Isoproterenol Potentiates Synaptic Transmission Primarily by Enhancing Presynaptic Calcium Influx via P- and/or Q-Type Calcium Channels in the Rat Amygdala

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The effects of selective β-adrenergic receptor agonist isoproterenol (Iso) on neuronal excitability and synaptic transmission were investigated in brain slices of rat amygdala. Iso (15 μM) produced a long-lasting enhancement of the EPSP that was not blocked by pretreatment with 20 μM D-2-amino-5-phosphonovalerate (D-APV) alone or D-APV in combination with kynuretic acid (1 mM). The sensitivity of postsynaptic neurons to the glutamate receptor agonist AMPA was unchanged by Iso pretreatment. Superfusion of Iso reversibly blocked the after-hyperpolarization (AHP) that followed a depolarizing current pulse and caused more action potential firing. Intracellular application of a selective inhibitor of the catalytic subunit of cAMP-dependent protein kinase A blocked the effect of Iso on the AHP, whereas Iso-induced potentiation was entirely normal in the same neuron. In addition, Iso decreased the magnitude of paired-pulse facilitation, which is consistent with a presynaptic mode of action.

Substituting the Mg^{2+} for Ca^{2+} in the medium completely abolished the Iso-induced enhancement of the EPSP. The effect of Iso also was blocked by low concentrations of ω-agatoxin-IVA, but not by nifedipine or ω-conotoxin-GVIA. These results suggest that Iso enhances synaptic transmission in the amygdala via a presynaptic site of action: the mechanism underlying the potentiating effect likely is attributable to an increased Ca^{2+} influx through P- and/or Q-type Ca^{2+} channels.

Key words: isoproterenol; glutamate receptor; synaptic transmission; long-term potentiation; calcium channel; amygdala

Norepinephrine (NE) is a putative neurotransmitter that is found throughout the mammalian CNS. The basolateral and central nuclei of the amygdala receive adrenergic innervations from neurons in the locus ceruleus and other brainstem nuclei (Jones and Moore, 1977; Fallon et al., 1978). These catecholaminergic fibers course through the median forebrain bundle and enter the amygdaloid complex primarily through the ventral amygdalofugal pathway.

The role of NE in modulating neuronal activity has been of interest for some time. Previous reports on the hippocampus have shown that NE can produce both excitatory and inhibitory effects on neuronal excitability, depending on the receptor subtypes activated (Mueller et al., 1982; Madison and Nicoll, 1986). Thus, NE increases the single-unit activity and evoked responses via action on β-adrenergic receptors (Heginbotham and Dunwiddie, 1991). However, activation of α-adrenergic receptors caused the attenuation of population spike (Mueller et al., 1982). Intracellular studies with both hippocampal and neocortical slices also showed that postsynaptic β-adrenergic receptors mediated the reduction of Ca^{2+}- and Na^{+}-dependent K^{+} currents (Madison and Nicoll, 1982; Foehring et al., 1989). However, no detailed intracellular analysis of β-adrenergic effect has been performed in the amygdala.

In the present study, we attempted to investigate the effects of isoproterenol (Iso), a β-adrenergic agonist, on the basolateral amygdala (BLA) neurons. We found that Iso exerts a dual effect on the BLA neurons: (1) Iso enhances cellular excitability by

blocking the spike frequency accommodation; and (2) Iso, via activation of the presynaptic β-adrenoreceptor, persistently enhances synaptic transmission by increasing transmitter release. Furthermore, by using antagonists selective for L-, N-, P-, or Q-type Ca^{2+} channels (Miller, 1987; Mintz et al., 1992; Luebke et al., 1993; Takahashi and Momiyama, 1993; Castillo et al., 1994; Wheeler et al, 1994), we first demonstrated that P- and/or Q-channels, but not L- or N-channels, likely were involved in Iso modulation of transmitter release at rat amygdalar excitatory synapses.

MATERIALS AND METHODS

Slice preparation and intracellular recordings. Experiments were performed on amygdaloid slices (500 μm thick) prepared from male adult Sprague–Dawley rats (125–200 gm). The rats were decapitated, and transverse slices were cut from tissue block of the brain using Vibroslice (Campden Instruments, Silbey, UK). The slices were placed in a beaker of artificial CSF (ACSF) oxygenated with 95% O_2/5% CO_2 and kept 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.

A single slice then 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. A bipolar stimulating electrode (Kopf Instruments SNE-200X, Bern, Germany) was placed in the ventral endopyriform nucleus, near the recording electrode (1–2.5 mm apart). Orthodromic stimuli were delivered with monophasic constant-voltage pulses at 0.033 Hz from a Grass stimulator with an isolation unit. Protein kinase A inhibitor (PKI; 45 μM/ml; activity, 1 μg of protein inhibits 4000 phosphorylating units of protein kinase A; Sigma, St. Louis, MO), dissolved in 3 M KCl, was applied intracellularly by allowing it to leak from the electrode for at least 40 min according to the method of Cerne et al. (1992).

Intracellular recording microelectrodes were pulled from 1.0 mm microfiber capillary tubing on a Brown-Flaming electrode puller (Sutter

Instruments, San Rafael, CA). The electrodes were filled with 3 M KCl with resistance ranging from 70 to 120 MΩ. The microelectrode tips were positioned into the basolateral subdivision of the amygdala. Electrical signals were amplified by using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) and recorded on a Gould 3200 chart recorder. Signals also were digitized and displayed on a Hewlett-Packard plotter. All data are expressed as mean ± SE. Statistical analysis was performed using the Student's t test, and $p < 0.05$ was considered statistically significant.

