Isoproterenol facilitates GABAergic autapses in fast-spiking cells of rat insular cortex

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Abstract

In the cerebral cortex, fast-spiking (FS) cells are the principal GABAergic interneurons, and potently suppress neural activities in the targeting neurons. Not a few FS neurons make synaptic contacts to themselves, i.e. autapses, which contribute to self-inhibition of FS neural activities. β-Adrenoceptors play a crucial role in the regulation of GABAergic synaptic inputs from FS to pyramidal (Pyr) cells, however, β-adrenergic functions on FS autapses remain unknown. To elucidate how the β-adrenoceptor agonist, isoproterenol, modulates inhibitory synaptic transmission in autapses of FS cells, the present study performed paired whole-cell patch-clamp recording from FS and Pyr cells in layer V of rat insular cortex. According to the previous report, isoproterenol (100 μM) induced pleiotropic effects on unitary IPSCs (uIPSCs) in FS→Pyr connections, whereas autapses in FS cells were invariably facilitated by isoproterenol. The facilitation of autapses by isoproterenol was accompanied by decreases in paired-pulse ratio of 2nd to 1st uIPSC amplitude and coefficient of variation of the uIPSC amplitude, suggesting that the β-adrenergic facilitation is likely to be mediated by presynaptic mechanisms. The discrepancy between isoproterenol-induced modulation of uIPSCs in FS autapses and that of FS→Pyr connections may reflect different presynaptic mechanisms of GABA release in each synapse.

Introduction

In the cerebral cortex, GABAergic neurons, which occupy 10-20% of cortical neurons (1), regulate neural activities by inducing inhibitory postsynaptic Cl^- currents. Electrophysiologically, GABAergic interneurons are divided into more than 4 classes according to their firing properties (2). Among these GABAergic neurons, fast-spiking (FS) cells are considered to play principal roles in suppressing postsynaptic neurons. FS cells were characterized by their unique afterhyperpolarization (AHP), i.e. large amplitude with a rapid repolarization, shorter duration of action potential, and extremely high frequent repetitive firing responding to a long depolarizing current pulse injection without spike adaptation (2-5). Interestingly, a large number of FS cells have autapses, self-synapses formed by the axon of a neuron on its own somata or dendrites (6). The FS autapses suppress the FS neural firing in a manner of self-inhibition. However, little physiological and pharmacological properties have been elucidated in the FS autapses in the cerebral cortex.

Activation of β-adrenoceptors has been well studied on glutamatergic synaptic transmission: β-adrenoceptor agonists increase glutamate release from presynaptic terminals (7-10). In contrast, the effects of isoproterenol are pleiotropic. Application of isoproterenol reduces the unitary inhibitory postsynaptic current (uIPSC) amplitude in non-FS to pyramidal cell (non-FS→Pyr) connections, whereas facilitates uIPSCs age-dependently in FS→Pyr connections, i.e. older animals (≥ postnatal days 24) rather than younger animals tend to show isoproterenol-induced facilitation of uIPSCs (5). These findings indicate that the functional roles of β-adrenoceptors in synaptic transmission are differently regulated between glutamatergic and GABAergic synapses.

The present study aimed to examine the effects of isoproterenol on uIPSCs recorded from FS autapses, and performed paired whole-cell patch-clamp recording from FS and Pyr

cells in layer V of the insular cortex (IC), and compared isoproterenol-induced modulation of uIPSCs between FS autapses and FS→Pyr connections.

Materials and Methods

All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Institutional Animal Care and Use Committee in the Nihon University. All efforts were made to minimize the number of animals used and their suffering.

