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Multiple Signal Transduction Pathways Mediated by 5-HT Receptors

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Abstract

Among human serotonin (5-HT) receptor subtypes, each G protein-coupled receptor subtype is reported to have one G protein-signaling cascade. However, the signaling may not be as simple as previously thought to be. 5-HT_{5A} receptors are probably the least well understood among the 5-HT receptors, but the authors found that 5-HT_{5A} receptors couple to multiple signaling cascades. When the 5-HT_{5A} receptors were expressed in undifferentiated C6 glioma cells, they modulated the level of second messengers. For example, activation of 5-HT_{5A} receptors inhibited the adenylyl cyclase activity and subsequently reduced the cAMP level, as previously reported. In addition to this known signaling via G_i/G_o, 5-HT_{5A} receptors are coupled to the inhibition of ADP-ribosyl cyclase and cyclic ADP ribose formation. On the other hand, activation of 5-HT_{5A} receptors transiently opened the K^+ channels, presumably due to the increase in intracellular Ca^{2+} after formation of inositol *(1,4,5)* trisphosphate. The K^+ currents were inhibited by both heparin and pretreatment with pertussis toxin, suggesting the cross-talk between G_i/G_o protein and phopholipase C cascade. Thus, the authors results indicate that 5-HT_{5A} receptors couple to multiple second messenger systems and may contribute to the complicated physiological and pathophysiological states. Although this multiple signaling has been reported only for 5-HT_{5A}/5-HT_1 receptors so far, it is possible that other 5-HT receptor subtypes bear similar complexity. As a result, in addition to the wide variety of expression patterns of each 5-HT receptor subtype, it is possible that multiple signal transduction systems may add complexity to the serotonergic system in brain function. The investigation of these serotonergic signaling and its impairment at cellular level may help to understand the symptoms of brain diseases.

Index Entries: 5-HT_{5A} receptors; G_i/G_o; adenylyl cyclase; cyclic AMP; ADP ribosyl cyclase; cyclic ADP ribose; IP3; K^+ channels.

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Introduction

Mammalian 5-hydroxytryptamine (5-HT; serotonin) receptors have seven subfamilies (5-$HT_{1–7}$), comprising at least 15 molecularly identified subtypes, some with splice variants and others with isoforms created by mRNA editing. Genomic structure, distribution, pharmacology, and functional responses attributed to each 5-HT receptor in the brain have been reviewed in detail previously *(1).* Briefly, the majority of the 5-HT receptors belong to the large family of receptors interacting with G proteins, except for the 5-HT_3 receptors, which are ligand-gated ion channel receptors. The 5-HT receptors belonging to the G protein-coupled receptor superfamily are characterized by the presence of seven transmembrane domains and the ability to alter G protein-dependent processes. The amino acid sequence of the membrane-spanning domains shows the least amount of variability compared with other cloned biogenic amine receptors. The 5-HT receptors can be divided into distinct subfamilies based on their coupling to second messengers (*see* Fig. 1). The 5-HT_1 receptor subfamily is coupled to inhibitory pathways; inhibition of adenylyl cyclase and regulation of ion channels. They are also coupled to stimulate phospholipase C and mitogen-activated protein kinase (MAPK) growth signaling pathway *(2).* The 5-HT_2 receptor subfamily contains three receptors that share striking amino acid homology and the same coupling with the activation of phospholipase C. In contrast, the 5-HT receptors that are positively coupled to adenylyl cyclase are a heterogenous group, including the 5-HT_4, 5-HT_6, and 5-HT_7 receptor subtypes. The 5-HT_5 receptor group contains two types, 5-HT_{5A} and 5-HT_{5B} receptors, and represents a new subfamily of 5-HT receptors that do not resemble the 5-HT_1 and 5-HT_2 subfamilies in amino acid sequence. The 5-HT_5 receptor was shown to be negatively coupled to adenylyl cyclase, similar to 5-HT_1 receptor subfamily. Although the 5-HT_5 receptor subfamily still remains probably the least well understood of all the 5-HT receptor family, it was recently shown that the cellular signaling attributed to the 5-HT_5 receptor was not restricted to the inhibition of adenylyl cyclase *(3),* but also extended to the inhibition of ADP-ribosyl cyclase and the formation of IP3. Activation of phospholipase C (PLC) was partly due to the cross talk between G_i/G_o and PLC, which appears to be mediated by βγ-subunits released form activated G_i/G_o. Activation of PLS might also partly be due to the activation of conventional G_q/G_{11} (Fig. 1, dotted line), because pretreatment of the cells with purtussis toxin did not completely block the 5-HT_{5A} receptor-induced currents. These multiple signalings suggest the activation of metabotropic effects and subsequent functions of 5-HT are not simple.

