Serotonin-mediated modulation of Na\(^+/K\(^+\) pump current in rat hippocampal CA1 pyramidal neurons

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Abstract

**Background:** The aim of this study was to investigate whether serotonin (5-hydroxytryptamine, 5-HT) can modulate Na\(^+/K\(^+\) pump in rat hippocampal CA1 pyramidal neurons.

**Results:** 5-HT (0.1, 1 mM) showed Na\(^+/K\(^+\) pump current (Ip) densities of 0.40 ± 0.04, 0.34 ± 0.03 pA/pF contrast to 0.63 ± 0.04 pA/pF of the control of 0.5 mM strophanthidin (Str), demonstrating 5-HT-induced inhibition of Ip in a dose-dependent manner in hippocampal CA1 pyramidal neurons. The effect was partly attenuated by ondasetron, a 5-HT\(_3\) receptor (5-HT\(_3\)R) antagonist, not by WAY100635, a 5-HT\(_1\)AR antagonist, while 1-(3-Chlorophenyl) biguanide hydrochloride (m-CPBG), a 5-HT\(_3\)R specific agonist, mimicked the effect of 5-HT on Ip.

**Conclusion:** 5-HT inhibits neuronal Na\(^+/K\(^+\) pump activity via 5-HT\(_3\)R in rat hippocampal CA1 pyramidal neurons. This discloses novel mechanisms for the function of 5-HT in learning and memory, which may be a useful target to benefit these patients with cognitive disorder.

Background

5-HT, as a neurotransmitter or neuromodulator in the central nervous system, plays a critical role in the control of blood pressure, body temperature, sleep, depression, anxiety, epilepsy [1-4]. Additionally, the modulation of the serotonergic system affects long-term potentiation (LTP) and long-term depression (LTD), the likely neurophysiologic derivate of learning and memory formation, which has been involved in the treatment of Alzheimer’s disease [5-8]. Some studies demonstrate that 5-HT\(_1\)AR-knockout animals show a deficit in hippocampal-dependent learning and memory, such as the hidden platform (spatial) version of the Morris water maze and the delayed version of the Y maze [9], while the stimulation of 5-HT\(_1\)AR mediates enhancement of LTP [10] and prevents the impairment of learning and memory [11,12]. Therefore, the stimulation of 5-HT\(_1\)AR may be useful in the symptomatic treatment of human memory disturbances. However, accumulated clinical reports support that the injection of 5-HT\(_3\)R antagonists facilitates the induction of LTP, and enhances the retention and consolidation of memory in hippocampal dependent tasks [13-15]. These clinical application of 5-HT\(_3\)R antagonists have been found to improve memory in schizophrenic or Alzheimer demented patients [16,17]. Therefore, 5-HT\(_3\)R also plays a critical role in cognitive function.

In addition to increasing neuronal excitability [18], inhibition of Na\(^+/K\(^+\) pump activity can induce LTD whereas depotentiate LTP [19], and then cause impairment of learning and memory and amnesia [20,21]. Herein, in the present study, we investigate if a relationship occurs between 5-HT and Na\(^+/K\(^+\) pump in hippocampal CA1 pyramidal neurons, which may provide new insights in the mechanisms responsible for the 5-HT-mediated modulation of learning and memory.

Results and discussion

5-HT-mediated inhibition of Ip in rat hippocampal CA1 pyramidal neurons

0.5 mM Str often did not recover completely in hippocampal CA1 slices even after prolonged washout in the present study, consistent with the previous study that Na\(^+/K\(^+\) pump inhibition by Str was effectively...
irreversible [22]. Furthermore, 10 μM Str did not recover completely in rat ventral midbrain slices [23]. Therefore, Str perfusion was applied one time in one brain slice.