Preparation of isolated amygdalar neurons and whole-cell recordings. Amygdalar neurons were isolated using a technique adapted from Kay and Wong (1986). Male Sprague–Dawley rats aged 2–3 weeks were decapitated, and serial transverse slices were cut. Amygdalar regions were dissected under a dissection microscope and then transferred to 10 ml of 1,4-piperazine(ethanesulfonic acid) (PIPES) saline solution in a spinner flask. The temperature was maintained at 32 ± 1°C, and 2–3 mg of protease XIV (Sigma) was added. The amygdalar minislices were stirred gently with a magnetic stirrer at a rate sufficient to prevent them from settling, and the solution was bubbled continuously with 95% O_2/5% CO_2. The PIPES saline solution contained (in mM): NaCl 120, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, glucose 25, and PIPES 20, pH 7.0.

One hour later, slices were washed with 10 ml of PIPES saline solution (3 times), transferred for storage to a holding chamber filled with continuously bubbled PIPES saline solution at room temperature, and kept at least 1 hr before recording. Slices were used within 2–6 hr. When needed, a slice was transferred to the 1 ml of external solution and the neurons were dissociated by trituration using a fire-polished Pasteur pipette with an ~1 mm tip diameter. The suspension of dissociated amygdalar neurons then was transferred to a 0.5–1 ml recording chamber mounted on an Olympus IMT-2 inverted microscope. Neurons were allowed to settle on poly-L-lysine-coated coverslips.

The whole-cell patch-clamp recording method was used to monitor membrane currents induced by pressure injections (10 psi, 1 sec duration) of AMPA (100 μM) in freshly isolated neurons. The pressure pipette was positioned ~50 μm from the neuronal soma. Patch pipettes for whole-cell recording were pulled from borosilicate glass and fire-polished with a resistance of 2–5 MΩ. Pipette solution contained (in mM): K-gluconate 120, tetraethylammonium chloride 20, HEPES 10, EGTA 2.25, $CaCl_2$ 1, $MgCl_2$ 2, Mg-ATP 4, and Na-GTP 0.3, pH 7.3. Whole-cell currents were recorded using a patch-clamp amplifier (EPC-7, List-Electronic, Darmstadt, Germany) and filtered at 10 KHz. Tetrodotoxin (300 nM) was added to the external solution to suppress spontaneous activity.

All drugs except nifedipine and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were dissolved in ACSF. Nifedipine and CNQX were dissolved in a dimethylsulfoxide (DMSO) stock solution and kept frozen until the day of experiment. The concentrations of DMSO in the perfusion medium ranged from 0.1 to 0.25%. In all three experiments, DMSO of these concentrations did not affect either the active or the passive properties of neurons. Nifedipine, kyurenate, D-2-amino-5-phosphonovalerate (D-APV), and isoproterenol were obtained from Sigma; ω-conotoxin-GVIA (ω-CgTX-GVIA), ω-conotoxin-MVIIC (ω-CgTX-MVIIC), ω-Agatoxin-IVA (ω-Aga-IVA), and CNQX were from Research Biochemicals (Natick, MA).

RESULTS

Orthodromic stimulation of the ventral endopyriform nucleus evoked an EPSP in BLA neurons. The EPSP is mediated via excitatory amino acids acting predominantly on AMPA receptors (Gean and Chang, 1992). Application of Iso (15 μM) persistently potentiated the EPSP amplitude to 198 ± 10% of control ($n = 8$). In another six experiments, we pretreated the slices with propranolol (10 μM) and found that propranolol blocked the effect of Iso (data not shown). Because NMDA receptors play an important role in β-adrenergic receptor activation-induced synaptic plasticity in other brain areas (Burgard et al., 1989; Stanton et al., 1989), we examined whether blockade of NMDA receptor prevented the development of Iso-induced potentiation. Figure 1*A1* shows that Iso (15 μM), administered in the presence of NMDA receptor antagonist D-APV (20 μM), increased the amplitude of EPSP to 202 ± 19% of control ($n = 13$; Fig. 1*A3*), which was not significantly different from that without D-APV preapplication.
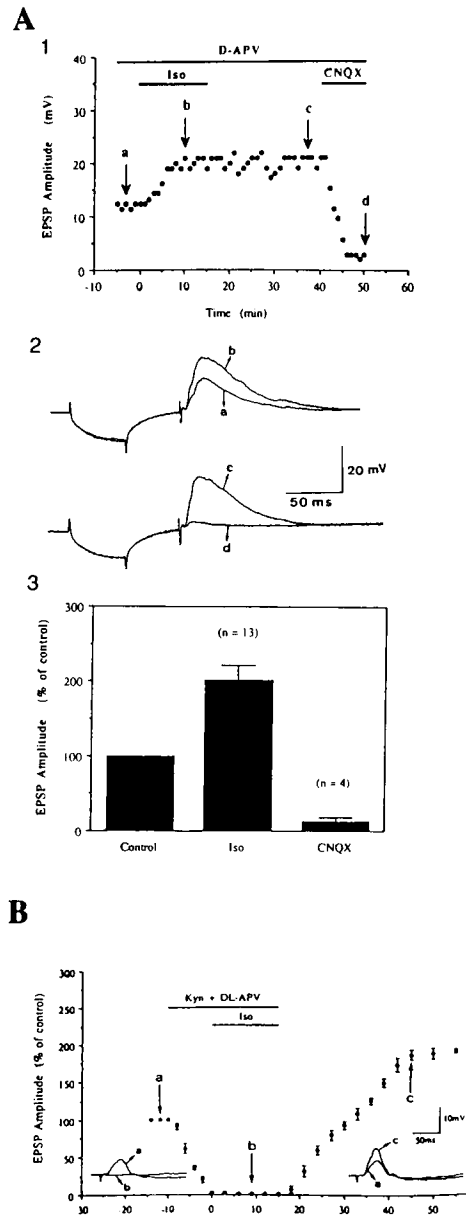
Figure 1. Long-term enhancement of the EPSP by Iso is both NMDA- and non-NMDA-receptor-independent. *A1*, The amplitude of the EPSP is plotted as a function of time. *Bars* denote periods of application of D-APV (20 μM), Iso (15 μM), and CNQX (10 μM). *A2*, Superimposed records of responses taken at different times indicated in *A1*. The EPSP was preceded by a transient hyperpolarizing current pulse (0.15 nA, 50 msec) passed through the recording electrode to monitor the input resistance. *A3*, Bar graph showing the average increase in the amplitude of EPSP by 15 μM Iso (202 ± 19% of control, $p < 0.001$; $N = 13$). In four experiments, CNQX (10 μM) was applied at the end of experiments to ensure that EPSP was mediated by the AMPA receptors. *B*, Blockade of postsynaptic glutamate receptors with Kyn and DL-APV failed to affect the Iso-induced potentiation. After the evoked responses had stabilized, Kyn (1 mM) and DL-APV (50 μM) were added to the bath. Once responses were blocked completely, Iso was applied for 15 min in the presence of Kyn and DL-APV and the drugs were then washed out. *Insets* show typical records taken before, during Kyn + DL-APV application, and after Iso in Kyn + DL-APV and subsequent washout of the antagonists.

