydrochloride (10 μM), a blocker of Ca^{2+}-activated potassium channels apamin (100 nM) were added to the perfusion solution. The choice of agent concentrations was based on the literature data (Holohean and Hackman, 2004; Hsiao et al., 1997). The time for replacing a bath solution was about 40–60 s. All reagents were purchased from Sigma-Aldrich or Tocris Bioscience.

Mean values were compared using a paired Student's *t*-test. Data are presented as mean ± SEM. For statistical analysis and graph plotting, Sigma Plot 11.0 and MS Excel were used.

RESULTS

Lumbar motoneurons were identified by the presence of the antidromic AP generated upon stimulation of the ventral rootlets IX and X. In all neurons, the input resistance and characteristics of evoked antidromic APs were measured. A

A comparison of properties of motoneurons in control and in the presence of 5-$HT_{1,2}$-receptor agonists

	Control	α-Me-5-HT	8-OH-DPAT
Number of motoneurons (*n*)	14	7	5
Resting membrane potential, mV	−67.2 ± 1.6	−63.7 ± 2.7 *	−66.5 ± 1.2
Input resistance, MΩ	7.3 ± 0.7	8.8 ± 0.8 *	7.0 ± 0.6
Amplitude of antidromic AP, mV	75 ± 2	73 ± 5	75 ± 2
Half-width of antidromic AP, ms	0.79 ± 0.03	1.1 ± 0.2 *	0.83 ± 0.02
Fast afterhyperpolarization (fAHP), mV	−12.9 ± 1.2	−8.6 ± 1.5 *	−10.7 ± 1.3
Medium afterhyperpolarization (mAHP), mV	−3.0 ± 0.2	−1.7 ± 0.5 *	−2.5 ± 0.4
Number of evoked antidromic APs per second	1.0 ± 0.0	10.0 ± 3.0 *	2.5 ± 0.5 (E) 0.5 ± 0.5 (I)

*—Statistically significant difference ($p < 0.05$, paired *t*-test). (E)—Excitatory effect, (I)—inhibitory effect.