In the present study, Ip densities affected by 5-HT (0.1, 1 mM) were 0.40 ± 0.04, 0.34 ± 0.03 pA/pF contrast to 0.63 ± 0.04 pA/pF of the control of 0.5 mM Str (Figure 1), demonstrating 5-HT inhibits Na⁺/K⁺ pump activity in hippocampal CA1 pyramidal neurons. Some studies have reported that 5-HT inhibits Na⁺/K⁺ pump in T sensory neurons of the leech [24] and kidney [25], then depresses the after-hyperpolarization. However, other studies have showed that 5-HT activates glial Na⁺/K⁺ pump activity in rat cerebral cortex and hippocampus [26, 27]. 5-HT stimulated synaptic membrane Na⁺/K⁺ pump from the rabbit cerebrum, but did not influence the activity of this enzyme in the other brain regions [28]. These studies suggested that the regulation of 5-HT-induced Na⁺/K⁺ pump activity may be attributable to tissue and cell specificity.

Furthermore, some studies reported that the application of 1 μM 5-HT prevented depotentiation but not LTP induced by high-frequency stimulation, whereas bath application of 100 μM 5-HT blocked the induction of tetanus-induced LTP [29], consistent with the previous study that 5-HT (30 μM) prevented LTP induced by a primed burst in rat hippocampal CA1 region [30]. Accordingly, different concentrations of 5-HT may have different modulation of learning and memory. Moreover, the inhibition of Na⁺/K⁺ pump activity can induce LTD whereas depotentiate LTP [19], and then cause impairment of learning and memory and amnesia [20, 21]. Herein, in the present study, 5-HT-mediated inhibition of Na⁺/K⁺ pump activity may disclose novel mechanisms in learning and memory. Further studies should be done to explore the mechanism.

5-HT mediated inhibition of Ip via 5-HT₃R not 5-HT₁AR

To identify the specific 5-HTR involved in the regulation of Ip, we focused on the 5-HT₁AR and 5-HT₃R that are abundant in all hippocampal layers and subregions [31-33]. In the present study, “WAY1000635 (a 5-HT₁AR antagonist) + 5-HT + Str” treatment yielded the similar result as “5-HT + Str” treatment (Figure 2), i.e., the application of the antagonist for 5-HT₁AR had no effect on 5-HT-mediated inhibition of Ip, suggesting that 5-HT₁AR was not involved in 5-HT-mediated inhibition of Ip. Some studies have reported that 5-HT₁AR mediates enhancement of LTP in hippocampal dentate gyrus [10] and prevents the impairment of learning and memory [11, 12], whereas inhibits LTP in the hippocampal CA1 field and visual cortex [34, 35], demonstrating the different effects of 5-HT₁AR on synaptic transmission in different tissues. Therefore, there still are some arguments about 5-HT₁AR-induced modulation of LTP. In the present study, 5-HT₁AR did not antagonize 5-HT-mediated inhibition of Ip, demonstrating that Na⁺/K⁺ pump may be not involved in 5-HT₁AR-mediated modulation of memory.

In the presence of ondasetron, a 5-HT₃R antagonist, 5-HT-mediated inhibition of Ip was blocked from 0.40 ± 0.04 to 0.61 ± 0.04 pA/pF (Figure 2), while m-CPBG, a 5-HT₃R specific agonist, mimicked the effect of 5-HT on Ip (Figure 2). These results show, for the first time, that the inhibition of 5-HT-mediated Ip is primarily mediated by 5-HT₃R in hippocampal CA1 pyramidal neurons. On the subcellular level, both presynaptic and postsynaptic 5-HT₃R can be found. Presynaptic 5-HT₃R is involved in mediating or modulating neurotransmitter release. Postsynaptic 5-HT₃R is preferentially expressed on interneurons [36, 37], and there is also 5-HT₃R in postsynaptic pyramidal neurons [38-42]. For example, electrophysiological studies in postsynaptic pyramidal
Figure 2 5-HT-mediated inhibition of Ip is mediated by 5-HT₃R, but not by 5-HT₁A in hippocampus. (A) The representative tracing of 0.5 mM Str-mediated Ip in the control. (B) The representative tracing of 5-HT (0.1 mM) -mediated inhibition of Ip. WAY100635, a 5-HT₁AR antagonist, alone did not affect Ip (P > 0.05) (C), and did not block 5-HT-mediated inhibition of Ip (D). Ondasetron, a 5-HT₃R antagonist, alone did not affect Ip (P > 0.05) (E), whereas attenuated 5-HT-mediated inhibition of Ip (F). (G) 5-HT-mediated inhibition of Ip is blocked by ondasetron, but not WAY100635. (H) The representative tracing of m-CPBG (0.1 mM)-mediated inhibition of Ip. (I) m-CPBG (0.1 mM) -mediated inhibition of Ip. Values significantly different by Student’s t-test from results are indicated as **P < 0.01.
neurons in the hippocampal CAl region or hippocampal primary cultures showed the activation of 5-HT$_3$R [38-42]. Furthermore, 5-HT$_3$R is a unique serotonin receptor as it acts as a ligand-gated ion channel, whereas all the other types of serotonin receptors belong to the G protein-coupled receptor superfamily, which may be the reason of 5-HT$_3$R, rather than 5-HT$_1$A, is the relevant 5-HTR for 5-HT-mediated inhibition of $I_p$ in the present study. This still deserves further investigations.