Slice preparations

Vesicular GABA transporters (VGAT)-Venus line A transgenic rats of either sex (postnatal day 17-46), in which the yellow fluorescent protein Venus (11) is expressed in almost all cortical GABAergic cells (12), were deeply anesthetized with sodium pentobarbital (75 mg/kg, i.p.) and decapitated. Tissue blocks including the IC around the intersection of the middle cerebral artery and rhinal fissure were rapidly removed and stored for 3 min in modified ice-cold artificial cerebrospinal fluid (M-ACSF) (in mM): 230 Sucrose, 2.5 KCl, 10 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$, and 10 D-glucose. Coronal slices were cut at 350 μm thickness using a microslicer (Linearslicer Pro 7, Dosaka EM, Kyoto, Japan). Slices were incubated at 32°C for 40 min in a submersion-type holding chamber which contained 50% M-ACSF and 50% normal ACSF (pH 7.35-7.40). Normal ACSF contained (in mM): 126 NaCl, 3 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2.0 $CaCl_2$, and 10 D-glucose. Slices were then placed in normal ACSF at 32°C for 1 h. Normal ACSF was continuously aerated with a mixture of 95% O_2 / 5% CO_2. Slices were thereafter maintained at room temperature until used for recording.

Cell identification and paired whole-cell patch clamp recording

The slices were transferred to a recording chamber which was perfused continuously with normal ACSF, humidified with 95% O_2 / 5% CO_2, at a rate of 1.0-1.5 ml/min. Paired

whole-cell patch-clamp recordings were obtained from fluorescent neurons and Pyr cells identified in layer V by a fluorescence microscope equipped with Nomarski optics (x 40, Olympus BX51, Tokyo, Japan) and an infrared-sensitive video camera (Hamamatsu Photonics, Hamamatsu, Japan). The distance between Venus-positive and Pyr cells was < 50 μm. Electrical signals were recorded by an amplifier (Axoclamp 700B, Axon Instruments, Foster City, CA), digitized (Digidata 1322A, Axon Instruments), observed on-line and stored on a computer hard disk using software (Clampex 9, Axon Instruments).

The composition of the pipette solution used for FS cell recordings had the following composition (in mM): 70 potassium gluconate, 70 KCl, 10 *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), 15 biocytin, 0.5 EGTA, 2 $MgCl_2$, 2 magnesium adenosine triphosphate (ATP), and 0.3 sodium guanosine triphosphate (GTP). Pyr cells were recorded using the following pipette solution (in mM): 120 cesium gluconate, 20 biocytin, 10 HEPES, 8 NaCl, 5 *N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX-314), 2 magnesium ATP, 0.3 sodium GTP and 0.1 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). The presence of QX-314 and cesium in the pipette solution precluded recording $GABA_B$-receptor-mediated IPSCs. Both pipette solutions had a pH of 7.3 and osmolarity of 300 mOsm. The liquid junction potential for current-clamp and voltage-clamp recordings were -9 and -12 mV, respectively, and voltage was corrected accordingly. Thin-wall borosilicate patch electrodes (2-5 MΩ) were pulled on a Flaming-Brown micropipette puller (P-97, Sutter Instruments, Novato, CA).

Recordings were obtained at 30-31°C. Seal resistance was > 5 GΩ and only data obtained from electrodes with access resistance of 6-17 MΩ and < 20% change during recordings were included in this study. Series resistance was 70% compensated. Repetitive firing in response to long (1 s) depolarizing current pulses was recorded to classify GABAergic interneuron

subtypes. uIPSCs were recorded from Pyr cells by applying depolarizing step voltage pulses (+80 mV, 2 ms, 0.05 Hz) to presynaptic Venus-positive cells. Pyr cells were voltage-clamed at -70 mV during uIPSCs recordings. For blocking $GABA_A$ receptors, 10 μM bicuculline methiodide (Tocris Cookson, Bristol, UK) were bath applied. Isoproterenol (100 μM, Research Biochemicals International, Natick, MA) was added directly to the perfusate. Membrane currents and potentials were low-pass filtered at 5-10 kHz and digitized at 20 kHz. All chemicals, unless specified otherwise, were purchased from Sigma-Aldrich (St. Louis, MO).

