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Cross-Modulation of Synaptic Plasticity by β-Adrenergic and 5-HT_{1A} Receptors in the Rat Basolateral Amygdala

Su-Jane Wang, Li-Liang Cheng, and Po-Wu Gean

Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan City, Taiwan 701

Neurotransmitter receptors are often colocalized in a neuron with other receptors, and activation of one receptor can either amplify or antagonize the response to a colocalized receptor. The aim of this study was to investigate the cross-regulation of synaptic transmission by β-adrenergic and serotonin 1A (5-HT_{1A}) receptors and to elucidate their underlying mechanisms. Stimulation of presynaptic β-adrenergic receptors with isoproterenol (Iso) in the basolateral amygdala resulted in a long-lasting increase in synaptic transmission. This effect was mimicked by forskolin, an activator for adenylyl cyclase and a cAMP analog. In addition, the effect of forskolin was blocked by catalytic and regulatory site antagonists for cAMP-dependent protein kinase (PKA), indicating a PKA-mediated mechanism. Application of 5-HT depressed the synaptic transmission and blocked Iso- and forskolin-induced potentiation. The effect of 5-HT was mimicked by the selective 5-HT_{1A} agonist 8-hydroxy-dipropylaminotetralin and was blocked by the selective 5-HT_{1A} antagonist 1-(2-methoxyphenyl)-4[4-(2-phthalimido)butyl]piperazine, indicating its mediation by 5-HT_{1A} receptors. To determine the locus of interaction, Sp-cAMPS, a membrane-permeable activator of PKA, was applied, and the potentiation produced by Sp-cAMPS was completely blocked in slices pretreated with 5-HT. These results suggest that the interaction between the intracellular signaling pathways activated by 5-HT_{1A} and β-adrenergic receptors occurs at a step downstream from cAMP production.

Key words: serotonin; isoproterenol; cAMP; protein kinase A; calcium channel; long-term potentiation; amygdala

Serotonin 1A (5-HT_{1A}) receptor belongs to a family of neurotransmitter receptors that act through G-proteins of the G_i/G_o class to inhibit adenylyl cyclase (Andrade et al., 1986; Taussig et al., 1993). In the amygdala, application of 5-HT and 5-HT_{1A} receptor agonists caused a depression of EPSP with no concomitant changes in the resting membrane potential or neuronal input resistance (Rainnie, 1995; Cheng et al., 1998). In addition, postsynaptic depolarization evoked by glutamate receptor agonists was unaltered in the presence of 5-HT, indicating an effect on the excitatory synapses rather than a change in the excitability of basolateral amygdala neurons (Cheng et al., 1998). On the other hand, activation of β-adrenergic receptors that are coupled positively to adenylyl cyclase through G_s proteins induced long-term enhancement of synaptic transmission in these same neurons (Huang et al., 1996).

Cross-talk between G-protein-coupled receptors has been demonstrated in a number of systems. For example, stimulation of α-adrenoceptors in hippocampal CA1 neurons strongly upregulated the effect of β-adrenoceptors on afterhyperpolarzing currents (I_{AHP}) (Pedarzani and Storm, 1996). Heterologous regulation has also been reported between $GABA_B$ and β-adrenoceptors in the hippocampus in which agonist stimulation of G_i-linked receptors may upregulate or downregulate β-adrenergic responses (Andrade, 1993; Gerber and Gahwiler, 1994). Similarly, in ileal and tracheal smooth muscles, antagonism of M_2 muscarinic receptors leads to an increase in the relaxant potency of β agonists (Fernandes et al., 1992). In the basolateral amygdala, functional 5-HT_{1A} and β-adrenergic receptors are present in the excitatory nerve endings (Huang et al., 1996; Cheng et al., 1998), which represent a useful system to investigate cross-talk between these receptors. Therefore, in this study, we investigated the effect of G_i-linked receptors on β-adrenoceptor-induced synaptic plasticity and elucidated their underlying mechanisms.

MATERIALS AND METHODS

Male Sprague Dawley rats, 4–6 weeks of age, were decapitated, and their brains were rapidly removed and placed in cold oxygenated artificial CSF (ACSF) solution. Subsequently, the brain was hemisected and cut transversely posterior to the first branch and anterior to the last branch of the superior cerebral vein. The resulting section was glued to the chuck of a Vibroslice tissue slicer (Campden Instruments, Silbey, UK). Transverse slices of 500 μm thickness were cut, and the appropriate slices were placed in a beaker of oxygenated ACSF at room temperature for at least 1 hr before recording. ACSF solution had the following composition (in mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, and glucose 11. The ACSF was bubbled with 95% O_2 and 5% CO_2 and had the pH of 7.4.

A single slice was transferred to the recording chamber, in which it was held submerged between two nylon nets and maintained at 32 ± 1°C. The chamber consisted of a circular well of a low volume (1–2 ml) and was perfused constantly at a rate of 2–3 ml/min. Intracellular recording microelectrodes were pulled from 1.0 mm microfiber capillary tubing on a Flaming–Brown electrode puller (Sutter Instruments, San Rafael, CA). The electrodes were filled with 4 M potassium acetate with resistance ranging from 70 to 130 MΩ. For chelating intracellular Ca^{2+}, the electrodes were filled with 50 mM BAPTA in addition to 3 M potassium acetate. When BAPTA-containing electrodes were used, loading of the cells with BAPTA was assayed by the blockade of Ca^{2+}-activated afterhyperpolarization and spike-frequency accommodation. The microelectrode tips were positioned into the basolateral subdivision of amygdala (BLA). Monosynaptic EPSPs were evoked in BLA neurons by electrical stimulation of afferents from the lateral nucleus of amygdala with a concentric bipolar stimulating electrode (SNE-100; Kopf Instruments, Bern, Germany). Electrical stimuli (150 μsec) were delivered at a frequency of 0.05 Hz. Extracellular recordings of field potentials were obtained using microelectrodes filled with 3 M NaCl (3–8 MΩ). The

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Correspondence should be addressed to Dr. Po-Wu Gean at the above address.