The increase persisted long after Iso was washed out. A hyperpolarizing current pulse (0.1 nA, 50 msec) was passed through the recording electrode to monitor the neuronal input resistance. As depicted in Figure 1*A2*, Iso had no measurable effect on either the

input resistance (43.3 ± 2.7 MΩ before and 43.0 ± 2.2 MΩ after the superfusion of Iso, $P > 0.1$; $n = 10$) or the resting membrane potential (−66.2 ± 1.1 mV before and −65.6 ± 1.1 mV after the application of Iso, $P > 0.1$; $n = 10$).

To address further the question of whether postsynaptic activation of non-NMDA (and NMDA) receptors is required for the effect of Iso, Iso was applied during the blockade of both NMDA and non-NMDA glutamate receptors. Kynuretic acid (Kyn), although having a lower affinity than CNQX, was used to block non-NMDA receptors because it can be washed out quickly. Consistent with previous reports, the EPSP was completely blocked in the Kyn (1 mM)- and DL-APV (50 μM)-containing solution. Iso (15 μM) then was added in the presence of glutamate receptor blockers. As shown in Figure 1B, after washout of the drugs, a clear potentiation of synaptic transmission occurred. The EPSP amplitude averaged 193 ± 3% ($n = 5$) of control 30 min after washout of Iso.

To determine whether the action of Iso was mediated by pre- or postsynaptic mechanisms, we examined the effect of Iso on the postsynaptic responses to exogenously applied AMPA. The experiments were performed in the presence of tetrodotoxin (0.5 μM). As illustrated in Figure 2A, application of AMPA produced a membrane depolarization (21.6 ± 4.8 mV; $n = 8$). Iso (15 μM) pretreatment did not affect the AMPA-induced depolarization (23.0 ± 4.5 mV, $P > 0.1$; $n = 8$; Fig. 2A2). In addition, Iso (15 μM) did not significantly affect currents induced by pressure application of AMPA (100 μM, 10 psi, duration 1 sec; 104 ± 4% of control; $n = 4$) in acutely dissociated amygdala neurons measured by whole-cell recording (Fig. 2B). Furthermore, Iso itself did not induce any change in holding current. These data indicate that the enhancement of EPSP by β-adrenergic receptor activation is not mediated by a postsynaptic action on the AMPA receptors.

It has been demonstrated repeatedly that the slow Ca^{2+}-activated K^+ current, I_{AHP}, underlies the spike frequency accommodation and slow afterhyperpolarization (AHP) in hippocampal and cortical neurons (Madison and Nicoll, 1984; Lancaster and Adams, 1986; Constanti and Sim, 1987). NE acting through β-receptor, cAMP, and cAMP-dependent protein kinase A (PKA) suppresses the I_{AHP} (Madison and Nicoll, 1982, 1986; Pedarzani and Storm, 1993). Therefore, we tested whether Iso-induced potentiation is caused by its inhibition of postsynaptic I_{AHP} by intracellular application of a specific PKI. The responses of a BLA neuron to a depolarizing current pulse consisted of an initial rapid train of action potentials that slowed or accommodated. The train of action potentials was followed by an AHP. Iso blocked the AHP and, consequently, the spike frequency accommodation was reduced in all cells tested ($n = 12$; Fig. 3A). PKI was applied intracellularly for at least 40 min before Iso administration. As depicted in Figure 3B, the effect on AHP usually seen after Iso either was markedly reduced or was completely abolished, whereas the effect on the EPSP persisted. In six neurons with PKI-filled microelectrodes, the amplitude of EPSP was increased to 200 ± 5% by Iso, whereas the AHP was unaffected (AHP of 7.2 ± 1.1 mV before and 7.2 ± 1.3 mV after the application of Iso; $n = 6$). These results suggest that blockade of I_{AHP} in the neuron being recorded is not required for the enhancement of EPSP.