Data analysis

uIPSCs were analyzed with Clampfit (pClamp 9, Axon Instruments). Amplitudes of uIPSCs were measured as the difference between the peak postsynaptic currents and the baseline currents taken from a 2 ms time window close to the onset of the uIPSCs. Average amplitude, paired-pulse ratio, and coefficient of variation (CV) of uIPSCs were calculated from 10-20 consecutive sweeps.

Data are presented as mean ± standard error of the mean (SEM). Comparisons of the uIPSC amplitude and PPR between control and isoproterenol application were conducted by paired *t*-test. CV in control and isoproterenol application was compared using Wilcoxon test. The level of $P < 0.05$ was adopted to indicate significance.

Results

Cell classification

Dual whole-cell patch clamp recording was performed from Venus-positive GABAergic and Venus-negative Pyr cells in layer V of the IC. As previously reported, Venus-positive cells were classified into FS and non-FS cells (5, 13-14). Among these GABAergic cells, FS cells are the only cell subtype that exhibited autapses (5), and therefore the present study focused on FS cells but not on non-FS cells.

FS cells were identified their characteristic repetitive spike firing properties: i.e. higher frequency of spike firing (> 100 Hz) in comparison to other cell subtypes including Pyr cells, large and short afterhyperpolarization, and less spike adaptation (5, 13-14; Fig. 1).

Identification of autapses

Fig. 2 shows an example of dual whole-cell recording from FS and Pyr cells. In this case, FS and Pyr cells were recorded under current- and voltage-clamp modes (holding potential = +10 mV), respectively. Intracellular depolarizing current pulse injection into the FS cell induced action potentials that were followed by depolarizing potentials (Fig. 2B, arrowheads). In response to the action potentials in the FS cell, outward synaptic currents were observed in the Pyr cell, which was recorded using the Cs-based low Cl^- internal solution (see Materials and Methods), indicating the synaptic contacts from the FS to the Pyr cells.

Because of the varied amplitude of these depolarizing potentials, they were considered to be mediated by synaptic transmission. The FS cell was recorded using the high concentration of Cl^-, which induced depolarizing synaptic potentials via autapses. Thus, these depolarizing potentials were likely to be mediated by autapses.

To confirm this possibility, both cells were recorded under voltage-clamp mode and

were examined whether uIPSCs in the FS and Pyr cells were blocked by the $GABA_A$ receptor antagonist, bicuculline (Fig. 2C). In the FS cell, the holding potential was set at -70 mV, and action currents (double arrowheads, Fig. 2D) followed by inward currents (Fig. 2CD, arrowheads) were observed in response to depolarizing voltage pulse injection (Fig. 2C, top trace). The action currents induced uIPSCs in the Pyr cell (Fig. 2C, bottom trace). Application of 10 μM bicuculline abolished both uIPSCs in FS and Pyr cells, indicating that these currents were mediated by $GABA_A$ receptors.

Effects of isoproterenol on inhibitory synaptic transmission

To examine the effects of a β-adrenoceptor agonist on uIPSCs obtained from FS autapses, 100 μM isoproterenol was bath applied. Dual recordings from FS and Pyr cells that exhibited FS autapses and FS→Pyr connections were performed, and profiles of isoproterenol-dependent modulation of autaptic uIPSCs were compared to those in FS→Pyr connections.

Figs. 3A and B showed an example of isoproterenol-induced synaptic facilitation in the FS autapse accompanied with a decrease in uIPSC amplitude of the FS→Pyr connection. The increase in the amplitude of autaptic uIPSCs was prominent in the first event, and the 3rd to 5th autaptic uIPSCs were less affected by isoproterenol, suggesting that the isoproterenol-induced facilitation of the autapse is likely mediated by presynaptic mechanisms.