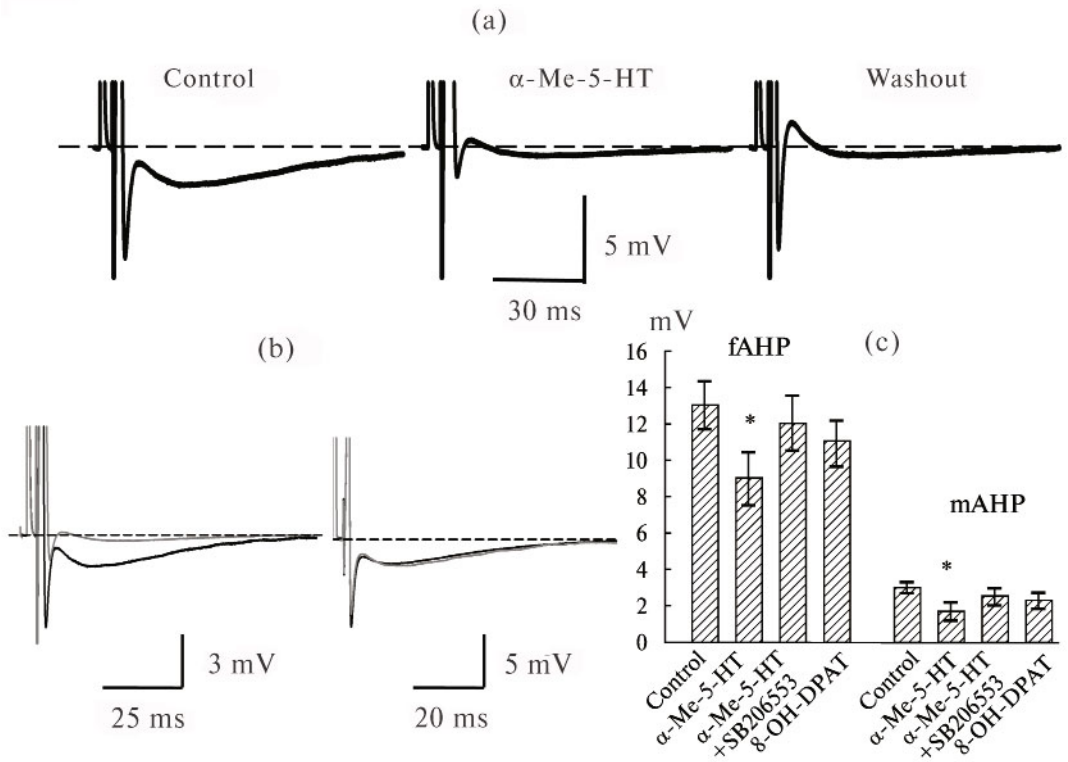

Fig. 2. Effect of 5-HT$_{1,2}$-receptor agonist and antagonist on fAHP and mAHP of antidromic AP. (a) From left to right: control, α-Me-5-HT application, washout. Application of 5-HT$_2$-receptor agonist α-Me-5-HT (10 μM) evokes reduction in fAHP and mAHP of antidromic AP, washout leads to mAHP recovery; (b) 5-HT$_2$-receptor antagonist SB 206553 (10 μM) (right) abolishes the agonist's effect (left). Control (black), agent (grey); (c) diagrams to illustrate a significant reduction in fAHP and mAHP amplitudes under the effect of 5-HT$_2$-receptor agonists and antagonist.

subsequent analysis included motoneurons with RMPs from –60 mV and above and the amplitude of antidromic APs of no less than 65 mV. Mean values of electrophysiological parameters of motoneurons in control and as affected by agonists of 5-HT$_{1,2}$-receptors are shown in Table.

Effect of a 5-HT$_{2A,B,C}$-receptor agonist α-Me-5-HT on electrophysiological properties of motoneurons. To reveal the effect of 5-HT$_2$-receptor activation on passive properties of the motoneuronal membrane, a 5-HT$_{2A,B,C}$-receptor agonist α-Me-5-HT (10 μM) was added to the perfusion solution against the background of spiking activity blocked by TTX (1 μM). α-Me-5-HT evoked membrane depolarization in lumber motoneurons by 0.7–3.4 mV (n = 5, paired t-test, $p < 0.05$, Fig. 1a, Table), while no depolarization developed after co-application of α-Me-5-HT and a specific 5-HT$_{2B,C}$-receptor antagonist SB 206553 (Fig. 1b) in all cells studied. This fact indicates that 5-HT$_{2B,C}$-receptors are expressed in the motoneuronal membrane. Upon application of α-Me-5-HT, the membrane input resistance increased by 20% (Table).

Effects of 5-HT$_2$-receptor activation on active properties of the motoneuronal membrane were studied on evoked antidromic APs. α-Me-5-HT application evoked an increase in the half-width of the antidromic AP as well as a decrease in the fAHP amplitude by 25–38% and about twofold in the mAHP amplitude (n = 7, $p < 0.05$, Table, Fig. 2). After washout, fAHP recovered up to the initial level, while mAHP did not (Fig. 2a).

A 5-HT$_{2A}$-receptor antagonist ketanserin (10 μM) and a selective 5-HT$_{2B,C}$-receptor antagonist SB 206553 (10 μM), when added to the perfusion solution in combination with an agonist α-Me-5-HT, abolished the effect of the latter (n = 5, Fig. 2b).

