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# Differential effects of the serotonin receptors on cultured rat cerebral cortical neurons

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**Abstract.** Effects of serotonin (5-HT) on cerebral cortical neurons were examined by patch clamp techniques. 5-HT produced a variety of responses such as outward (19/73 patches/neurons), slow inward (15/73 patches/neurons), fast inward (8/73 patches/neurons), and mixed currents (initially fast inward deflection followed by an outward response: 2/73 patches/neurons), with a latency of 12 sec, 15 sec, 0 sec, and 0 sec respectively, at a holding potential of −60 mV in whole-cell patches. The fast inward currents were again evoked by a selective 5-HT3 receptor agonist, 1-(m-chlorophenyl)-biguanide hydrochloride (CPBG). In the cell-attached patch clamp configuration, 5-HT inside the patch pipette elicited single channel currents with slope conductances of 42 pS and 132 pS (4/42 patches/neurons). CPBG inside the patch pipette evoked inward single channel currents with a lower slope conductance of 41 pS (3/23 patches/neurons). In contrast, application of 5-HT or a 5-$HT_2$ receptor agonist, $\alpha$-methyl-5-hydroxytryptamine-maleate, outside the patch pipette induced outward single channel currents with a major slope conductance of 140 pS (8/30 patches/neurons) or 135 pS (6/20 patches/neurons), respectively. These results indicate that the outward and fast inward currents may be mediated respectively by the 5-$HT_2$ receptor, which is coupled to a G-protein, and by the 5-$HT_3$ receptor, which contains the non-selective cation channel, and that the mixed type may be caused by both the 5-$HT_2$ and 5-$HT_3$ receptors.
**Key words.** Serotonin; ligand-gated receptor; G-protein-coupled receptor; patch-clamp; cerebral cortical neuron.

Serotonin (5-HT) is a neurotransmitter that exerts both excitatory and inhibitory effects in central and peripheral nervous systems [1]. The many physiological and behavioral effects of 5-HT reflect its interaction with a large number of functionally diverse receptor subtypes. The 5-HT receptors are currently divided into eight subgroups: 5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, 5-$HT_5$, 5-$HT_6$, 5-$HT_7$, and others [2, 3]. The 5-$HT_3$ receptor is characterized as a ligand-gated receptor channel and the remaining ones are linked to a G-protein. The 5-$HT_1$ receptor is involved in inhibition of adenylate cyclase, while 5-$HT_4$, 5-$HT_6$, and 5-$HT_7$ receptors stimulate adenylate cyclase [2]. It has been suggested that the 5-$HT_2$ receptor stimulates phospholipase C [2]. A possible mechanism for the inhibitory effect would be an increase in potassium conductance [4]. In contrast, the excitatory effect may be caused by a decrease in potassium conductance [5] or rapid depolarization through the 5-$HT_3$ receptor [6]. A multiplicity of responses to 5-HT, such as outward, fast inward and slow inward currents, are observed in cultured mouse hippocampal and striatal neurons [7]. Another study demonstrates that 5-HT produced outward, inward and outward mixed, or long-lasting inward currents, in dissociated rat hippocampal pyramidal neurons [8]. The 5-HT receptors coupled to each current, however, largely remain unknown.

We show here that 5-HT produces four different kinds of current responses in cultured rat cerebral cortical neurons, such as outward ($I_{out}$), slow inward ($I_{si}$), fast inward ($I_{fi}$), and mixed type ($I_{mix}$). The results suggest that the $I_{out}$ and $I_{fi}$ are mediated by the 5-$HT_2$ and 5-$HT_3$ receptor, respectively, and the $I_{mix}$ by the 5-$HT_2$ and 5-$HT_3$ receptors.