Figs. 3C and D shows another example of simultaneous recording of FS autapse and FS→Pyr connection. This pair showed isoproterenol-induced synaptic facilitation in the FS autapse without changing the 1st uIPSC amplitude of the FS→Pyr connection. Similar to the pair shown in Figs. 3A and B, the increase in the amplitude of autaptic uIPSCs was prominent in the first event.

Isoproterenol-induced facilitation of autapses accompanies increases in PPR and CV

As previously reported (5), FS→Pyr connections showed pleiotropic modulation of uIPSC amplitude: facilitation in 4/9 pairs, suppression in the same number of pairs, and no effect in 1/9 pair (Fig. 4A). In contrast, the FS autapses showed relatively consistent facilitation by isoproterenol (129.1 ± 10.6%, n = 9, $P < 0.05$, paired *t*-test). The isoproterenol-induced facilitation of autaptic uIPSCs was partially recovered by 10 min washout (Fig. 4B).

The facilitation of autaptic uIPSCs by isoproterenol was accompanied by decreases in PPR (0.73 ± 0.04 to 0.62 ± 0.04, n = 9; $P < 0.01$, paired *t*-test) and CV (0.35 ± 0.10 to 0.16 ± 0.03, n = 9; $P < 0.01$, Wilcoxon test), suggesting that presynaptic mechanisms are involved in the isoproterenol-induced facilitation of autapses.

Discussion

The principal findings of this study are: (1) isoproterenol consistently increased the amplitude of uIPSCs obtained from FS autapses; (2) this facilitation was accompanied by decreases in PPR and CV; and (3) the simultaneously recorded FS→Pyr connections showed pleiotropic modulation of uIPSC amplitude by isoproterenol. Thus, it is possible to propose that FS autapses have their intrinsic mechanisms of GABA release in comparison to those in FS→Pyr connections.

The isoproterenol-induced facilitation of uIPSCs in FS autapses was accompanied by a decrease in CV and PPR, suggesting modulation of presynaptic GABA release mechanisms. β-adrenoceptors are coupled to Gs proteins, which activate cAMP-dependent protein kinase (PKA) and its downstream signaling pathways, including p42/p44 mitogen-activated protein kinase (MAPK). These kinases facilitate glutamate release from presynaptic terminals in the cerebral cortex (9), and therefore, similar mechanisms may be involved in FS autapses.

It is worth noting that the same FS cell often showed opposite effects of isoproterenol on uIPSCs as shown in Fig. 3A and B. This suggests that the presynaptic terminals are not homogenously regulated by β-adrenoceptors. A possible explanation for different profiles of the isoproterenol-induced uIPSC modulation is the variability of Ca^{2+} channel subtypes. At present, however, it remains controversial what types of voltage-gated calcium channels (VGCCs) exist in FS interneuronal synaptic terminals. Zaitsev et al. (16) have reported that GABAergic terminals of FS cells express only P/Q-type Ca^{2+} channels in the cerebral cortex, similar to those in the hippocampus (17). In contrast, Ali and Nelson (18) report that N-type Ca^{2+} channels play a major role in releasing GABA in FS cells in the cerebral cortex. Further identification of Ca^{2+} channel distribution in FS cells including FS autapses will help to elucidate the mechanisms of divergence of GABAergic synaptic responses.

Autapses in FS cells regulate their own neural activities: suppression of spike firing by hyperpolarizing the membrane potential (6). The enhancement of autaptic uIPSCs by isoproterenol may contribute to further suppression of FS neuronal activities. In addition, autapses play a critical role in regulation of spike-timing precision in FS and Pyr cells (19). This autaptic mechanism on spike firing is essential for well-timed spike firing under synaptic noise. The present results suggest that noradrenalin controls spike-timing via β-adrenoceptors, which may increase output from layer V Pyr cells.