The addition of α-Me-5-HT likewise increased

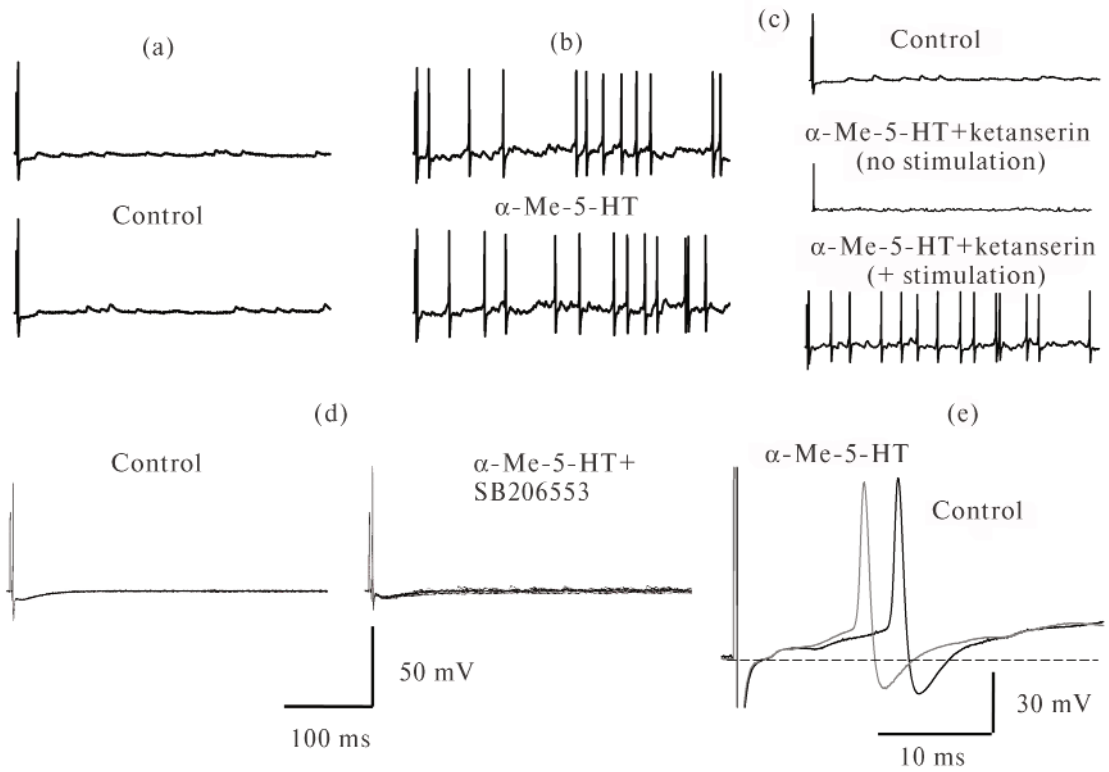

Fig. 3. Effect of 5-HT_2-receptor agonist α-Me-5-HT and antagonists ketanserin and SB 206553 on the number of antidromic APs. (a) Control (2 separate runs); (b) α-Me-5-HT application (10 μM) (2 separate runs); (c) top down: control, α-Me-5-HT and ketanserin co-application without stimulation and with stimulation (single runs); for (a)–(c): calibration 50 mV, scan 1 s; (d) control (left), α-Me-5-HT and SB 206553 co-application (10 μM), superimposition of 10 runs; (a)–(c) and (d)—two different motoneurons. Note the absence of repetitive discharges during α-Me-5-HT and SB 206553 co-application; (e) effect of α-Me-5-HT on the latency of the first AP evoked by stimulation of the dorsal rootlet.

the number of evoked antidromic APs by several times (Figs. 3a, 3b; AP were recorded for 1 s, $n = 5$). Upon co-application of α-Me-5-HT and a 5-HT_{2A}-receptor antagonist ketanserin, the same effect was achieved (Fig. 3c), whereas co-application of α-Me-5-HT and a 5-$HT_{2B,C}$-receptor antagonist SB 206553 led to no changes in the number of antidromic APs compared to control (Fig. 3d).