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stimulus intensity was adjusted individually for each experiment to produce field potential amplitude that was 40–50% of the maximal response. Experimental treatments were not initiated until the response had been stable for at least 20 min. The strength of synaptic transmission was quantified by measuring the amplitude of field potentials. The amplitude of field potential was measured as the difference between negative peak and the average value of the following positive peak. Electrical signals were amplified by using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) and recorded on a Gould 3200 chart recorder. All data were expressed as mean ± SE. Statistical analysis was performed using the Student's t test, and $p < 0.05$ was considered statistically significant.

5-HT and forskolin were obtained from Sigma (St. Louis, MO), and other chemicals were purchased from Research Biochemicals (Natick, MA).

RESULTS

PKA mediates isoprotenol-induced synaptic potentiation

Previous studies from this laboratory have demonstrated that administration of the β-adrenergic agonist isoproterenol (Iso) to the BLA neurons resulted in long-term enhancement of synaptic transmission (Huang et al., 1996). We tested whether β-adrenergic response is mediated by stimulation of adenylyl cyclase, resulting in an increase in intracellular cAMP by application of forskolin, a direct activator of adenylyl cyclase. Figure 1*A* shows that forskolin (25 μM) produced an effect similar to that of Iso. In seven cells, the amplitude of EPSP was increased to 188% of baseline, which remained potentiated for at least 30 min after washout of the drug (control, 4.8 ± 0.6 mV; 30 min after treatment with forskolin, 9.0 ± 0.9 mV, $n = 7$, $p < 0.001$). The sustained enhancement of EPSP could be caused by a slow washout of Iso or forskolin. To examine this possibility, a β-receptor blocker propranolol was applied during the washing period. In three neurons tested, propranolol (1 μM) did not affect Iso-induced potentiation (data not shown), suggesting that the long-term effect was not caused by a continued activation of receptors. Moreover, extracellular field recordings were made because it could be maintained for a long period of time. Electrical stimulation of the lateral nucleus resulted in a biphasic response, presumably a presynaptic fiber volley followed by a postsynaptic potential. Bath application of the ionotropic glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) abolished the latter component, demonstrating that this component was mediated by excitatory synaptic transmission (Fig. 1*B*). Figure 1*C* shows that forskolin induced a long-term enhancement of field potentials. The amplitude of field potentials was 191 ± 15% ($n = 7$) of control 105 min after application of forskolin. To test the involvement of cAMP-independent action of forskolin, 1,9-dideoxy-forskolin (25 μM), which has no effect on adenylyl cyclase but does mimic many cAMP-independent actions of forskolin (Laurenza et al., 1989), was applied. In four neurons, 1,9-dideoxy-forskolin did not significantly affect the amplitude of field potentials (104 ± 8%).

As a further test of the involvement of cAMP, the effect of membrane-permeable cAMP analog Sp-cAMPS (25 μM) on the EPSP was investigated. Figure 2*A* shows that superfusion of Sp-cAMPS produced a reversible depression of EPSP, presumably caused by an action at adenosine A_1 receptors (Dunwiddie and Hoffer, 1980; Pockett et al., 1993), and no LTP was seen after washout of the drug. Sp-cAMPS was therefore coapplied with 3-isobutyl-1-methylxanthine (IBMX, 50 μM), an adenosine A_1 receptor antagonist and phosphodiesterase inhibitor. IBMX alone increased the amplitude of EPSP to 146 ± 10% ($n = 6$) of control, which was completely reversible on washout of the IBMX. As shown in Figure 2*B*, concurrent application of Sp-cAMPS and IBMX did not result in a depression such as that caused by Sp-cAMPS alone. By contrast, they induced a long-term enhancement of EPSP (177 ± 15% of control; $n = 7$; $p < 0.001$).

Finally, a catalytic site antagonist for PKA was applied to confirm the involvement of PKA in the action of Iso. In these experiments, slices were presoaked in 1 μM KT 5720 for at least