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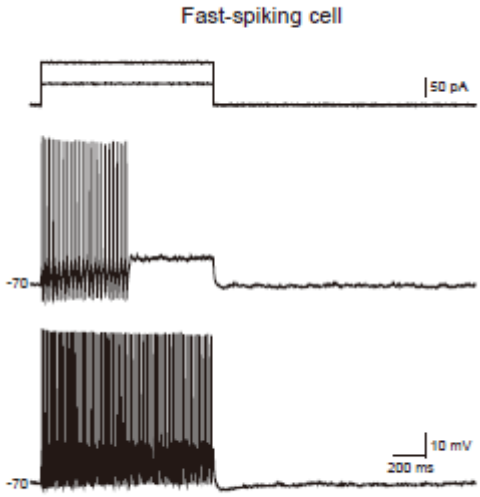

Fig. 1 An example of repetitive spike firing in a Venus-positive FS cell. Repetitive firing induced by a depolarizing current pulse injection (1 s). The resting membrane potential was -70 mV. The FS cell exhibited large and fast afterhyperpolarization and high frequency spike firing without spike adaptation.

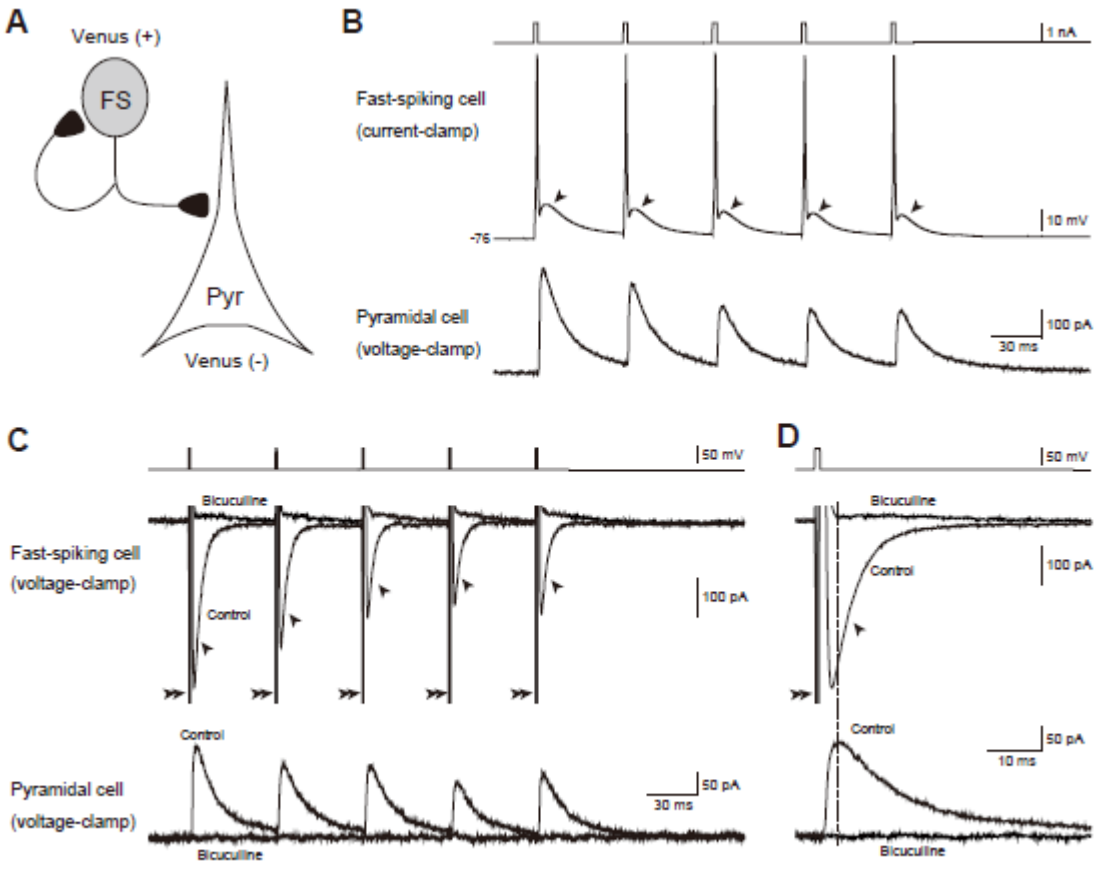

Fig. 2 uIPSC recording from an FS and Pyr cell pair. ***A***: Scheme of dual recording from FS and Pyr pair. ***B***: uIPSCs recorded from postsynaptic Pyr cell (*bottom*) in response to presynaptic action potentials (*middle*) evoked by depolarizing current pulse injection (*top*) to FS cells in ***A***. Five pulses were applied to the presynaptic cell at 20 Hz. Note the depolarizing humps (arrowheads) mediated by autapses. The presynaptic FS cell and postsynaptic Pyr cell were recorded under current- and voltage-clamp condition, respectively. The holding potential of postsynaptic cell was 0 mV. ***C***: Bath application of bicuculline (10 μM) completely blocked uIPSCs in the FS autapses (arrowheads) and the FS→Pyr connection. The presynaptic FS cell and postsynaptic Pyr cell were recorded under voltage-clamp condition. ***D***: Time expanded traces of the 1st uIPSCs shown in ***C***. The FS cell induced action current (double arrowheads) followed by autaptic uIPSC (arrowhead). In the Pyr cell, uIPSC was elicited by presynaptic action current. The latency of the uIPSC peak in FS was shorter than that in the Pyr cell.