We failed to measure the latency for the antidromic AP because it was masked by the stimulus artifact, but we could do this for the AP evoked by the dorsal rootlet stimulation. The average latency for the AP evoked by the dorsal rootlet stimulation (counting from the stimulus artifact) in control was 25 ± 6 ms, with the minimum latency for the first spike of 12 ms and the maximum latency for the last spike of 67 ms. After 3 min of α-Me-5-HT application, the average latency was 18.6 ± 1.4 ms, the minimum latency for the first spike was 8 ms (Fig. 3e), and the maximum latency for the last spike was 29 ms. This finding may also indicate an increased excitability of the motoneuronal membrane.

Effect of 5-HT_1-receptor agonists on electrophysiological properties of motoneurons. In contrast to α-Me-5-HT, a 5-$HT_{1A/7}$-receptor agonist 8-OH-DPAT evoked no membrane depolarization and did not change the input resistance, however the mAHP amplitude decreased in three cells out of five (to a far lesser extent than after α-Me-5-HT application; Table, Fig. 4a). Apparently, even this insignificant decrement was sufficient enough to evoke an increase in the number of antidromic APs within the first minutes of 8-OH-DPAT application. However, the inhibitory effect developed later on, leading to a complete suppression of APs. A 5-$HT_{1B,D}$-receptor agonist sumatriptan ($n = 4$) had no detectable effect on the shape of afterhyperpolarization.