Some studies indicate that overexpression of the 5-HT$_3$R in mouse forebrain results in enhanced hippocampal-dependent learning and attention involved in fear conditioning [43], whereas most reports show that 5-HT$_3$R antagonists can facilitate LTP and enhance the retention and consolidation of memory in hippocampal dependent tasks [13]. Furthermore, the complete abolishment of 5-HT innervation in the hippocampus increases LTP in vivo [44]—which would suggest that, on balance, 5-HT may exert a negative influence on LTP via 5-HT$_3$R and then impair learning and memory [14,15]. Clinically application of 5-HT$_3$R antagonists have been found to improve memory in schizophrenic or Alzheimer demented patients [16,17]. It is, however, not clear whether this effect is specific to LTP, or secondary to other changes.

Some studies reported that inhibition of Na$^+$/K$^+$ pump activity can induce LTD whereas depotentiate LTP [19], and then cause impairment of learning and memory and amnesia [19-21,45-47]. Moreover, The initial stationary phase of the LTP was followed by a decrease in Na$^+$/K$^+$ pump activity of neurons and an augmentation of Na$^+$/K$^+$ pump activity in the glial cells [48]. These studies supported that there may be some relationship between Na$^+$/K$^+$ pump and LTP. The present results show that 5-HT can suppress $I_p$ in hippocampal CA1 neurons via 5-HT$_3$R, consistent with the previous study that 5-HT$_3$R partly mediated the decrease of Na$^+$/K$^+$ pump activity induced by cocaine in neuronal-like cells [49], suggesting that inhibition of Na$^+$/K$^+$ pump activity may be involved in 5-HT$_3$R-induced modulation of learning and memory. This provides new insights for the possible synaptic role of 5-HT via 5-HT$_3$R in cognitive function and neuronal development through Na$^+$/K$^+$ pump, which may be a useful target to benefit these patients with cognitive disorder.

**Conclusion**

5-HT inhibits neuronal Na$^+$/K$^+$ pump activity via 5-HT$_3$R in hippocampal CA1 pyramidal neurons, which may disclose novel mechanisms for the function of 5-HT in learning and memory.

**Methods**

**Solutions and chemicals**

Str, ondasetron and WAY-100635 were purchased from Sigma (St. Louis, MO, USA). 5-HT and tetrodotoxin (TTX) were purchased from Alexis (San Diego, CA, USA). m-CPBG and other chemicals were purchased from Alfa Aesar (Ward Hill, MA, USA). Str was dissolved in DMSO and further diluted 1:1000 in artificial cerebrospinal fluid (ACSF) containing (mM): NaCl 119, KCl 5.4, MgCl$_2$ 1.3, NaH$_2$PO$_4$$\cdot$2H$_2$O 1, D-Glucose 11, NaHCO$_3$ 26.2, CaCl$_2$ 2.5. Control solutions of 1:1000 DMSO had no effect on membrane current. TTX was dissolved in dilute acetic acid (PH 4.8-4.9). 5-HT, ondasetron, m-CPBG and WAY-100635 were dissolved in sterile water and stored as stock solutions. All stock solutions were stored as frozen aliquots at -20°C.