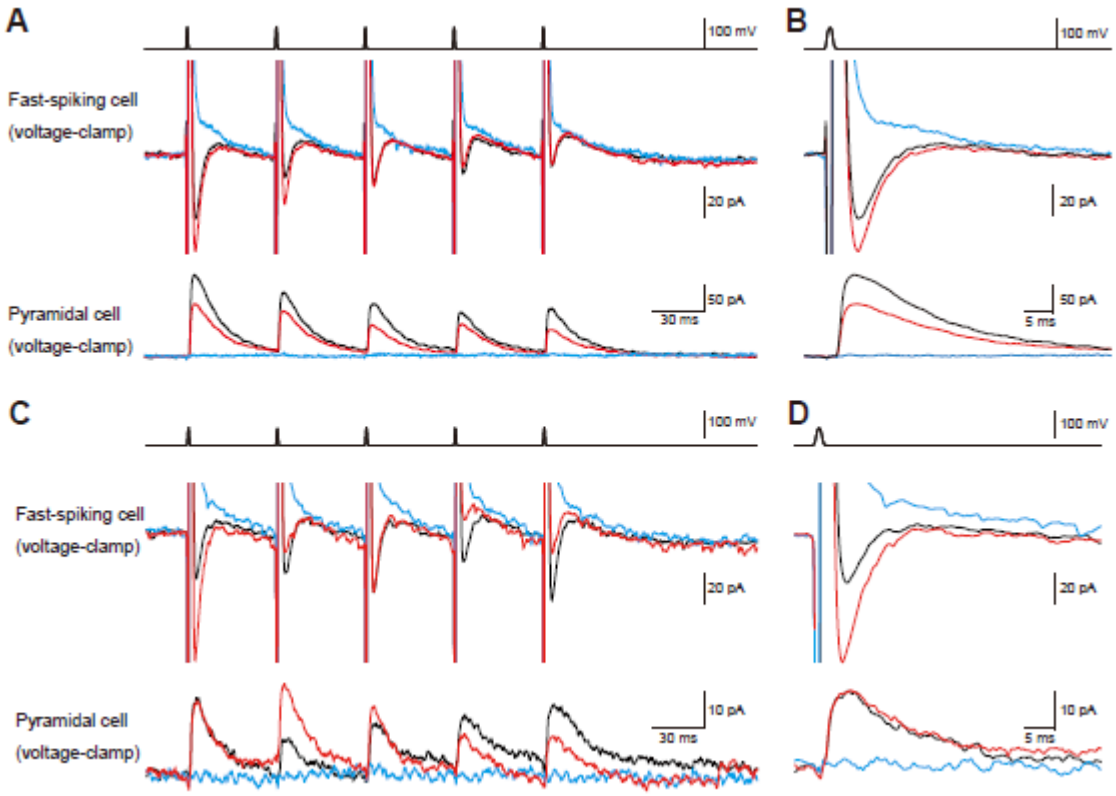

Fig. 3 Facilitative effects of isoproterenol on autaptic uIPSCs obtained from FS cells. ***A***: An example of the effects of 100 μM isoproterenol on 5 consecutive autaptic (*middle*) and FS→Pyr uIPSCs (*bottom*). Average of 10 traces in control (*black*), during isoproterenol application (*red*) and 10 μM bicuculline (*blue*) are shown. Note facilitation of autaptic uIPSC amplitude by isoproterenol in contrast to suppression of uIPSCs in the FS→Pyr connection. ***B***: Time expanded traces of the 1st uIPSCs in ***A***. ***C***: Another example of the effects of 100 μM isoproterenol on autaptic (*middle*) and FS→Pyr uIPSCs (*bottom*). Note facilitation of autaptic uIPSC amplitude