Paired-pulse facilitation (PPF) has been studied extensively and is attributed to an increase in the amount of transmitter released in response to a second stimulus. If Iso-induced potentiation involves presynaptic mechanisms, then it might be associated with a decrease in PPF, because manipulations that enhance transmit-
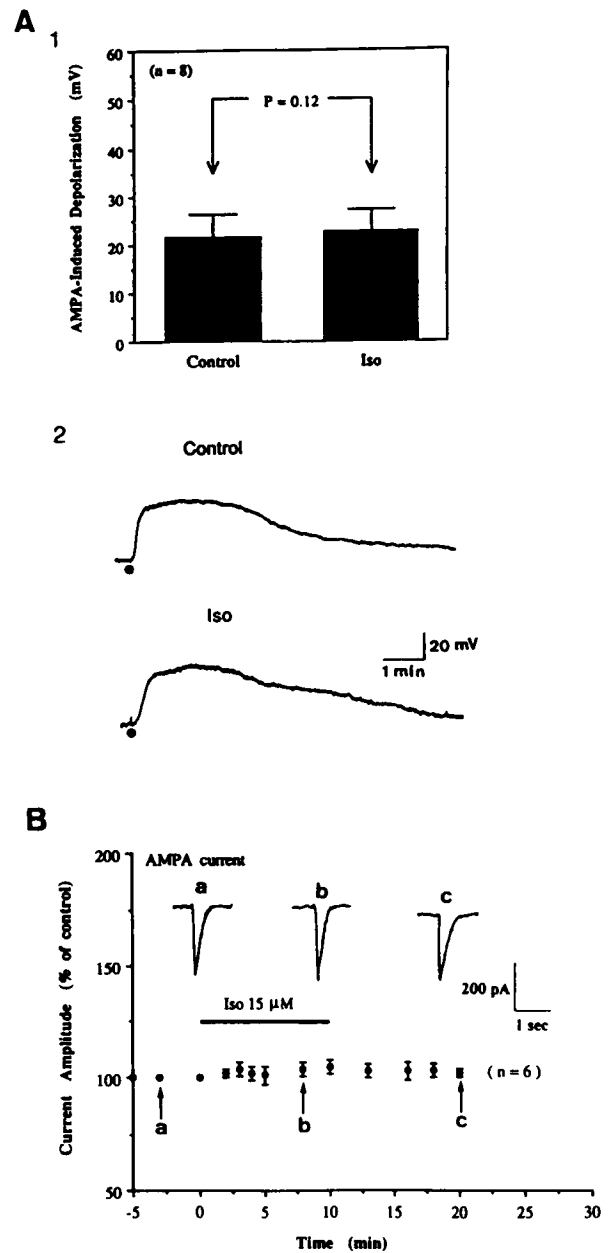
Figure 2. Effect of Iso on AMPA-induced membrane responses. *A1*, Graphic analysis of the effect of Iso pretreatment on the AMPA-induced membrane depolarization. *A2*, Superfusion of AMPA (10 μM, 1 min) evoked a membrane depolarization. Pretreatment of the slice with Iso (15 μM) for 20 min did not affect AMPA-induced depolarization significantly. The experiment was performed in the presence of tetrodotoxin (0.5 μM). *B*, Representative traces showing the lack of effect of Iso (15 μM) on the amplitude of AMPA (100 μM, 10 psi, 1 sec)-evoked currents. Holding potential, −60 mV.

ter release usually decrease the magnitude of PPF (Manabe et al., 1993; Schulz et al., 1994). Therefore, we compared the magnitude of PPF before and after the treatment with Iso. Synaptic responses to a pair of stimuli were recorded with an interstimulus interval of 60 msec (Fig. 4A), and PPF is expressed as the percent increase in the second response compared with the first. Figure 4B is a summary of 12 experiments in which initial PPF is plotted on the x-axis and the change in PPF induced by Iso is plotted on the y-axis. It is clear that in all neurons tested Iso decreased the magnitude of PPF. In addition, there was a significant inverse

A

1 Control   Isoproterenol   Wash

2

B   PKI electrode

Control   Iso

EPSP

AHP
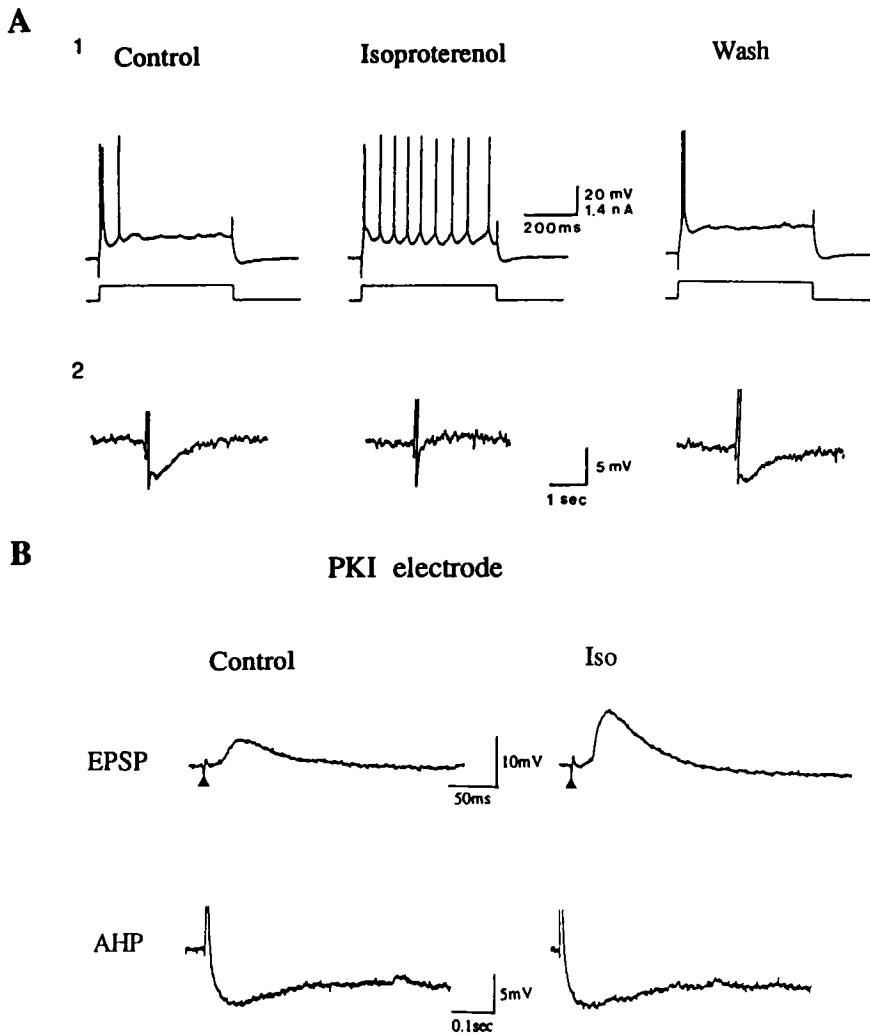
Figure 3. Intracellular application of PKI prevented the effect of Iso on the AHP without blocking the Iso-induced potentiation. *A*, Blockade of the AHP and spike frequency accommodation by Iso. The responses in the *middle column* were obtained 10 min after adding 15 μM Iso to the bathing solution. The records in *Wash* were obtained 20 min after returning to the control solution. *A1*, Records of the response of a BLA neuron to depolarizing current pulses. The current trace is positioned below the voltage trace. *A2*, The AHP after the depolarizing pulse. *B*, Representative traces recorded in a neuron impaled with 3 M KCl + PKI (45 μM/ml)-filled microelectrodes showing that the lack of effect of Iso on the AHP was not blocked by Iso; in the same cell, however, Iso was effective in potentiating the EPSP.