## Materials and methods

The cerebral cortex was removed from the brain of neonatal rats under ether anesthesia. The tissues were incubated in 0.25% trypsin in $Ca^{2+}$-, $Mg^{2+}$-free saline for a few min at room temperature and then mechanically dissociated by triturating with a Pasteur pipette. The dissociated cells were plated on collagen-coated cover-slips and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, 7.5 mM $NaHCO_3$, 25 μg/ml insulin, 100 μg/ml transferrin, 60 μM putrescine, 20 nM progesterone, 30 nM sodium selenate, 5 mM HEPES buffer (pH 7.0), 100 mg/ml streptomycin [9]. Cells were cultured at 37 °C in a humidified atmosphere of 95% air and 5% $CO_2$. Cultured neurons were used 7–10 days after plating. The cells were bathed at room temperature (20–22 °C) in a standard extracellular solution (in mM): 145 NaCl,

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5 KCl, 2.4 $CaCl_2$, 1.8 glucose, and 10 HEPES, pH 7.4. The basic patch electrode-filling solution was (in mM): 150 KCl, 10 EGTA, and 10 HEPES, pH 7.2. Membrane currents from the whole-cell voltage clamp were recorded using an Axopatch-200A amplifier (Axon Instrument, Inc., USA). After formation of whole-cell patches, series resistance (Rs) compensation was made up to about 95%. The drugs were applied to cells for 1 sec using an air pressure microinjector (PV 830 Pneumatic Picopump, World Precision Instruments, Inc., USA). The currents were filtered at 5 kHz, stored on a magneto-optical disk (MK128D, Mitsubishi-Kasei, Inc., Japan), and analysed with a laboratory computer using pClamp software (Axon Instrument, Inc.; version 6). Single channel currents were recorded in the cell-attached patch clamp configuration using an Axopatch-200A amplifier. The currents were filtered at 2 kHz, digitized at 1 kHz, and analysed using pClamp software. The patch electrode was filled with the same extracellular solution as used in whole-cell patches. 5-HT or 5-HT receptor agonists were added to the patch electrode-filling solution or bath-applied to cells after gigaseal formation.

## Results

To see whether the cultured cells were neurons, 500 msec voltage pulses were applied to the cells from $-200$ mV to $+40$ mV in 20 mV steps before application of 5-HT, and the cells with inward $Na^+$-currents were used for the experiments (data not shown). 5-HT (50 μM) produced a variety of responses at a holding potential of $-60$ mV; $I_{out}$ (19/73 patches/neurons), $I_{si}$ (15/73 patches/neurons), fast $I_{fi}$ (8/73 patches/neurons), and $I_{mix}$ (2/73 patches/neurons) (fig. 1 and table 1). 5-HT was applied to cells at concentrations ranging from 1 μM to 100 μM, and 50 μM was the concentration producing the maximal response to 5-HT in this study. The $I_{out}$ was induced with a latency of $12 \pm 3$ sec, and slowly desensitized (fig. 1 and table 1). The $I_{si}$ was

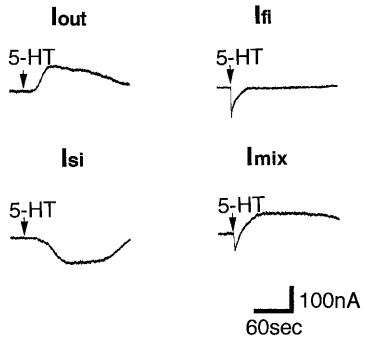


Figure 1. Whole-cell membrane currents induced by 5-HT. 5-HT (50 μM) was applied to cells (arrow) for 1 sec. Typical evoked currents are illustrated. Inward and outward whole-cell currents correspond to downward and upward deflections, respectively. The holding potential was $-60$ mV.

Table 1. Different responses induced by 5-HT or CPBG in cultured cerebral cortical neurons.

| | $I_{out}$ | $I_{si}$ | $I_{fi}$ | $I_{mix}$ | No response |
|---|---|---|---|---|---|
| 5-HT | 19/73 | 15/73 | 8/73 | 2/73 | 29/73 |
| CPBG | 0/44 | 5/44 | 0/44 | 0/44 | 39/44 |

evoked with a latency of $15 \pm 6$ sec and the currents slowly decayed (fig. 1 and table 1). The latency of more than 10 sec in the $I_{out}$ and $I_{si}$ suggests that these currents were activated through a G-protein-coupled 5-HT receptor. In contrast, the $I_{fi}$ was induced without latency (fig. 1 and table 1). Similarly, a 5-$HT_3$ receptor agonist, 1-(m-chlorophenyl)-biguanide hydrochloride (CPBG) ($IC_{50} = 1.5$ nM) at concentrations of 100 nM and 1 μM also produced this type of current with a frequency of 11% (table 1), suggesting that the $I_{fi}$ was mediated by the 5-$HT_3$ receptor, which forms the non-selective cation channel. Regarding the $I_{mix}$, 5-HT initially induced sharp inward currents without latency followed by outward currents (fig. 1 and table 1), indicating that at least two different kinds of 5-HT receptors, possibly the 5-$HT_3$ receptor and a G-protein-coupled 5-HT receptor, might be expressed in a single neuron.