by isoproterenol without effect on uIPSCs in the FS→Pyr connection. ***D***: Time expanded traces of the 1st uIPSCs in ***C***.

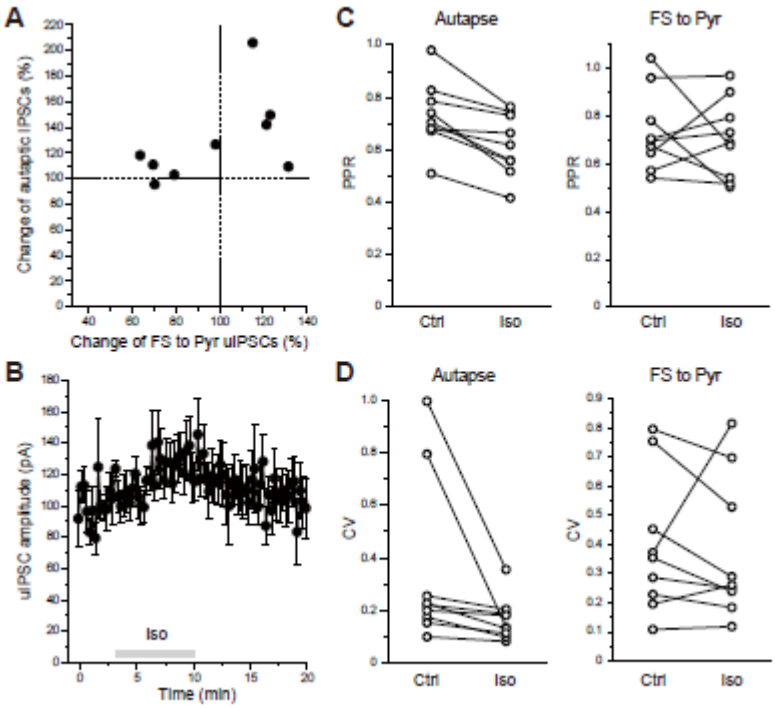

Fig. 4 Profiles of isopreoterenol-induced facilitation of autaptic uIPSCs. ***A***: Changes of autaptic uIPSC amplitude by isoproterenol, normalized with control values, are plotted against those of uIPSC amplitude in FS→Pyr connections. ***B***: Time course of the amplitude of autaptic uIPSCs before, during, and after 100 μM isoproterenol application (n = 9). ***C***: PPR in control and during isoproterenol application in autapses and FS→Pyr connections. ***D***: CV in control and during isoproterenol application in autapses and FS→Pyr connections.