5-HT_5 Receptors

The two members of the 5-HT_5 receptors subfamily, 5-HT_{5A} and 5-HT_{5B} receptors, were identified in mice *(4,5)* and subsequently in rats *(6,7).* A cDNA encoding the 5-HT_{5A} receptors has been cloned from human tissues *(8)* and mapped on chromosome 7q34-q36 *(9),* while the 5-HT_{5B} receptors do not seem to be functionally expressed in humans *(10).* Pharmacologically, the 5-HT_5 receptors resemble the 5-HT_1 receptor subfamily, displaying high affinities for the agonists, 5-carbamidotryptamine (5-CT) and ergot derivatives, such as lysergic acid diethylamide (LSD) and ergotamine *(8,11).* However, for various reasons, 5-HT_5 receptors may represent a distinct subfamily. In contrast to 5-HT_1 receptor genes, 5-HT_5 receptor genes contain an intron in the region encoding the third intracellular loop *(4).* Furthermore, 5-HT_5 receptors exhibit a rather low affinity for 5-HT and the percentage of amino acid sequence homology in the transmembrane domains to other 5-HT receptors is less than 50% *(12).*

Many physiological roles of 5-HT_{5A} receptors have been suggested; 5-HT_{5A} receptors might be involved in learning, memory and

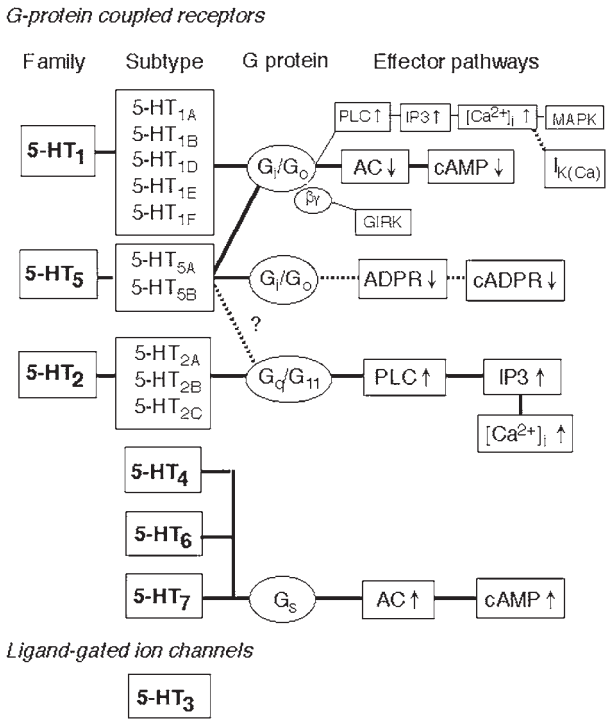

Fig. 1. Mammalian 5-HT receptor subtypes and their signal transduction pathways. The dotted lines show the proposed new signal cascade. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; GIRK, G protein-gated inwardly rectifying K^+ channel; ADPR, ADP-ribosyl cyclase; cADPR, cyclic adenosine diphosphate ribose; PLC, phospholipase C.

emotional behaviors *(4,6,13)*, motor coordination and control *(4,13,14)*, the acquisition of adaptive behavior under stressful situations *(15)*, and arterial chemoreception *(16)*. In 5-HT_{5A} receptor knockout mice, increased exploratory activity (but no change in anxiety-related behaviors) were observed, while stimulatory effect of LSD on exploratory activity was attenuated, suggesting that some of the psychotropic effects of LSD may be mediated by 5-HT_{5A} receptors *(17)*. Furthermore, the allelic variation in the human 5-HT_{5A} receptor gene may be involved in susceptibility to schizophrenia and affective disorders *(18,19)*, though the controversial result has also been reported that polymorphisms analyzed in the 5-HT_{5A} receptor gene do not play a major role in the pathogenesis of affective disorders *(20)*.