To investigate the characteristics of 5-HT-induced currents further, single channel current recording was carried out. 5-HT elicited single channel currents at concentrations ranging from 1 μM to 50 μM in the cell-attached patch clamp configuration. The currents induced by 1 μM 5-HT were analysed in the present study, since higher concentrations of 5-HT gave bursts. 5-HT (1 mM) inside the patch pipette evoked single channel currents with two different kinds of slope conductances: $132 \pm 15$ pS and $42 \pm 7$ pS (4/42 patches/neurons) (fig. 2). CPBG (100 nM) inside the patch pipette evoked single channel currents and the current/voltage (I/V) relation had a major slope conductance of $41 \pm 5$ pS with a reversal potential of around 0 mV (3/23 patches neurons) (fig. 2). This slope conductance was in good agreement with the lower conductance of the currents induced by 5-HT. In addition, the currents were never produced by application of CPBG outside the pipette (data not shown). These results may imply that 5-HT produced single channel currents with a lower slope conductance through the 5-$HT_3$ receptor. Otherwise, bath application of 5-HT (1 μM) elicited single channel currents with a major slope conductance of $140 \pm 23$ pS (8/30 patches/neurons) in patches using the patch electrode-filling solution without 5-HT (fig. 2). Furthermore, single channel currents with similar slope conductance ($135 \pm 11$ pS: 6/20 patches/neurons) were again induced by bath application of a 5-$HT_2$ receptor agonist, α-methyl-5-hydroxytryptamine maleate (αMe5HT), at concentrations of 1 μM and

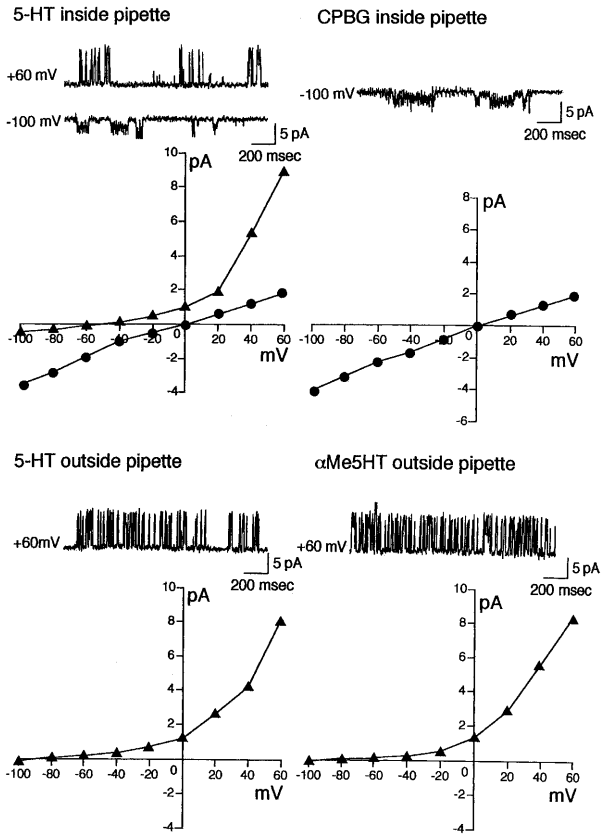


Figure 2. Single channel currents elicited by 5-HT and 5-HT receptor agonists. Cell-attached patches were made. In some patches, 5-HT (1 μM) or CPBG (100 nM) was added to the patch electrode-filling solution. In other patches using the patch electrode-filling solution without 5-HT or 5-HT receptor agonists, 5-HT (1 μM) or αMe5HT (1 μM) was bath-applied outside the patch pipette during recording. Patch potential represents the voltage loaded on the inside membrane. Inward and outward single channel currents correspond to downward and upward deflections, respectively. The current/voltage (I/V) relations from −100 mV to +60 mV are shown beneath each illustrated current. The slope conductances obtained with CPBG and with 5-HT inside the patch pipette were measured by the I/V relations from −100 mV to +60 mV (closed circle) and other slope conductances were measured by linear regression fitted to the I/V relations from +20 mV to +60 mV (closed triangle). Closed circles and closed triangles represent $I_{fi}$ and $I_{out}$, respectively.