correlation between the initial PPF and the change in PPF induced by Iso, as demonstrated by the best-fit linear regression line (Fig. 4*B*, $y = -0.65x + 0.1$, $r^2 = 0.91$; $n = 12$). Thus, a larger initial PPF was associated with a greater decrease in PPF, whereas smaller initial PPF was associated with less of a decrease. This result is consistent with a presynaptic mode of action.

Previous hippocampal studies have shown that β-adrenergic activation potentiated the function of voltage-dependent Ca^{2+} channels (Fisher and Johnston, 1990), and this increase in channel activity might contribute to enhancement of transmitter release. To investigate the role of presynaptic Ca^{2+} entry in Iso-induced potentiation, we substituted Mg^{2+} for Ca^{2+} in the medium to compensate for the increased Ca^{2+} influx and then examined the action of Iso under this condition. As shown in Figure 5, the EPSP was completely blocked by Ca^{2+}-free solution and, in each of 11 experiments, application of Iso failed to induce a potentiation. The amplitude of EPSP measured 40 min after the application of Iso was $102 \pm 2\%$ ($p > 0.1$; $n = 11$). These findings indicate the importance of presynaptic Ca^{2+} in both transmitter release and Iso-induced potentiation. We have undertaken, therefore, a pharmacological analysis of the effect of selective Ca^{2+} channel blockers on the Iso-induced potentiation.

Nifedipine (20 μM), a selective L-type Ca^{2+} channel blocker, affected neither the synaptic transmission nor the Iso-induced potentiation. In eight neurons, Iso produced an average of 103 ±

16% increase in EPSP amplitude in the presence of nifedipine, which was not significantly different from the potentiation induced by Iso alone (Table 1). Application of N-type Ca^{2+} channel blocker ω-CgTX-GVIA at a saturating dose of 1 μM suppressed the evoked EPSP by an average of $83.3 \pm 6.3\%$ ($p < 0.001$; $n = 7$; Table 1). The amplitude of EPSP was increased to pretoxin levels by increasing the stimulus intensity, and then Iso (15 μM) was applied. Unexpectedly, the amplitude of EPSP was increased to $238 \pm 16\%$ of control ($n = 7$), which was significantly larger than that observed when slices were treated with Iso alone (Table 1).

ω-Aga-IVA, a peptide purified from the venom of funnel web spider, has been reported to specifically block the P-type Ca^{2+} channel at very low concentrations (Mintz et al., 1992; Luebke et al., 1993; Randall and Tsien, 1995). ω-Aga-IVA blocks P- and Q-type Ca^{2+} channels when the concentrations are raised to ≥20 nM (Randall and Tsien, 1995). We have used a series of concentrations in our experiments to define the involvement of P- and Q-type channels in synaptic transmission and Iso-induced potentiation. As shown in Table 1, application of ω-Aga-IVA depressed EPSP amplitude in a concentration-dependent manner. ω-Aga-IVA at 1 and 5 nM, concentrations that selectively block P-type Ca^{2+} channels, depressed the EPSP amplitude slightly by 9.8–19.6%. However, raising the concentrations of ω-Aga-IVA to 20–200 nM reduced the EPSP amplitude by 31.3–88.3%. These