5-HT_{5A} Receptor Distribution

The 5-HT_{5A} receptors were initially reported to be expressed predominantly by astrocytes *(14)*. However, the expression of 5-HT_{5A} receptor mRNA in cultured glial cells and C6 glioma cells is negligibly low compared to the expression level in the whole rat brain (Noda et al., unpublished data). *In situ* hybridization histochemistry in human brain showed that the main site of 5-HT_{5A} receptors mRNA expression were the cerebral cortex, hippocampus, and cerebellum suggesting that 5-HT_{5A} receptors may have an important role in high cortical, limbic, and cerebeller function *(13)*. Expression in the olfactory bulb and medial habenula was also reported in mice *(17)*. In rat brain, 5-HT_{5A}-like immunoreactivity was widely detected

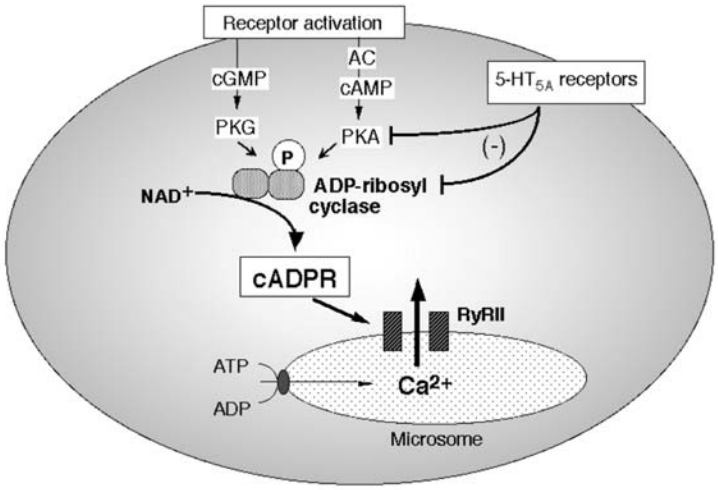

Fig. 2. Signal cascade leading to inhibition or activation of ADP-ribosyl cyclase from 5-HT_{5A} receptors and other receptors. Phosphorylation (P) of the cytosolic or membrane-bound form of ADP-ribosyl cyclase increases enzymatic activity via cGMP-dependent protein kinase (PKG) and cAMP-dependent protein kinase (PKA). The inhibition of ADP-ribosyl cyclase by the activation of 5-HT_{5A} receptor may also be due to the possible inhibition of PKA via inhibition of adenylyl cyclase (AC) and cAMP formation. The activation of 5-HT_{5A} receptors may compete with other receptor activation which activate the formation of cyclic ADP ribose (cADPR), and in turn elicit Ca^{2+} release via type II ryanodine receptors (RyRII).

(21), including hypothalamic and amygdaloid nuclei, which have significant serotonergic input. These results indicate that 5-HT_{5A} receptors show broad distribution in the whole brain, mediating a wide range of serotonergic effects. Immunoreactivity was also detected in the suprachiasmatic nucleus, suggesting a possible role in the regulation of circadian rhythms *(22,23).* Besides the expression in the brain, RT-PCR showed that 5-HT_{5A} receptors were also expressed in carotid body, petrosal ganglion, and the superior cervical ganglion in rat, suggesting that 5-HT_{5A} receptors play fundamental roles in arterial chemoreception *(16).* Furthermore, while the physiological meaning was not suggested, 5-HT_{5A} receptors as well as 5-HT_{2C} receptors were shown in human-resting lymphocytes by RT-PCR. The presence of these receptors in an easily available tissue can be considered potentially useful for future quantitative analyses in patients with different psychiatric conditions *(24).*

Activation of Multiple Signal Transduction Cascades by 5-HT_{5A} Receptors

The mouse, rat, and human 5-HT_{5A} receptors have been investigated in various mammalian cells using transfection method and functional coupling to effectors was analyzed. Inhibition of adenylyl cyclase via coupling to PTX-sensitive G_i/G_o has been reported *(25–27).* Recently, it was reported that the human 5-HT_{5A} receptors were able to couple to the inwardly rectifying K^+ channel, GIRK, when expressed in Xenopus oocytes *(10).* More recently, in addition to the inhibition of cAMP accumulation, other signal transduction pathways and affective second messengers were found *(3).*

One is the inhibition of ADP-ribosyl cyclase activity (*see* Fig. 2), which was measured in the membrane preparation. This result indicates that the inhibition by 5-HT_{5A} receptors was