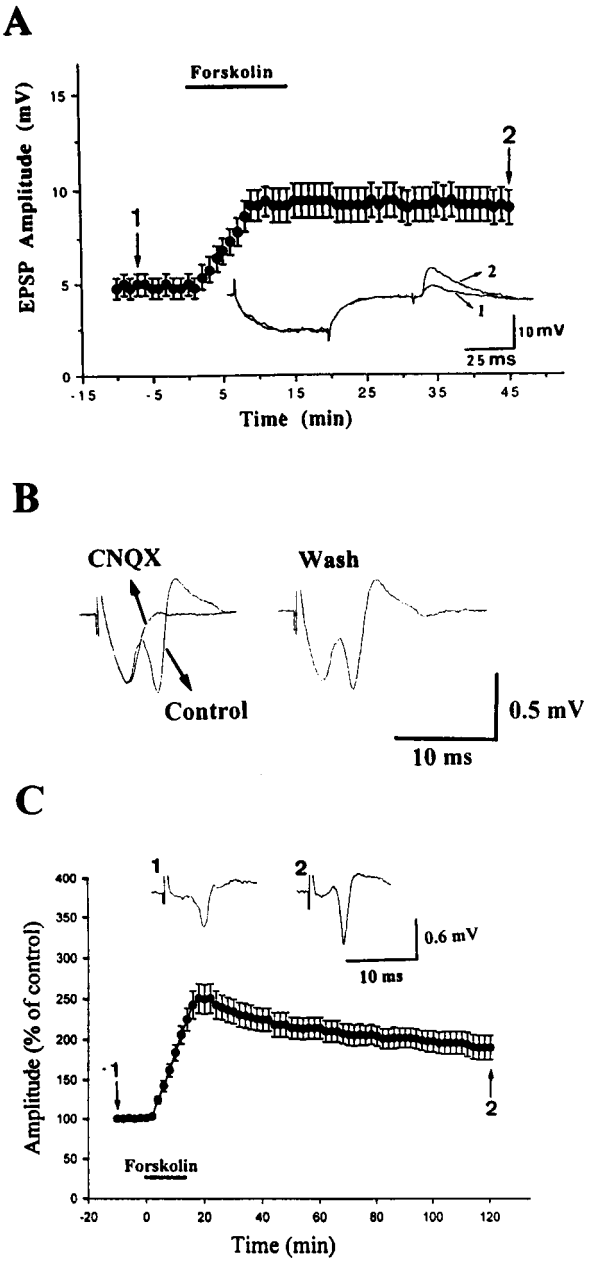


Figure 1. Long-term potentiation of EPSP induced by forskolin in BLA neurons. *A*, The amplitude of EPSP was plotted as a function of time. *Bar* denotes period of application of forskolin (25 μM). *Inset* shows superimposed records taken at different times as indicated. The EPSP was preceded by a transient hyperpolarizing current pulse (0.2 nA, 50 msec) passed through the recording electrode to monitor input resistance. *B*, Extracellular recordings of a typical biphasic potential, demonstrating that the latter event was sensitive to CNQX (10 μM). *C*, LTP of extracellularly recorded potentials induced by forskolin (25 μM).

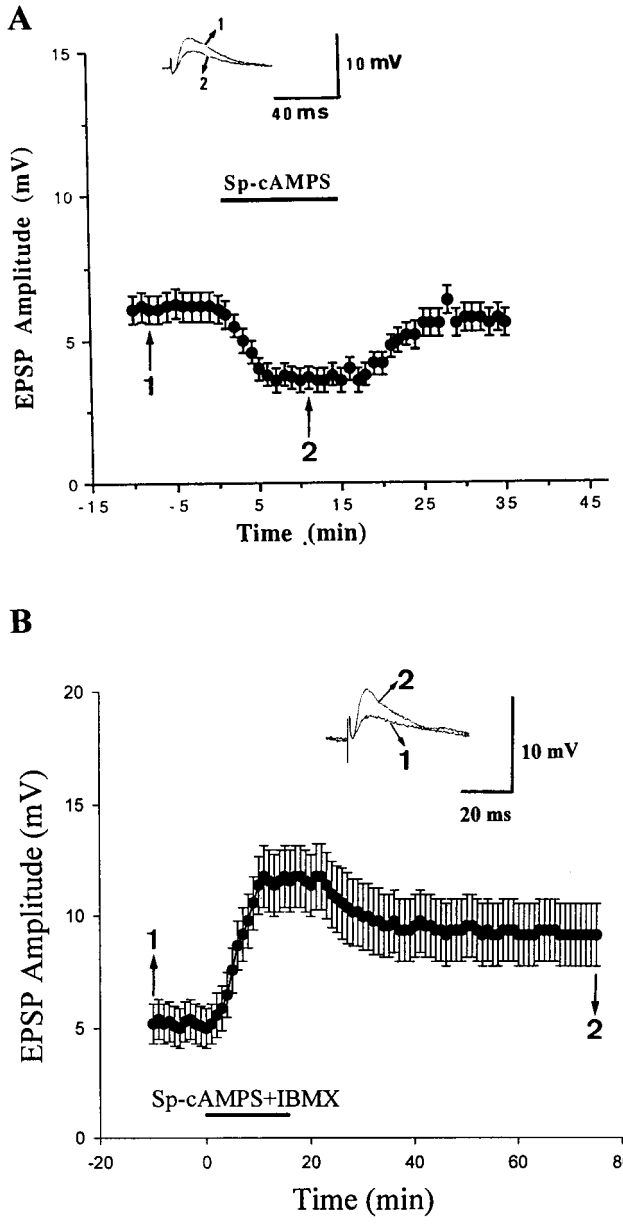


Figure 2. Effect of Sp-cAMPS on the EPSP. *A*, Application of Sp-cAMPS (25 μM) caused a depression of EPSP that returned to control level after washout of the drug without initiating LTP. *B*, The depression caused by Sp-cAMPS was prevented by IBMX (50 μM) and, in the presence of IBMX, Sp-cAMPS induced LTP.

1 hr before being transferred to the recording chamber where the drug was maintained at the same concentration. Figure 3 shows that Iso-induced potentiation was completely blocked in KT 5720-pretreated slices. In control slices, the EPSP amplitude was increased to 194% of baseline by Iso (15 μM) (EPSP amplitude was 6.7 ± 0.6 mV before and 13.0 ± 0.9 mV 30 min after treatment with Iso, $n = 7$), whereas in KT 5720-pretreated slices the EPSP amplitude was 6.0 ± 1.3 mV before and 6.0 ± 1.4 mV ($n = 7$) in the presence of Iso. The difference in the effect of Iso between two groups was statistically significant ($p < 0.01$, unpaired t test). Similar result was obtained when slices were preincubated with 25 μM Rp-cAMPS, a PKA regulatory site antagonist (EPSP amplitude was 6.2 ± 0.9 mV before and 7.0 ± 1.0 mV in the presence of Iso, $n = 6$) (Fig. 3).