A

Control

Iso

20 mV

50 ms

B

$y = 0.10740 - 0.65403x \quad R^2 = 0.912$
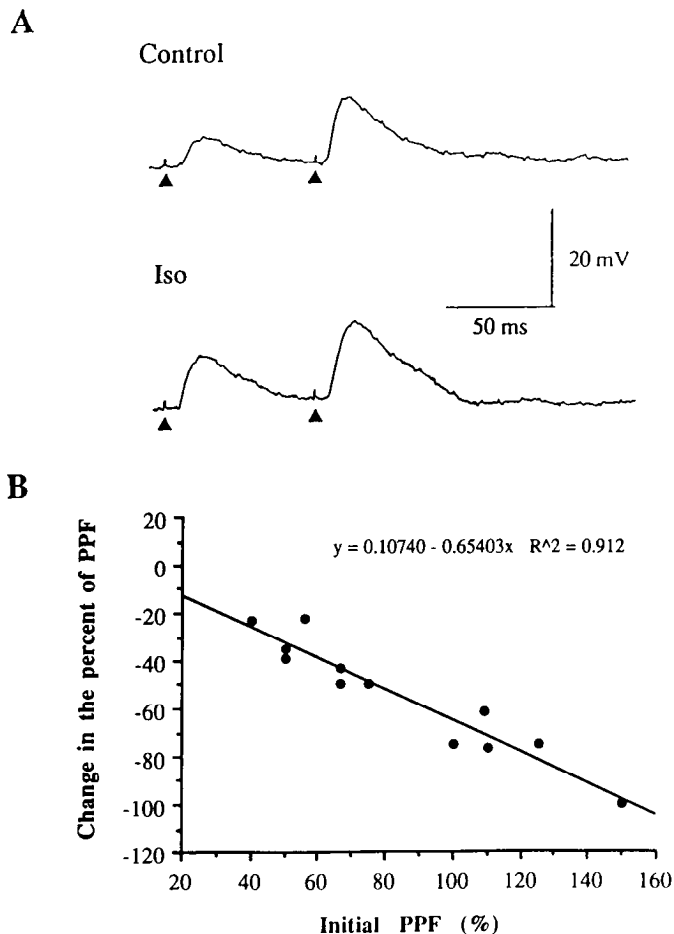
Initial PPF (%)

Figure 4. Isoproterenol decreases PPF. *A*, Sample records of EPSPs evoked by paired stimuli (60 msec interval) in control condition and after the application of Iso (15 μM). *B*, The initial PPF was plotted on the x-axis, and the change in PPF was plotted on the y-axis. The change in PPF was calculated as final PPF minus initial PPF. In all neurons tested, Iso decreased the magnitude of PPF. The best-fit regression line was plotted ($y = -0.65x + 0.1$), and there was a significant inverse correlation between initial PPF and the change in PPF induced by Iso ($r = 0.912$).

results suggest that ω-Aga-IVA-sensitive Ca^{2+} channels that participate in synaptic transmission are primarily Q-type, with a small contribution from P-type. More importantly, after 10–15 min of treatment with 5–200 nM ω-Aga-IVA, subsequent Iso application failed to potentiate the EPSP (Fig. 6, Table 1). ω-Aga-IVA at 1 nM also significantly reduced the magnitude of Iso-induced increase in EPSP amplitude. Iso produced an average of 98.0 ± 2.9% ($n = 8$) increase in control, whereas it only caused a 36.2 ± 3.7% ($p < 0.005$, $n = 4$, unpaired t test) increase in the presence of ω-Aga-IVA. The inhibition of EPSP amplitude by ω-Aga-IVA was compared with its effect on Iso-induced potentiation (Table 1). The potency for inhibition of EPSP was approximately two orders of magnitude lower than for the inhibition of Iso-induced potentiation (IC_{50} values were ~100 and 1 nM, respectively).

Finally, we tested the effect of a broad-spectrum Ca^{2+} channel blocker, ω-CgTX-MVIIC, on the Iso-induced potentiation. Application of 1 μM ω-CgTX-MVIIC reduced the amplitude of EPSP by an average of 87.3 ± 2.3% ($n = 6$). The amplitude of EPSP was restored to its predrug level by increasing the stimulus intensity, and then Iso was applied. As shown in Figure 7, pretreating the slices with ω-CgTX-MVIIC completely abolished the
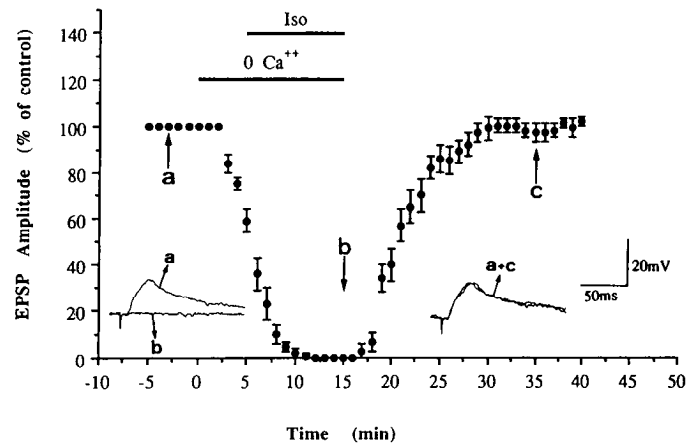
Figure 5. The Iso-induced increase in EPSP amplitude is blocked by Ca^{2+}-free solution. The percent change of EPSP amplitude was plotted against time. *Bars* denote the periods of delivery of 15 μM Iso and Ca^{2+}-free solution. *Insets* are superimposed traces taken at different times as indicated.

effect of Iso (99.0 ± 1.9% of control; $n = 6$). This result further strengthens the hypothesis that Iso enhances synaptic transmission by increasing presynaptic Ca^{2+} influx.