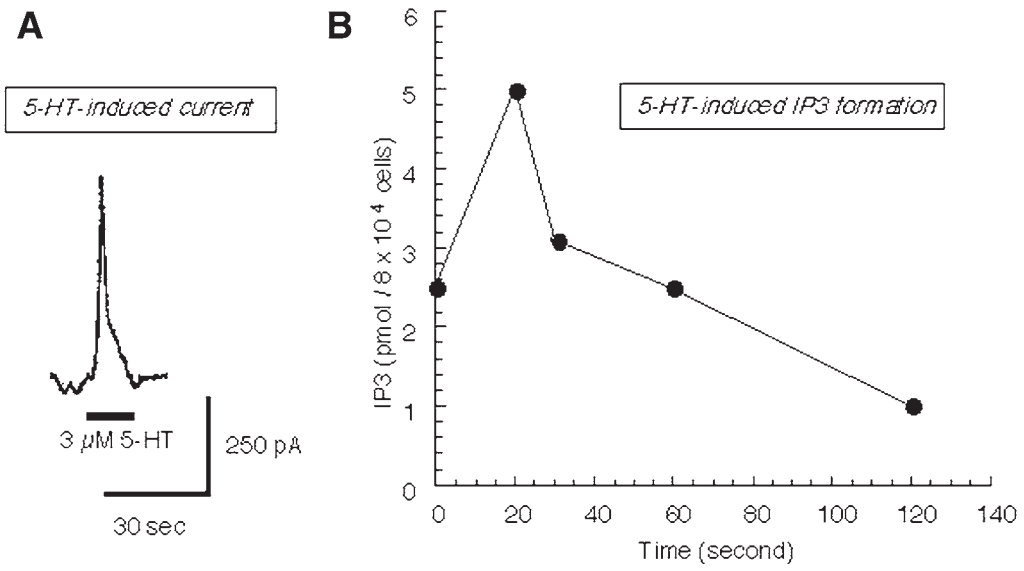

Fig. 3. Outward current and transient increase in IP3 formation after activation of 5-HT_{5A} receptors. **(A)** Whole-cell patch-clamp recording showed the outward membrane currents induced by 3 μ*M* 5-HT at the holding potential of –40 mV in 5-HT_{5A} receptor-transfected C6 glioma cells *(2).* **(B)** Determination of IP3 in 5-HT_{5A} receptor-transfected C6 glioma cells were performed as described previously *(3,42).*

considered to be rather direct on the membrane-bound form of ADP-ribosyl cyclase. This inhibition of ADP-ribosyl cyclase was nullified by treatment of the cells with pertussis toxin, suggesting the involvement of G_i/G_o. The consequence of the inhibition of ADP-ribosyl cyclase was presumably a decrease in the cyclic adenosine diphosphate ribose (cADPR) level. Cyclic ADPR is produced from NAD^+ by ADP-ribosyl cyclase and targets type-II ryanodine receptors to release Ca^{2+} *(28–30).* Therefore, cADPR is suggested as an intracellular Ca^{2+} modulating second messenger *(31).* Its modulatory role was shown in neurotransmitter release *(32–34)* and insulin release *(35),* and inducing the long-term synaptic depression in hippocampus *(36).*

The formation of cADPR is regulated by the phosphorylation of ADP-ribosyl cyclase, dependent on either cAMP- or cGMP-dependent protein kinase. As for the latter signaling, the NO/cGMP-mediated pathway to ADP-ribosyl cyclase has been suggested to play a key role in inducing long-term depression *(37),* while cGMP-independent inhibition of ADP-ribosyl cyclase by nitric oxide was observed in airway smooth muscle *(38).* On the other hand, activation of ADP-ribosyl cyclase by cAMP-dependent protein kinase was suggested in the heat-stress signaling cascade *(39).* Since inhibition of adenylyl cyclase activity and cAMP accumulation by 5-HT_{5A} receptors was observed, cAMP-dependent protein kinase was also predicted to be inhibited, which in turn might attenuate ADP-ribosyl cyclase activity (Fig. 2). Therefore, the dual inhibitions of ADP-ribosyl cyclase due to both direct and indirect effect of 5-HT_{5A} receptors may affect cellular functions related to cADPR.

Electrophysiological measurements using the patch-clamp technique in whole-cell configuration showed pronounced 5-HT-induced transient outward currents in 5-HT_{5A} receptor-expressing C6 glioma cells (*see* Fig. 3A). The current–voltage relationships obtained at the peak by applying 50 ms step pulses were

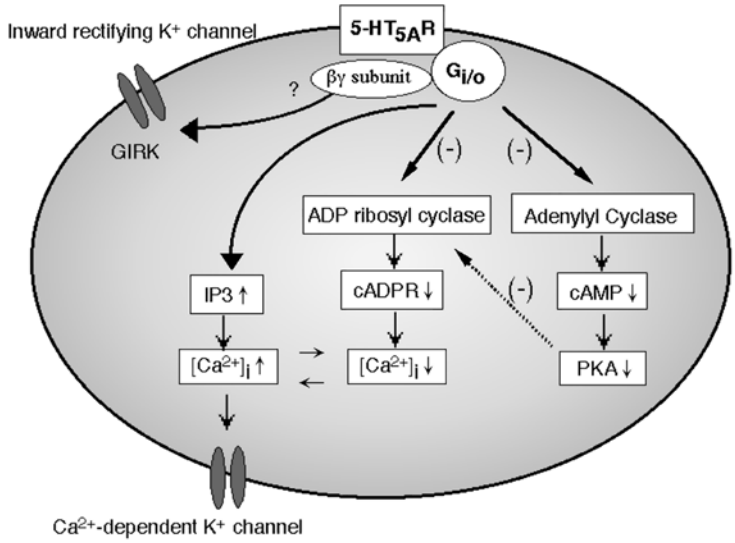

Fig. 4. Summary of proposed signaling mediated by 5-HT_{5A} receptors.