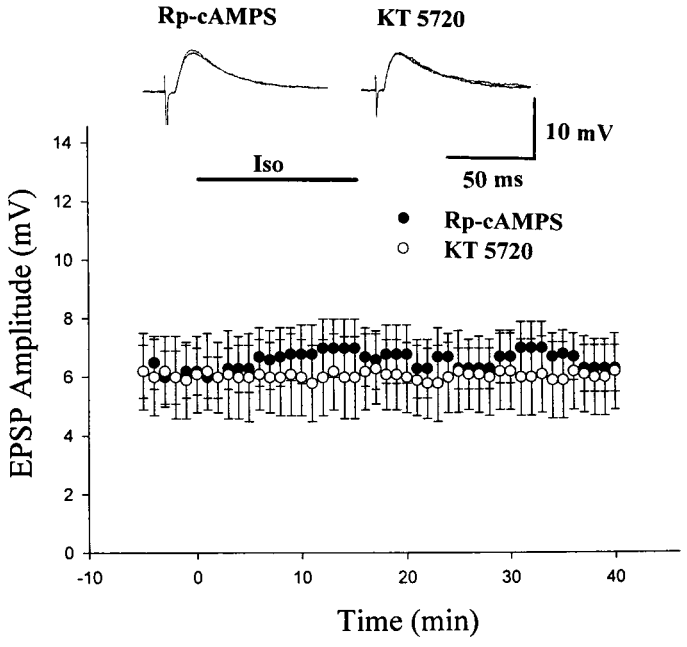


Figure 3. Blockade of Iso-induced potentiation by PKA inhibitors. Slices were incubated for at least 1 hr in 25 μM Rp-cAMPS or 1 μM KT 5720 before being transferred to the recording chamber where the same concentration of drugs was maintained. Iso-induced potentiation normally observed in control slices was blocked in the Rp-cAMPS- or KT 5720-treated slices.

Blockade of Iso- and forskolin-induced potentiation by 5-HT

We examined the influence of 5-HT on Iso-induced potentiation because a subtype of 5-HT receptors (5-HT_{1A}) has been shown to reduce forskolin-stimulated cAMP formation in the hippocampus (De Vivo and Maayani, 1986). Figure 4*A* shows that superfusion of 5-HT (10 μM) depressed the EPSP amplitude to 52% of control (control, 8.8 ± 1.4 mV; in the presence of 5-HT, 4.6 ± 0.5 mV, $n = 6$). Iso was then added to the bath but failed to enhance the EPSP. In the presence of 5-HT, the EPSP amplitude in Iso remained 100 ± 10% ($n = 6$), which was significantly different from those in the absence of 5-HT (194 ± 13%; $n = 7$; $p < 0.001$). Similarly, in the presence of 5-HT, forskolin also failed to affect the EPSP (103 ± 7%; $n = 6$; Fig. 4*B*).

We sought to clarify which 5-HT receptor subtype was involved in the inhibition of synaptic transmission and forskolin-induced potentiation. Figure 5*A* shows that the depression of EPSP produced by 5-HT was blocked by the selective 5-HT_{1A} receptor antagonist 1-(2-methoxyphenyl)-4[4-(2-phthalimido)butyl] piperazine (NAN-190, 2 μM). In the presence of 5-HT and NAN-190, forskolin potentiated the EPSP amplitude to 196 ± 13% ($n = 6$) of control, which was not significantly different from that without 5-HT + NAN-190. Furthermore, the selective 5-HT_{1A} receptor agonist 8-hydroxy-dipropylaminotetralin (8-OH-DPAT, 10 μM) reversibly depressed the EPSP amplitude to 53 ± 5% of baseline and, in the presence of 8-OH-DPAT, forskolin failed to affect the EPSP amplitude (103 ± 10%; $n = 6$; Fig. 5*B*).

Cross-talk mechanism between 5-HT and β-adrenergic receptors

We intended to identify the level in the second messenger cascade at which the cross-talk between 5-HT and β-adrenergic receptors

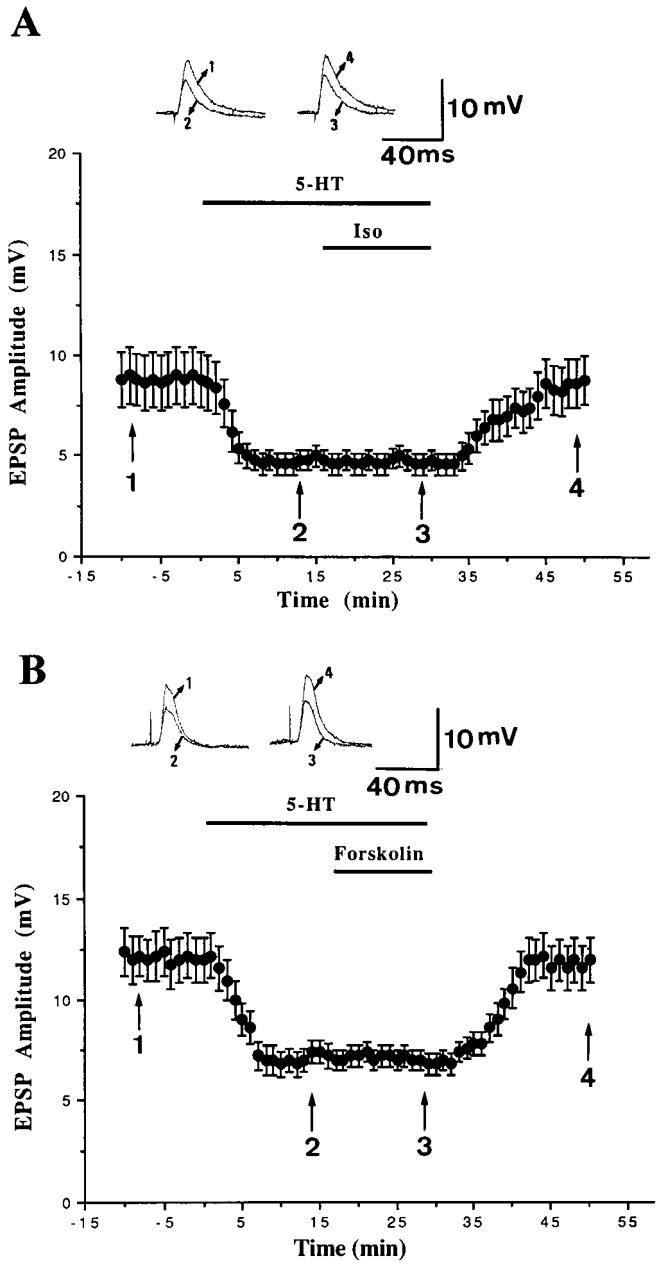


Figure 4. Antagonism of Iso- and forskolin-induced potentiation by 5-HT. Application of 5-HT (10 μM) reduced synaptic responses. Subsequent addition of Iso (15 μM) (*A*) or forskolin (25 μM) (*B*) in the presence of 5-HT failed to potentiate the EPSP.