DISCUSSION

Iso-induced synaptic enhancement

The results of this study provide evidence that Iso enhances synaptic transmission in the amygdala primarily by increasing neurotransmitter release from presynaptic terminals. Three lines of evidence support this conclusion. First, Iso enhances synaptic transmission with negligible changes in resting membrane potential or input resistance of postsynaptic neurons, and the enhancement is accompanied by a decrease in PPF. Second, postsynaptic responses induced by AMPA in the slice preparation and in acutely dissociated cells were not altered by Iso. Third, Iso-induced potentiation is blocked in Ca^{2+}-free medium or by a specific type of Ca^{2+} channel antagonist, but was unaffected by blockade of postsynaptic non-NMDA and NMDA receptors. These results are consistent with the findings of Chavez-Noriega and Stevens (1994) and Gereau and Conn (1994); they showed that Iso, acting via cAMP cascade, can produce an enhancement of excitatory transmission in area CA1 of the rat hippocampus by increasing transmitter release. Furthermore, in the mossy fiber–

Table 1. Effects of Ca^{2+} channel antagonists on the EPSP amplitude and Iso-induced potentiation

Experiment	% Inhibition of EPSP amplitude	% Inhibition of Iso-induced enhancement	n
Nifedipine (20 μM)	4.2 ± 2.9	−5.1 ± 1.9	8
ω-CgTX-GVIA (1 μM)	83.3 ± 6.3**	−40.8 ± 9.6*	7
ω-Aga-IVA (1 nM)	9.8 ± 0.8*	63.1 ± 1.4**	4
ω-Aga-IVA (5 nM)	19.6 ± 2.4*	95.9 ± 3.7**	5
ω-Aga-IVA (20 nM)	31.3 ± 2.2**	96.7 ± 2.1**	5
ω-Aga-IVA (100 nM)	57.6 ± 1.2**	97.5 ± 1.9**	5
ω-Aga-Iva (200 nM)	88.3 ± 4.4**	98.1 ± 1.7**	3
ω-CgTX-MVIIC (1 μM)	87.3 ± 2.3**	99.2 ± 0.6**	6

Values are means ± SEM. n is the total of cells tested with each agent under those particular conditions. * $p < 0.005$ vs control. ** $p < 0.001$ vs control.
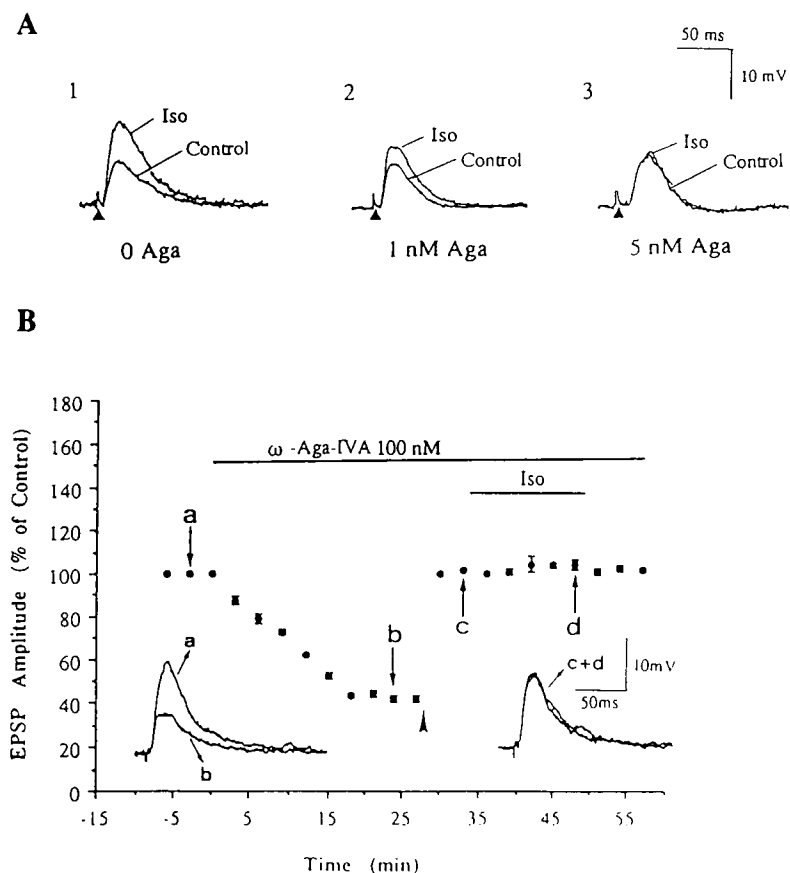
Figure 6. Pretreatment with ω-Aga-IVA blocked the Iso-induced enhancement of synaptic transmission. *A*, Comparison of the effect of Iso in the absence and in the presence of ω-Aga-IVA (*Aga*; 1 and 5 nM) on the EPSP. In the presence of ω-Aga-IVA, the effect of Iso (15 μM) was reduced or completely blocked. *B*, Time course of the effects of ω-Aga-IVA (100 nM) on EPSP and Iso-induced potentiation. ω-Aga-IVA reduced the EPSP amplitude to ~42% of its baseline. The amplitude of EPSP was restored to its control level by increasing the stimulus intensity (*arrowhead*), and then Iso (15 μM) was applied. Under this condition, Iso did not potentiate the EPSP. *Bars* denote the delivery of 100 nM ω-Aga-IVA and 15 μM Iso.

CA3 pathway, brief applications of a membrane-permeable analog of cAMP, Sp-cAMPS, or an adenylyl cyclase activator, forskolin, produce a lasting synaptic potentiation that is induced presynaptically and does not require activation of NMDA receptors (Weisskopf et al., 1993; Huang et al., 1994).

## A role for presynaptic Ca^{2+} entry in Iso-induced potentiation

We have examined the effect of blocking synaptic transmission, either presynaptically by removing extracellular Ca^{2+} or postsynaptically by blocking the action of glutamate with glutamate receptor antagonists, on the action of Iso. Iso-induced potentiation was blocked entirely when synaptic transmission was abolished in the Ca^{2+}-free medium. However, blockade of synaptic responses with Kyn and D-APV did not affect the effect of Iso. Because the EPSP was blocked completely in the presence of glutamate receptor antagonists, the membrane potential during application of Iso was well below threshold for activation of high-voltage-activated Ca^{2+} channels. These results suggest that Ca^{2+} entry into presynaptic terminals, but not into postsynaptic cells via glutamate receptors or voltage-dependent Ca^{2+} channels, is required for Iso-induced potentiation.