almost linear with reversal potential at around –100 mV, and were not inward-rectifying. The 5-HT-induced outward current was observed in 84% of the patched 5-HT_{5A} receptor-expressing cells and was concentration-dependent. The 5-HT-induced current was inhibited when intracellular K^+ was replaced with Cs,$^+$ but was not significantly inhibited by typical K^+ channel blockers. On the other hand, the 5-HT-induced current was significantly attenuated by BAPTA in the patch pipet. Similarly, depleting the intracellular Ca^{2+} store by application of caffein or thapsigargin also blocked the 5-HT-induced current. From these results, the 5-HT-induced outward currents were most likely Ca^{2+}-dependent K^+ currents. Since the ryanodine-sensitive Ca^{2+} release was presumably inhibited, a possible Ca^{2+} source was inositol *(1,4,5)* trisphosphate (IP3)-sensitive Ca^{2+} release from sarcoplasmic reticulum. In fact, the 5-HT-induced outward currents were inhibited by heparin, a blocker of IP3 receptors *(40).* The formation of IP3 was confirmed by radioligand-binding assay, showing the transient increase in IP3 formation within 20 s (*see* Fig. 3B). The time course of IP3 formation was in accordance with the observed membrane current change (Fig. 3A). Interestingly, the 5-HT-induced outward currents were also inhibited by pretreatment of the cells with pertussis toxin, though not completely. This result suggests that the formation of IP3 was partly due to the activation of G_i/G_o, as observed in muscarinic 2 and 4 (m2 and m4) acetylcholine receptor subtypes *(41,42).* The contribution of the coupling of 5-HT_{5A} receptors to Gq was also possible, as in the conventional IP3-signaling cascade. The transient increase in IP3 and consequent increase in intracellular Ca^{2+} may have an important physiological meaning when the 5-HT_{5A} receptors are activated, because many cellular functions are Ca^{2+}-dependent.

It was concluded that in addition to the inhibition of adenylyl cyclase and ADP-ribosyl cyclase activities—which leads to the inhibition of Ca^{2+} release from ryanodine-sensitive stores—5-HT_{5A} receptors regulate intracellular Ca^{2+} mobilization due to the IP3-sensitive Ca^{2+} store. The difference in the time-course of these contradicting signalings for the intracellular Ca^{2+} concentration might result in transient membrane outward currents. These multiple signal transduction systems were summarized

in Fig. 4. These new aspects of multiple signaling induced by a single 5-HT receptor subtype may contribute to a better understandings of diverse 5-HT function in the brain.

Physiological and Pathophysiological Implication of Multiple Signaling

Here the authors described multiple coupling preferences of 5-HT_{5A} receptors at cellular level. However, it is not easy to assess the physiological effects due to the activation of 5-HT_{5A} receptors in the brain. One reason is that some 5-HT receptors, including 5-HT_{5A} receptors, still lack selective ligands and antagonists. Also, the cellular functions of 5-HT_{5A} are partly similar to those of 5-$HT_{1A/1D}$ receptors and it is thus difficult to distinguish them. Moreover, the signaling cascade of one 5-HT receptor subtype may not be restricted to one, as observed in 5-HT_{5A} receptors, making it more difficult to define the functional responses attributed to each receptor in the brain. Furthermore, the regional distributions of each 5-HT receptor subtype have a wide variety and are largely overlapped. Given these complex viewpoints, it is difficult to clarify the physiological and pathophysiological role of each 5-HT receptor subtype. However, 5-HT receptor polymorphism found in patients would make it possible to investigate the pathophysiological role of 5-HT receptors in brain diseases. The total 5-HT response is most likely in good harmony and finely tuned, and once the balance is destroyed it may be easy to induce symptoms of the central nervous system (CNS), including physical and psychological disorders *(43,44)*. The signaling study obtained in recent works may help to understand the mechanisms of serotonergic dysfunction and will contribute to therapeutic strategy.

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