10 μM (fig. 2), suggesting that the currents with a higher slope conductance are mediated by the 5-$HT_2$ receptor.

## Discussion

Molecular cloning of the 5-HT receptors demonstrates the extreme diversity of subtypes within the family. With the exception of the 5-$HT_3$ receptor, the others have been shown to be members of the G-protein coupled receptor superfamily [2]. In this study, longer latency to the current onset in the $I_{out}$ and $I_{si}$ indicates that these currents are evoked via a G-protein-regulated signaling pathway. Bath-application of 5-HT outside the patch pipette evoked single channel currents in the cell-attached patch clamp configuration, further supporting this idea. Additionally, the finding that 5-HT and αMe5HT induced outward single channel currents with a similar slope conductance may imply that the receptor responsible for the $I_{out}$ is a 5-$HT_2$ receptor. The 5-$HT_2$ receptor has three components, each of which is involved in stimulation of phospholipase C [2], and the 5-$HT_{2c}$ receptor is preferentially expressed in the brain [10]. 5-HT produces potassium currents through the 5-$HT_2$ receptor by interaction with $Ca^{2+}$/calmodulin kinase II, which may be activated by inositol 1,4,5-triphosphate-regulated $Ca^{2+}$ release from intracellular calcium stores, in CA1 neurons from rat hippocampus [8]. Thus, the $I_{out}$ appears to be regulated by phospholipase C-mediated phosphatidylinositol signalling, although the interaction with $Ca^{2+}$/calmodulin kinase II is unproven. It is not clear which 5-HT receptor causes the $I_{si}$. The 5-$HT_1$ receptor family contains five members so far, each coupled to the inhibition of adenylate cyclase activity [11]. The 5-$HT_{1A}$ receptor is reported to be linked to inward rectifying potassium currents in the hippocampus [4, 12]. Taken together, the $I_{si}$ may be mediated by the 5-$HT_{1A}$ receptor. We are currently carrying out further experiments to address this question. The 5-$HT_3$ receptor is known to produce inward whole-cell currents, causing rapid excitation in the hippocampus [13]. Also, extracellular recordings of hippocampal slices suggest that the 5-$HT_4$ receptor, which is involved in stimulation of adenylate cyclase activity [2], exerts an excitatory effect [14]. In the current study, 5-HT and CPBG evoked inward currents without latency in the whole-cell patch clamp configuration. Moreover, CPBG inside the patch pipette produced single channel currents with a reversal potential of 0 mV, although the currents were not induced by application of CPBG outside the pipette. This finding indicates that the receptor responsible for the $I_{fi}$ is directly coupled to an ion channel, a 5-$HT_3$ receptor, but is not a 5-$HT_4$ receptor linked to a G-protein.

In the $I_{mix}$, 5-HT evoked biphasic currents with fast inward and slow outward deflections. 5-HT inside the patch pipette elicited single channel currents with two different kinds of slope conductances in a single neuron, each of which is consistent with that of the currents induced by CPBG and αMe5HT. This finding suggests that the $I_{mix}$ is mediated by the 5-$HT_3$ receptor, involving a fast inward current, and the 5-$HT_2$ receptor, involving a slow outward current. Thus, 5-HT may have both excitatory and inhibitory effects in some hippocampal neurons.

In conclusion, the results presented here demonstrate that 5-HT produces a variety of responses in cerebral cortical neurons. The $I_{out}$ appears to be regulated by the 5-$HT_2$ receptor, while the $I_{fi}$ seems to be mediated by the 5-$HT_3$ receptor. In some cases, the 5-$HT_2$ and 5-$HT_3$ receptors may be co-expressed in a single neuron, causing the $I_{mix}$.

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