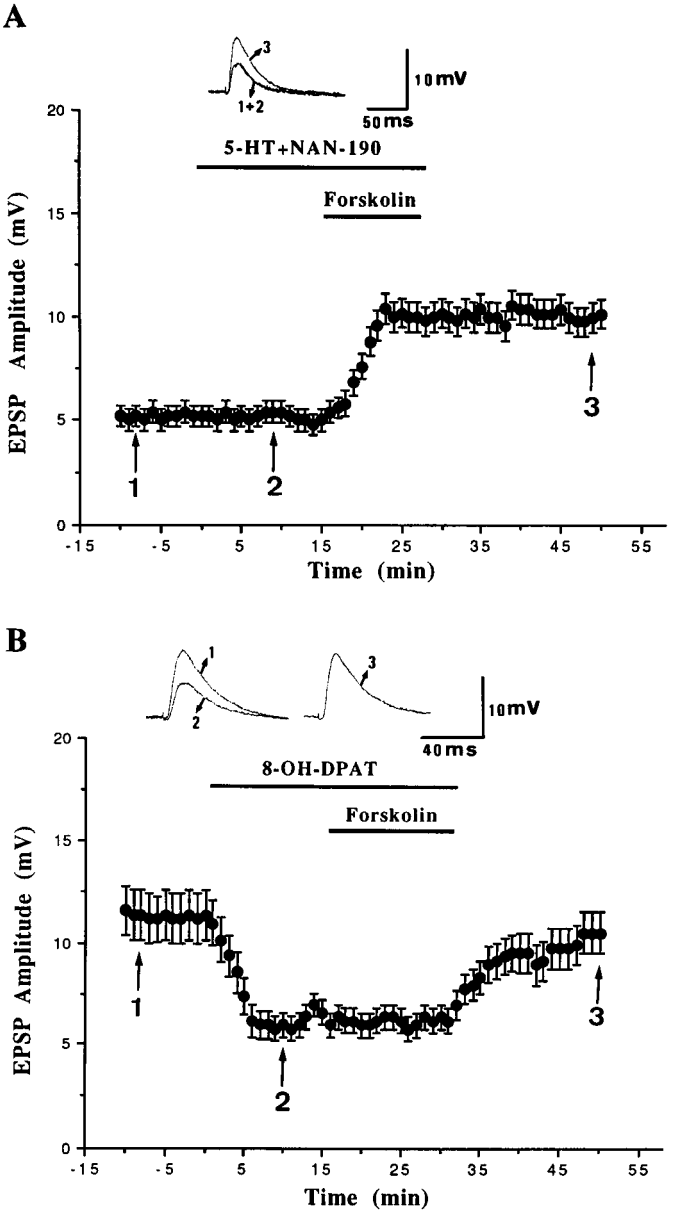


Figure 5. The effect of 5-HT is blocked by the selective 5-HT_{1A} receptor antagonist and is mimicked by the selective 5-HT_{1A} agonist. *A*, In the presence of NAN-190 (2 μM), 5-HT did not affect the EPSP significantly. Subsequent application of forskolin (25 μM) induced potentiation. *Inset* shows superimposed traces taken at the time points indicated. *B*, Application of 8-OH-DPAT (10 μM) mimicked 5-HT in reducing synaptic responses. Subsequent addition of forskolin (25 μM) in the presence of 8-OH-DPAT failed to potentiate the EPSP.

took place. If the cross-talk occurred at the enzyme adenylyl cyclase, then 5-HT could not block the effect of cAMP analog. On the other hand, if the cross-talk took place at the level downstream from cAMP production, then 5-HT should be able to inhibit the effect of cAMP analog. These experiments were performed in the presence of IBMX to prevent the degradation of cAMP analog and to block adenosine A_1 receptors. Figure 6 shows that Sp-cAMPS + IBMX no longer induced potentiation in slices pretreated with 5-HT; EPSP amplitudes were 10.2 ± 0.6 mV for control and 9.6 ± 0.6 mV ($n = 6$) 40 min after washout of Sp-cAMPS + IBMX. These results indicate that the action is downstream of cAMP production; likely at the Ca^{2+} channels or the release processes.

Thus, it is possible that 5-HT may block Iso-induced potentiation by inhibiting Ca^{2+} influx. This hypothesis is supported by our recent report showing that activation of 5-HT_{1A} receptors in the amygdala depressed synaptic transmission primarily by inhibiting presynaptic Ca^{2+} channels (Cheng et al., 1998). If this is the case, we predicted that (1) buffering intraterminal Ca^{2+} with chelator should be able to block Iso-induced potentiation; (2) 5-HT should be able to depress synaptic transmission in forskolin-treated slices because its action is downstream of cAMP production; and (3) neurotransmitters that have been shown to inhibit Ca^{2+} channels are expected to reduce forskolin-induced potentiation too.