Furthermore, the effect of Iso on the EPSP is unlikely attributable to a direct action on the Ca^{2+}-activated K^+ currents underlying the AHP because a reduction in the AHP reversed when the Iso was washed out of the bath, whereas the enhancement of EPSP persisted during the entire experimental period. In addition, the inhibition of AHP caused by Iso was markedly retarded after PKI was applied intracellularly; in the same cell, however, the enhancement of EPSP remained intact.

Transmission at excitatory synapses of the amygdala involves N-, P-, and Q-type Ca^{2+} channels, and the Iso-induced potentiation is blocked selectively by P- and/or Q-type antagonists

Neurotransmitter release is initiated by an elevation of intracellular Ca^{2+} concentration caused by depolarization of the presynaptic nerve terminal and an influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (VDCCs) (Llinas et al., 1981). We have observed that the stimulus-evoked EPSP was not affected by nifedipine, suggesting that L-type Ca^{2+} channels normally do not participate in transmitter release (Jones and Heinemann, 1987; Lovinger et al., 1994; Wu and Saggau, 1994). After exposure to 1 μM ω-CgTX-GVIA or 200 nM ω-Aga-IVA, the EPSP was reduced by 83 and 89%, respectively. This finding suggests that both ω-CgTX-GVIA- and ω-Aga-IVA-sensitive Ca^{2+} channels support the synaptic transmission in the amygdala. More detailed analysis of the dose dependence of ω-Aga-IVA for inhibition of EPSP revealed that Q-type channels may make up a major portion of the ω-Aga-IVA-sensitive presynaptic VDCCs.

The Iso-induced enhancement of EPSP did not change significantly with nifedipine pretreatment. By contrast, Iso potentiated synaptic transmission to a greater extent in the presence of ω-CgTX-GVIA. A possible explanation is that Iso had no effect on N-channel-mediated transmission, because removal of this component by ω-CgTX-GVIA would leave transmission proportionally more sensitive to Iso. In the presence of low concentrations of ω-Aga-IVA, the potentiating effect of Iso was reduced or diminished. Because the IC_{50} of ω-Aga-IVA on Iso-induced potentiation matches the high-affinity K_d value (~2 nM) and the IC_{50} (1–3 nM) for the block of P-type Ca^{2+} channels in cerebellar

A

B
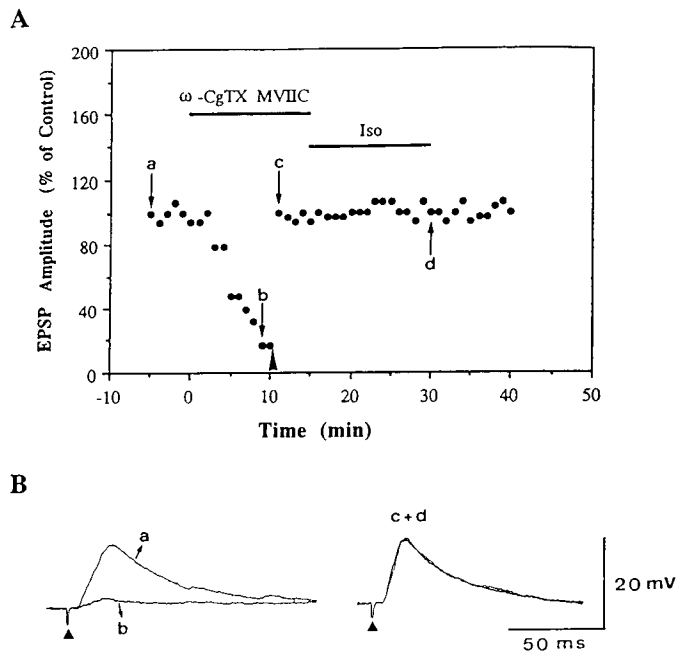
Figure 7. Blockade of the effect of Iso by ω-CgTX-MVIIC. *A,* Typical experiment showing the effects of ω-CgTX-MVIIC (1 μM) on the EPSP and Iso-induced potentiation. In this cell, ω-CgTX-MVIIC reduced the EPSP amplitude to 17% of its baseline. The amplitude of EPSP was restored to its control level by increasing the stimulus intensity (*arrowhead*), and then Iso (15 μM) was applied. Under this condition, Iso failed to affect the EPSP. *B,* Superimposed traces taken at different times indicated in *A.*

Purkinje neurons and granule cells (Mintz et al., 1992; Randall and Tsien, 1995), it appears that P-type Ca^{2+} channels are responsible for the action of Iso. However, these results do not necessarily rule out the involvement of Q-current in Iso action, because the distinction between P- and Q-type channels in the present study was based completely on their differential sensitivities to ω-Aga-IVA, and the IC_{50} of ω-Aga-IVA for Q-type channels in the amygdala is not known.

Functional implications

There is general consensus that the amygdala is intimately involved in neuronal plasticity such as epilepsy, learning, and memory (Gloor, 1992; Kesner, 1992; McGaugh et al., 1992). It has been shown that post-training intra-amygdala and peripheral administration of adrenergic agonists enhances the retention of an inhibitory avoidance task. Propranolol, a β-adrenergic antagonist, not only blocked this memory-enhancing effect (Liang et al., 1986), but it also blocked its own impaired rats' retention of an inhibitory avoidance response (Gallagher et al., 1981). In addition, microinjections of cAMP analogs into the amygdala produced electrographic manifestations of seizures (Gessa et al., 1970). The present study demonstrates that Iso blocks an AHP, causing more action potential firing. In addition, Iso produces a long-lasting enhancement of EPSP. These actions may form the cellular basis for the excitatory effects of Iso in the amygdala.

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