**Brain hippocampal slice preparation and loading**

Sprague-Dawley rats of 12-14 days were deeply anesthetized with sodium pentobarbital (45 mg kg$^{-1}$, i.p.) and then rapidly decapitated. Our experiments were approved by Animal Care Committee of Hebei Medical University. Appropriate experimental procedures were taken to minimize pain or discomfort. The brain was quickly removed from the skull and transverse hippocampal slices (300 μm thick) were obtained by cutting with a vibroslice MA752 (Campden Instruments, Loughborough, UK) in ice-cold ACSF well-saturated with 95% O$_2$ and 5% CO$_2$ (PH 7.3-7.4). These slices were pre-incubated in oxygenated ACSF at room temperature (22-25°C) for 1 h.

**Figure 3** The location visualization and electrophysiological characteristics of rat hippocampal CA1 pyramidal neurons. A. Schematic representation of the location of the hippocampal CA1 slice. B. The hippocampal CA1 location as visualized with ×10 infrared video microscopy. C. The hippocampal CA1 pyramidal neurons as visualized with ×40 water immersion lens of infrared video microscopy. Patch pipette is visible during whole-cell recording from the recorded neuron on right. D. Action potentials of the hippocampal CA1 pyramidal neuron during a depolarizing current pulse from the resting potential of -60 mV. Note the spike frequency adaptation. The whole cell recording under the current clamp method was used. The amplitude and the time of injected currents were shown on top of membrane potential trace.
Figure 4 I-V curve of recorded neurons. A, Schematic representation of the protocol of the recorded neurons; B, Representative current traces recorded from a typical hippocampal CA1 pyramidal neuron. Cell was held at -60 mV and stepped from -100 mV to +105 mV in 5 mV interval for 1-s duration, followed by a step to -60 mV once. C and D, I-V plots were constructed from the values of traces.
Electrophysiological recordings of Na\(^+\), K\(^+\)-pump currents

The hippocampal slice containing CA1 pyramidal neurons was transferred to a submerged recording chamber and continuously superfused with oxygenated ACSF (containing 2 mM BaCl\(_2\), 0.2 mM CdCl\(_2\) and 0.5 μM TTX) at a rate of 2 ml/min \(^{-1}\) at room temperature [50]. Only one cell was measured from each brain slice. Hippocampal CA1 pyramidal neurons were visualized by their location [51,52] using infrared differential interference contrast video microscopy and a \(\times40\) water immersion lens (Zeiss Axioskop), as shown in Figure 3A, B, C. In addition to the discrimination by the location, we determined pyramidal neurons by electrophysiological characteristics. We used current-clamp method to record the action potentials of hippocampal CA1 pyramidal neurons held at 0 pA and elicited the action potential by current injection for 1 s. Hippocampal CA1 pyramidal neurons, which were recorded with an EPC-10 amplifier (HEKA Instruments), usually exhibited spike frequency adaptation in response to a depolarizing 10 amplifier (HEKA Instruments), usually exhibited spike frequency adaptation in response to a depolarizing

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15. Hong E, Meneses A: Systemic injection of p-chloroamphetamine eliminates the effect of the 5-HT3 compounds on learning. Pharmacol Biochem Behav 1996, 53:765-769.

16. Levkovitz Y, Arens G, Mendlovic S, Treves I, Fennig S: The effect of Ondansetron on memory in schizophrenic patients. Brain Res Bull 2005, 65:291-295.

17. Dyksen M, Kuskowski M, Love S: Ondansetron in the treatment of cognitive decline in Alzheimer dementia. Am J Geriatr Psychiatry 2002, 10:212-215.

18. McCarren M, Alger BE: Sodium-potassium pump inhibitors increase neuronal excitability in the rat hippocampal slice: role of a Ca2+ + dependent conductance. J Neurophysiol 1987, 57:496-509.

19. Reich CG, Mason SE, Alger BE: Novel form of LTD induced by transient, partial inhibition of the Na+, K-pump in rat hippocampal CA1 cells. J Neurophysiol 2004, 91:239-247.