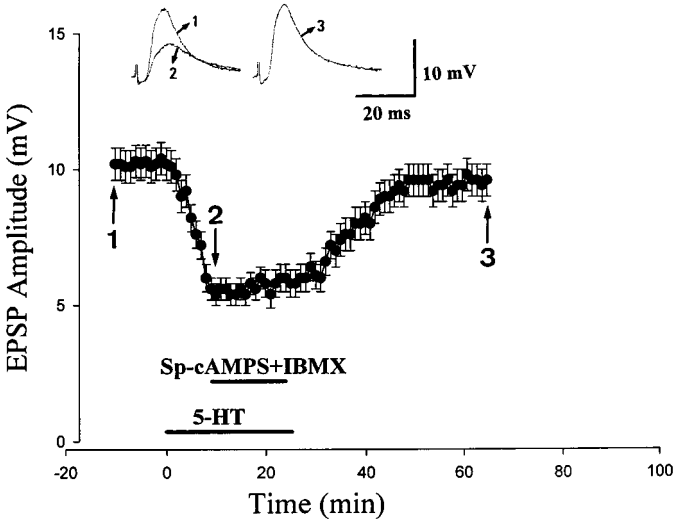
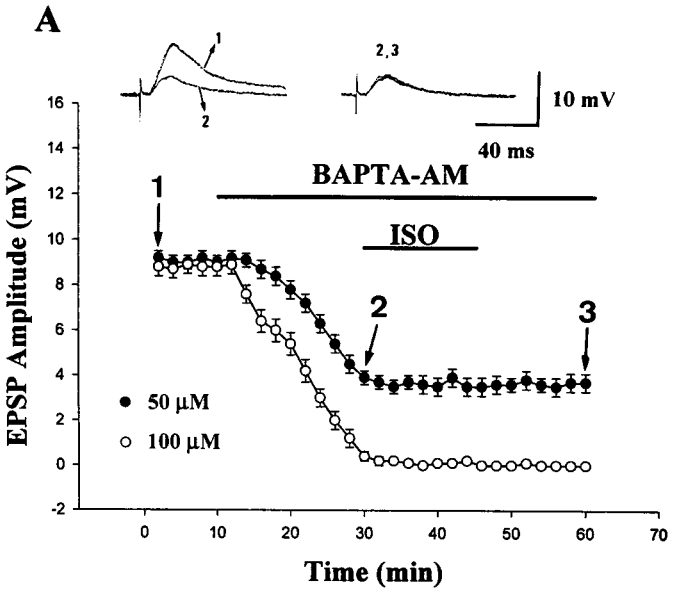


Figure 6. Antagonism of Sp-cAMPS-induced LTP by 5-HT. Application of 5-HT (10 μM) depressed the EPSP. Subsequent application of Sp-cAMPS (25 μM) + IBMX (50 μM) failed to potentiate the EPSP.



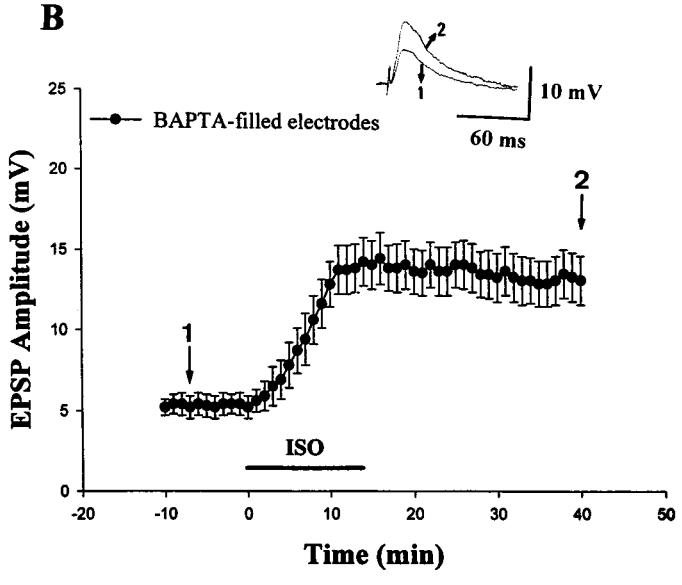


Figure 7. A rise in intraterminal Ca^{2+} is required for the Iso-induced potentiation. *A*, Superfusion of BAPTA-AM concentration-dependently depressed the EPSP and abolished Iso-induced potentiation. The amplitude of EPSP was plotted against time. *Bars* denote the periods of delivery of BAPTA-AM and 15 μM Iso. *B*, The effect of Iso was not affected by intracellular BAPTA. Electrodes were filled with BAPTA (50 mM) as described in Materials and Methods.

First, as demonstrated in Figure 7*A*, application of membrane-permeant Ca^{2+} chelator BAPTA-AM, which concentration dependently decreased the amplitude of EPSP, virtually abolished Iso-induced potentiation. To differentiate presynaptic or postsynaptic sites of action, we loaded the recorded postsynaptic neuron with BAPTA salt. After impalement, the cells were allowed to stabilize for at least 30 min to allow the cell to fill with BAPTA, which was manifested by blockade of slow afterhyperpolarization. Baseline responses were then obtained for a further 10 min before superfusing Iso. As shown in Figure 7*B*, Iso still induced a potentiation under this condition (control, 5.2 ± 0.5 mV; 25 min after treatment with 15 μM Iso, 13.0 ± 1.5 mV, $n = 6$, $p < 0.001$), suggesting a rise in intraterminal Ca^{2+} is required for the action of Iso.

Second, as shown in Figure 8, when forskolin (50 μM) was superfused into the bath, field potentials increased gradually. Subsequent addition of 5-HT (30 μM) reduced the slope of field potentials by 61 ± 2% ($n = 6$), not significantly different from that observed without forskolin pretreatment (63 ± 6%; $n = 6$). Thus, PKA pretreatment did not affect the action of 5-HT. Thirdly, we examined the effects of activation of adenosine A_1 and $GABA_B$ receptors, two receptors known to inhibit Ca^{2+} channels (Pfrieger et al., 1994; Wu and Saggau, 1994, 1995) on the forskolin-induced potentiation. Superfusion of N^6-cyclopentyladenosine (CPA, 0.5 μM), an adenosine A_1 receptor agonist, depressed the EPSP amplitude to 62 ± 6% of baseline ($n = 6$). However, it can be seen from Figure 9, the effect of forskolin (25 μM) was completely blocked in the presence of CPA. Similar experiments were performed for baclofen, a $GABA_B$ receptor agonist. Figure 9 shows that application of baclofen (5 μM) nearly abolished the EPSP. Subsequent addition of forskolin (25 μM) no longer induced potentiation.

DISCUSSION

In the present study, we intend to accomplish two goals. First, we wish to identify the intracellular mechanism by which Iso produces long-term enhancement of EPSP. Second, we wish to illuminate any convergence between the 5-HT-induced EPSP inhibition and Iso-induced enhancement.