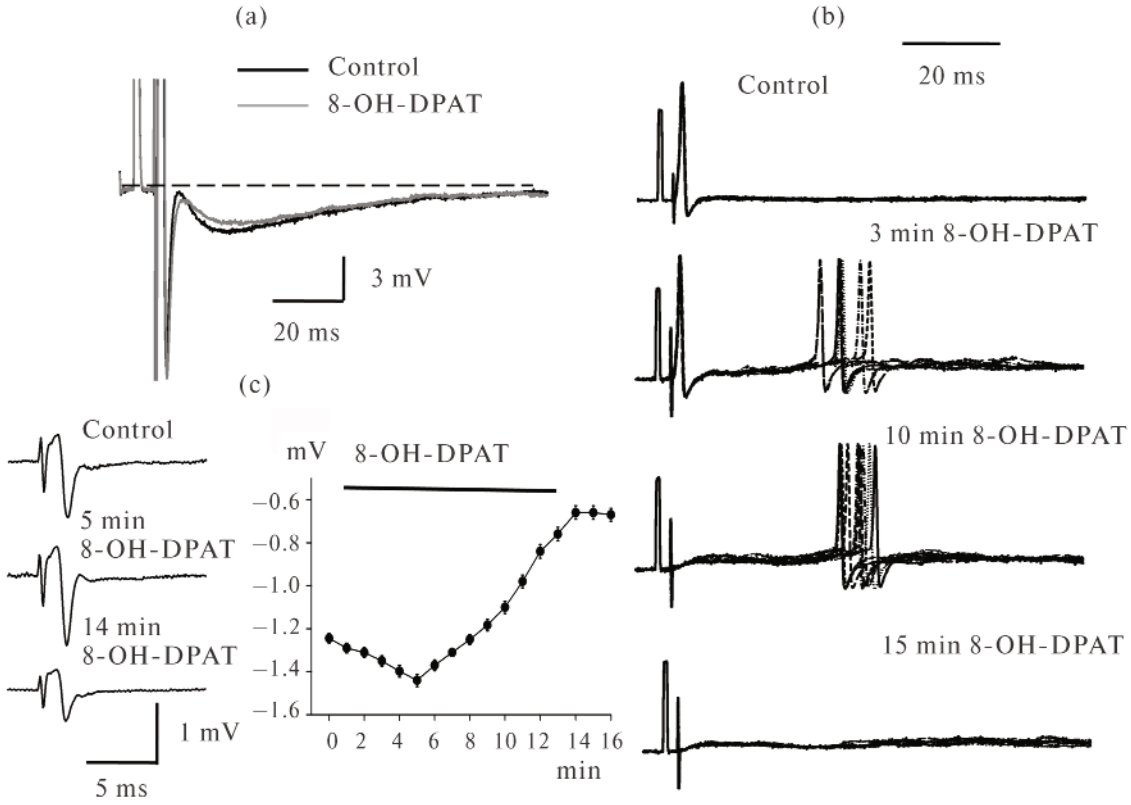

Fig. 4. Effect of 5-HT$_{1A,7}$-receptor agonist 8-OH-DPAT on electrophysiological parameters of motoneuron. (a) Effect on fAHP and mAHP amplitude of antidromic AP; (b) time-dependent effect of 8-OH-DPAT on antidromic AP frequency and generation time. Top down: control, 3, 10 and 15 min of 8-OH-DPAT application (10 μM), calibration 50 mV; (c) (left)—effect of 8-OH-DPAT on the field antidromic potential in control and after 5 and 14 min of application; (c) (right)—plot of the dependence between average amplitude of antidromic field potential (*y* axis) and agonist application time (*x* axis) for individual motoneuron.

At the same time, 8-OH-DPAT exerted a time-dependent effect on the number of spikes and the time of their generation: within the first 3–7 min of its application, there was observed an increase in the number of APs, while 10 min thereafter the latent period of the first evoked AP increased by 40–60 ms compared to control, and after 15 min APs completely disappeared (Fig. 4b). Thus, 8-OH-DPAT initially increased the excitability of motoneurons and then decreased it. An analogous time-dependent effect of 8-OH-DPAT application we observed when recording the antidromic field potential: within the first 3–5 min, the amplitude of the field potential increased and then, after 15 min, decreased (Fig. 4c).

Excitability of motoneurons is increased via modulation of Ca^{2+}-activated potassium channels. It is well known that mAHP is mediated by Ca^{2+}-activated potassium channels (SK-channels) that are blocked by apamin [22, 24, 29, 30]. A decrease in the mAHP amplitude may be due both to attenuation of the current flowing through SK-channels caused by their negative modulation and augmentation of cationic currents through calcium and potassium channels which mask mAHP and provide afterdepolarization. To reveal the mechanism of action of 5-HT receptor agonists on afterprocesses, we used a pharmacological approach. Apamin application (100 nM) reduced the mAHP amplitude and also revealed afterdepolarization. After apamin and α-Me-5-HT co-application, no additional effects were observed (Fig. 5). Thus, activation of 5-HT$_{2A,B,C}$-receptors by an agonist α-Me-5-HT led to attenuation of the current flowing through SK-channels.

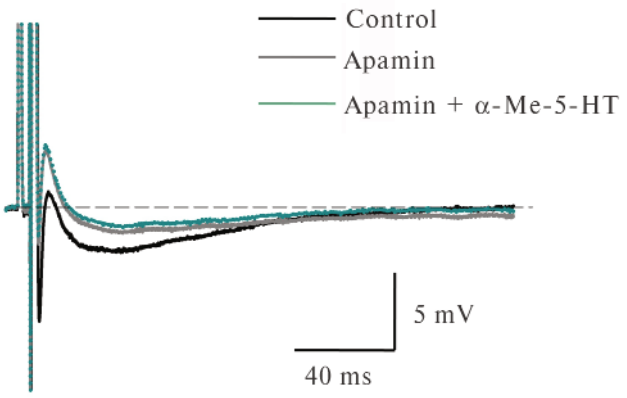

Fig. 5. Effect of Ca^{2+}-activated potassium channel blocker apamin on AHP (black—control, grey—apamin, green—apamin + α-Me-5-HT mixture). α-Me-5-HT has no effect during co-application with apamin.

DISCUSSION

Several types of serotonin receptors (5-$HT_{1A,B,D}$, 5-$HT_{2A,B,C}$ and 5-HT_{5A}) are expressed in the membrane of mammalian spinal motoneurons (Perrier et al., 2013). In our experiments, application of a 5-$HT_{2A,B,C}$-receptor agonist α-Me-5-HT evoked membrane depolarization which did not develop after co-application of α-Me-5-HT with a specific 5-$HT_{2B,C}$-receptor antagonist SB 206553. Here we also showed that α-Me-5-HT reduces the mAHP amplitude and increases the number of antidromic APs. This effect was abolished by application of an antagonist SB 206553. A 5-HT_{1A}-receptor agonist 8-OH-DPAT exerted a time-dependent effect on the number of antidromic APs, evoking initially a short-time excitation later followed by an inhibition. Thus, our electrophysiological and pharmacological data indicate the presence of 5-HT_{1A}- and 5-$HT_{2B,C}$-receptors on the postsynaptic membrane of frog spinal motoneurons.