20. Moseley AE, Williams MT, Schaefer TL, Bohanen CS, Neumann JC, Behbehani MM, Vothees CV, Lingrel JB: Deficiency in Na+, K-ATPase alpha isoform genes alters spatial learning, motor activity, and anxiety in mice. J Neurosci 2007, 27:616-626.

21. Sato T, Tanaka K, Ohnishi Y, Teramoto T, Irifune M, Nishikawa T: The distribution of 5-HT1A receptor is expressed in a subpopulation of GABAergic neurons in the rat central nervous system. Brain Res Mol Brain Res 2003, 114:171-175.

22. Baker PF, Willis JS: Sodium-potassium pump inhibitors increase neuronal membrane ATPase by biogenic amines. Neurochem Res 2005, 30:151-159.

23. Shen KZ, Johnson SW: Sodium pump evokes high density pump currents in rat midbrain dopamine neurons. J Physiol 1998, 512(2 Pt 2):459-467.

24. Catarsi S, Brunelli M: Serotonin depresses the after-hyperpolarization through the inhibition of the Na+/K+ electrogenic pump in T sensory neurons of the leech. J Exp Biol 1991, 155:261-273.

25. Stepp LR, Novakowski MA: Effect of 5-hydroxytryptamine on sodium- and potassium-dependent adenosine triphosphatase and its reactivity toward ouabain. Arch Biochem Biophys 1997, 337:43-53.

26. Pena-Rangel MT, Mercado R, Hernandez-Rodriguez J: Regulation of glial Na+/K+ ATPase by serotonin: identification of participating receptors. Neurochem Res 1999, 24:643-649.

27. Mercado R, Hernandez J: Regulatory role of a neurotransmitter (5-HT) on sodium-potassium pump evokes high density pump currents in rat midbrain dopamine neurons. J Physiol 1998, 512(2 Pt 2):459-467.

28. Catarsi S, Brunelli M: Serotonin depresses the after-hyperpolarization through the inhibition of the Na+/K+ electrogenic pump in T sensory neurons of the leech. J Exp Biol 1991, 155:261-273.

29. Logan JG, O'Donovan DJ: The effects of ouabain and the activation of neural membrane ATPase by biogenic amines. J Neurochem 1976, 27:185-189.

30. Normann C, Clark K: Selective modulation of Ca2+ influx pathways by 5-HT regulates synaptic long-term plasticity in the hippocampus. Brain Res 2005, 1037:187-193.

31. Coradetti R, Ballerini L, Pugliese AM, Pepeu G: Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of rat hippocampal slices. Neurosci 1996, 46:511-518.

32. Burnet PW, Eastwood SL, Lacey K, Harrison PJ: The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. Brain Res 1995, 675:157-168.

33. Parker RM, Bames JM, Ge J, Barber PC, Barnes NM: Autoradiographic distribution of [3H]-5-zacopride-labelled 5-HT3 receptors in human brain. Journal of the Neurological Sciences 1996, 144:199-127.

34. Tecott LH, Marois AV, Stahl ML, Julius D: Nervous system distribution of the serotonin 5-HT3 receptor mRNA. Proc Natl Acad Sci USA 1993, 90:1430-1434.

35. Edagawa Y, Saito H, Abe K: 5-HT1A receptor-mediated inhibition of long-term potentiation in rat visual cortex. Eur J Pharmacol 1998, 349:221-224.

36. Morate M, Battenberg E, de Lecca L, Sanna PP, Bloom FE: Cellular and subcellular immunolocalization of the type 3 serotonin receptor in the rat central nervous system. Brain Res Mol Brain Res 1996, 36:231-260.

37. Morales M, Battenberg E, de Lecca L, Bloom FE: The type 3 serotonin receptor is expressed in a subpopulation of GABAergic neurons in the rat neocortex and hippocampus. Brain Res 1996, 731:199-202.

38. Yakei JL, Jackson MB: 5-HT3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. Neuropharmacology 1988, 1:615-621.