PKA-mediated synaptic enhancement

Our previous report has shown that when Iso was perfused into the bath, the amplitude of EPSP began to increase in 2–3 min. The Iso effect was long-lasting and was blocked by the antagonist propranolol; besides, it did not affect the resting membrane potential or neuronal input resistance (Huang et al., 1996). The long-latency of Iso action and its sustained effect suggested that a chain of biochemical steps involving second messengers might be the mechanism leading to the enhancement. In the present study, we have demonstrated that forskolin but not 1,9-dideoxy-forskolin mimics the effect of Iso. Similarly, activation of PKA by

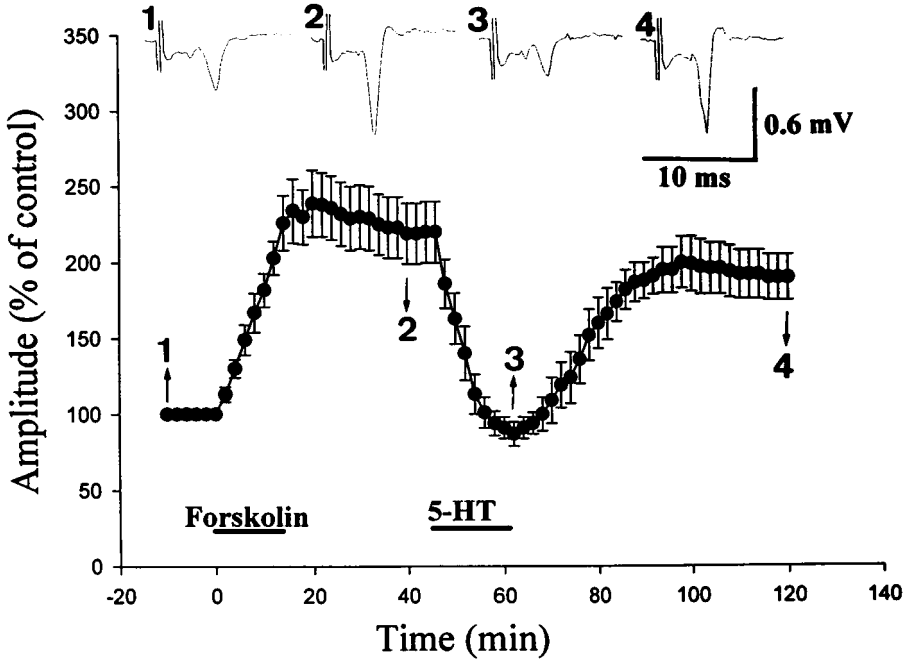


Figure 8. Forskolin pretreatment did not affect the action of 5-HT. As forskolin (50 μM) was perfused into the bath, the amplitude of field potential gradually increased. Thirty minutes after application of forskolin, 5-HT (30 μM) was added, which still exerted an average of 61 ± 2% inhibition.

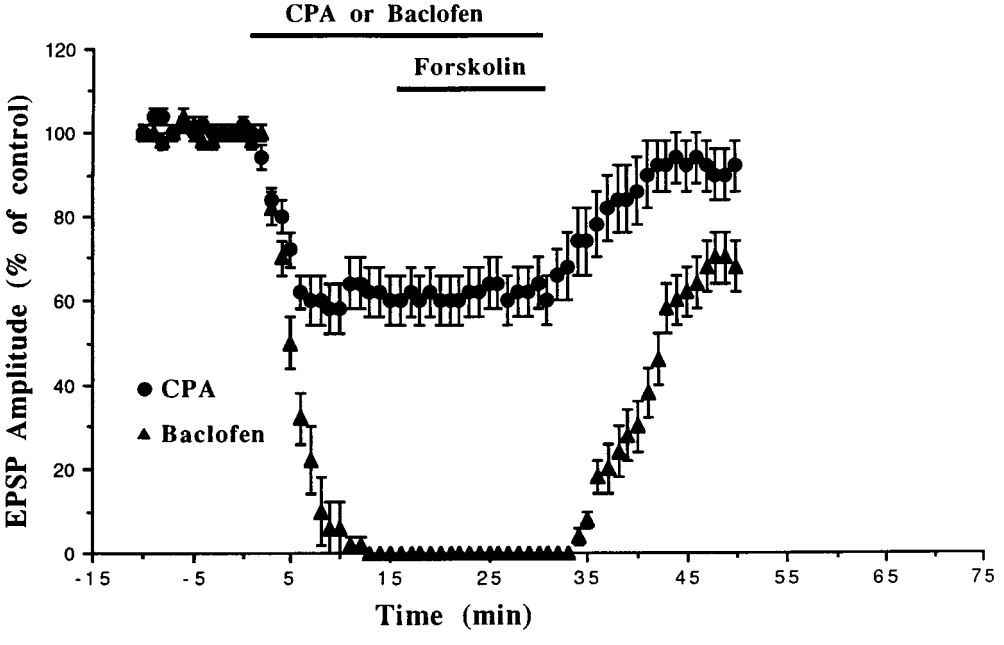


Figure 9. Antagonistic effect of 5-HT on the forskolin-induced LTP is mimicked by adenosine A_1 or $GABA_B$ receptor agonists. Application of CPA (0.5 μM) or baclofen (5 μM) depressed the EPSP. Subsequent addition of forskolin (25 μM) in the presence of these agonists failed to potentiate the EPSP.

Sp-cAMPS results in a potentiation. Furthermore, the effect of forskolin was blocked by KT 5720 or Rp-cAMPS, the respective PKA catalytic and regulatory site inhibitors. These results suggest that PKA mediates the enhancement of EPSP by Iso in the amygdala.