One of the well-known mechanisms of action of 5-HT is a reduction in the mAHP amplitude mediated via Ca^{2+}-activated apamin-sensitive potassium channels (SK-channels) [24, 30]. Pharmacological tests demonstrated that 5-HT, when added to the extracellular medium, inhibited mAHP in spinal motoneurons [24], hypoglossal motoneurons [19], trigeminal motoneurons [20], and jaw-closing motoneurons [31]. mAHP plays an important role in establishing the spike frequency in motoneurons due to delaying the generation of the following AP. Thus, mAHP modulation is an evident target for the fine adjustment of monto-neuronal activity. Perrier and Delgado-Lezama reported that 5-HT, which is released synaptically from the raphe nuclei, also inhibits motoneuronal mAHP [32]. As a result, the spike frequency notably increases. The outcomes of most relevant studies carried out to date are consistent with the fact that this effect of 5-HT is underlain by activation of 5-HT_{1A}-receptors [19, 24, 25].

Our results well agree with the experiments conducted on guinea pig motoneurons [20] in which it was shown that the addition of 10 μM 5-HT to a bath reduces the maximum mAHP amplitude by 51% due to decreased Ca^{2+}-activated potassium current underlying mAHP. In our experiments, the mAHP amplitude was reduced by a 5-$HT_{2A,B,C}$-receptor agonist α-Me-5-HT but not a 5-HT_{1A}-receptor agonist 8-OH-DPAT, as was the case in spinal motoneurons of turtle [3, 24], rat [19] and lamprey [25]. In a lamprey study, application of a 5-HT_2-receptor agonist α-CH_3-5-HT likewise reduced the mAHP amplitude, with this effect abolished by spiperone, an antagonist of 5-HT_{2A}- and D_2 type dopamine receptors, but not ketanserin, a specific 5-HT_{2A}-receptor antagonist of [25]. Probably, this difference indicates that the types of 5-HT receptors involved in the modulation of motoneuronal membrane properties may vary in different animal species. It is also reasonable to assume that following a transection of serotonergic fibers coming out of the raphe nuclei (after 4–30 h from the moment of transection until registration) expression of 5-HT_2-receptors increases, e.g., as it was shown for mouse 5-HT_{2C}-receptors when spinal injury caused an increase in the number and density of 5-HT_{2C}-receptors in the ventral raphe of the lumbar spinal cord segment [33].

In the present work, we found out that a 5-HT_{1A}-receptor agonist 8-OH-DPAT had a time-dependent effect on the number of APs, evoking an initial excitation followed later by an inhibition (Fig. 4). In a paper by Cotel et al., it was demonstrated that activation of 5-HT_{1A}-receptors inhibits the generation of APs [34]. Thus, it is possible to suggest a joint modulation of the accommodative properties of motoneurons by the two types of 5-HT re-

ceptors, 5-$HT_{2B,C}$ and 5-HT_{1A}. If the activation of 5-$HT_{2B,C}$-receptors augments the frequency of repetitive discharges, then the later activation of 5-HT_{1A}-receptors exerts an inhibitory effect. Our data are in compliance with the hypothesis that one subtypes of 5-HT receptors (specifically 5-HT_2) intensify phosphorylation and thus exert an excitatory effect, whereas others (specifically 5-HT_1) inhibit phosphotylation and hence have an inhibitory effect in the CNS [35].

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

All applicable international, national and institutional principles of handling and using experimental animals for scientific purposes were observed.

This study did not involve human subjects as research objects.

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