Iso-induced potentiation was blocked in Ca^{2+}-free solution or by specific P/Q type Ca^{2+} channel blockers (Huang et al., 1996), suggesting that potentiation required Ca^{2+} entry into presynaptic terminals. Of the known adenylyl cyclases, type 1 calmodulin-sensitive adenylyl cyclase (AC1) is stimulated directly by Ca^{2+} and is synergistically stimulated by combination of Ca^{2+} and receptor activation. In view of the fact that AC1 is neurospecific, expressed in the amygdala (Xia et al., 1991), and is important for synaptic plasticity (Mons and Cooper, 1995; Xia and Storm, 1997; Storm et al., 1998; Villacres et al., 1998), it is hypothesized that entry of Ca^{2+} into the presynaptic terminal activates AC1, resulting in an increase in intracellular cAMP and activation of PKA, which subsequently phosphorylates Ca^{2+} channels and causes a persistent increase in glutamate release.

PKA-mediated synaptic potentiation has been described in several brain areas, including hippocampal mossy fiber-CA3 synapses and cerebellar parallel fiber–Purkinje cell synapses (Huang et al., 1994; Weisskopf et al., 1994; Salin et al., 1996). It is suggested that modulation of these synapses by PKA occurs via presynaptic mechanisms that do not alter Ca^{2+} entry; PKA may directly modulate the secretory processes, or the silent release sites are activated by PKA (Trudeau et al., 1996; Chen and Regehr, 1997; Chavis et al., 1998). This study is one of a few examples in which modulation of presynaptic Ca^{2+} channels contributes to synaptic enhancement (McGehee et al., 1995).

Blockade of β-adrenergic response by 5-HT$_{1A}$

5-HT$_{1A}$ receptors are representative of a family of neurotransmitter receptors known to couple functionally to $G_{i/o}$ proteins to inhibit adenylyl cyclase (Andrade et al., 1986). We have shown that activation of presynaptic 5-HT$_{1A}$ receptors reduces transmitter release in the basolateral amygdala (Cheng et al., 1998). Previous biochemical studies have shown that activation of G_i-linked receptors inhibited forskolin-stimulated adenylyl cyclase (Cooper et al., 1980; Fredholm et al., 1985; Taissig et al., 1993) and, on the other hand, enhanced the ability of β-adrenergic receptors to generate cAMP (Tang et al., 1991). It was suggested that activation of these G_i-linked receptors led to liberation of βγ complex, which alone had no effect on adenylyl cyclase but potentiated $G_{s\alpha}$-stimulated adenylyl cyclase II activity. This hypothesis was supported recently by the electrophysiological studies showing that 5-HT$_{1A,}$ GABA$_B$, and α-adrenergic receptor agonists enhanced β-adrenergic-mediated reduction in the I_{AHP} in rat hippocampal CA1 neurons (Andrade, 1993; Pedarzani and Storm, 1996). In the present study, we have shown that 5-HT inhibited synaptic transmission and completely blocked Iso-induced potentiation. Blockade of Iso-induced potentiation by 5-HT could be prevented by NAN-190, indicating its mediation by 5-HT$_{1A}$ receptors. Our data suggest antagonistic interaction between 5-HT and β-adrenergic receptors. Interestingly, Sp-cAMPS alone depressed the EPSP without inducing potentiation after its washout. Only in the presence of IBMX did Sp-cAMPS induce LTP. These results suggest that Sp-cAMPS may act as an adenosine A_1 agonist and further support the antagonistic relationship between adenosine A_1 and β-adrenergic receptors. Finally, our results, although in contrast to the hypothesis suggested by Andrade (1993), are consistent with the report of Gerber and Gahwiler (1994), who showed that the action of Iso on I_{AHP} was curtailed significantly by GABA$_B$ or adenosine A_1 receptor agonists in rat hippocampal CA3 neurons.

Cross-talk mechanism

In principle, the cross-talk described in the present study might occur at any steps involving the modulation of β-adrenergic receptor, G-proteins, adenylyl cyclase, cAMP metabolism, PKA, Ca^{2+} channels, or the release processes. However, the enhancement of EPSP induced by activation of β-receptor, direct stimulation of adenylyl cyclase by forskolin, or application of cAMP analog was completely blocked in the presence of 5-HT. These results indicate that the interaction between the intracellular signaling pathways activated by 5-HT and Iso occurs at a step downstream from cAMP production. Indeed, several lines of evidence support this hypothesis. First, buffering intraterminal Ca^{2+} with membrane-permeant Ca^{2+} chelator blocked Iso-induced potentiation. This finding coupled with our previous observation that 5-HT inhibits glutamate release via a presynaptic blockade of Ca^{2+} influx suggests that signaling pathways activated by these two receptors may converge at presynaptic Ca^{2+} channels. Second, If 5-HT action depends on adenylyl cyclase (AC) inhibition, there should be a simple summation of agonist and AC inhibitor effects. Because forskolin produced a 88% increase in EPSP amplitude, and 5-HT decreased EPSP amplitude by 48%, the sum result would be an increase of ~40%. In fact, pretreatment with 5-HT completely blocked the forskolin enhancement. More importantly, 5-HT depressed the normal and forskolin-enhanced EPSP to the similar extent (~50%), indicating that 5-HT directly targets Ca^{2+} channels. Moreover, an important note from Figure 8 is that delayed application of 5-HT in forskolin-pretreated slices depressed the EPSP in a reversible manner. These results indicate that 5-HT blocks the induction but not the expression of forskolin-induced potentiation.

Finally, adenosine A_1 and GABA$_B$ receptor agonists, which have been shown to depress transmitter release by inhibiting presynaptic Ca^{2+} channels (Pfrieger et al., 1994; Wu and Saggau, 1994, 1995), also blocked forskolin-induced potentiation.

Functional implication

The functional role of cross-talk explored here is not clear. Because cAMP induces or enhances epileptiform activity (Yokoyama et al., 1989; Boulton et al., 1993), and elevation of cAMP levels are measured in amygdala-kindled animals (Mori, 1983), it is expected that 5-HT$_{1A}$ receptors may have physiological consequence of reducing the response to prolonged excitatory synaptic inputs and act as an anticonvulsant in the amygdala.

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