Adrenergic regulation of GABA release from presynaptic terminals in rat cerebral cortex

Masanori Terakado

Nihon University Graduate School of Dentistry,
Major in Pharmacology
(Directors: Prof. Noriaki Koshikawa and Assoc. Prof. Masayuki Kobayashi)

Abstract

The α_1-adrenoceptor agonist, phenylephrine, and the β-adrenoceptor agonist, isoproterenol, have an opposite effects on evoked EPSPs (eEPSPs) in the cerebral cortex. Suppressive effects of phenylephrine on eEPSPs are mediated by modulation of postsynaptic glutamate receptors, whereas enhancement of eEPSPs by isoproterenol is due to facilitation of glutamate release from presynaptic terminals. The present study aimed to assess the effects of phenylephrine and isoproterenol on release probability of γ-aminobutyric acid (GABA) using whole-cell patch clamp method from layer V pyramidal neurons in the visuocortical slice preparations. The present study recorded evoked IPSCs (eIPSCs) by repetitive electrical stimulation (100 μs duration, 10 stimuli at 33 Hz), and miniature IPSCs (mIPSCs). The effects of phenylephrine (100 μM) depended on the amplitude of eIPSCs: phenylephrine decreased paired-pulse ratio (PPR) of eIPSCs with smaller amplitude (< ~600 pA), but increased PPR with larger amplitude. Phenylephrine also showed amplitude-dependent modulation on mIPSCs, an increase in the frequency of smaller mIPSC events (< ~20 pA) and decrease of larger events. These findings suggest that α_1-adrenoceptor activation facilitates GABA release from a subpopulation of GABAergic terminals that induce smaller amplitude of IPSCs in postsynaptic neurons. On the other hand, isoproterenol (100 μM) consistently decreased PPR of eIPSCs and increases the frequency of mIPSCs, suggesting that presynaptic β-adrenoceptors facilitates release probability from most GABAergic terminals. The complicate adrenoceptor modulations on GABAergic synaptic transmission by α_1-adrenoceptor and β-adrenoceptor activation may be due to the presence of pleiotropic subtypes of GABAergic interneurons in the cerebral cortex.

Introduction

Noradrenaline contributes to physiological functions of inhibitory system in the cerebral cortex. Komatsu and Yoshimura (1) demonstrated that noradrenaline controls the maintenance of long-term potentiation (LTP) of inhibitory postsynaptic potentials (IPSPs) in the visual cortex, which may play a role for experience-dependent refinement of visual responsiveness. In the entorhinal cortex, noradrenaline reduces epileptiform discharges induced by bicuculline, a $GABA_A$ receptor antagonist (2). In the cerebral cortex, 10-20% of neurons are non-spiny smooth neurons, which are thought to be GABAergic neurons (3). Anatomical studies have reported that at least 19 types of smooth neurons are present in the cerebral cortex (4,5). Recent electrophysiological studies have revealed that GABAergic interneurons can be divided into more than 4 classes according to their firing properties (6). These findings indicate that GABAergic interneurons have multiple subtypes, which may exhibit different electrophysiological and pharmacological responses to noradrenaline.

An activation of α_1-adrenoceptors suppresses glutamatergic synaptic transmission through postsynaptic mechanism (7). In terms of the mechanisms of adrenergic modulation on GABAergic synaptic transmission, less information is available. Regarding α-adrenergic receptor-mediated effects on inhibitory postsynaptic currents (IPSCs), it has been reported that epinephrine increases the frequency of $GABA_A$ receptor-mediated spontaneous IPSCs (sIPSCs) through α-adrenergic receptors in rat sensorimotor cortex (8,9), and this facilitatory effects on sIPSCs was mediated by depolarizing the resting membrane potential of several types of GABAergic interneurons via α_1-adrenoceptor activation (9). Although several studies have shown an increase of miniature IPSC (mIPSC) frequency by noradrenaline through α_1-adrenoceptors (9,10), Bennett et al. (8) reported that adrenaline produces heterogeneous effects on amplitude of evoked IPSCs (eIPSCs), i.e. an enhancement of eIPSCs in ~33% of pyramidal neurons and decrease in ~40 % of neurons, suggesting pleiotropic

modulation of inhibitory synaptic transmission by α_1-adrenoceptors agonists. The reason for these seemingly controversial findings remains an open issue.

It is well known that β-adrenergic receptor agonists presynaptically facilitate glutamate release in the cerebral cortex (11-14), and activation of β-adrenoceptors induces depolarization of the resting membrane potential or blockade of spike accommodation with an increase in firing frequency (7, 15-17). However, little known is about the effects of β-adrenoceptor activation on inhibitory synaptic transmission in the cerebral cortex.

To elucidate the presynaptic mechanism of IPSC modulation via β-adrenoceptors, the present study examined eIPSCs and mIPSCs by whole-cell patch clamp technique from layer V pyramidal neurons in rat visuocortical slice preparations, and found different actions of phenylephrine and isoproterenol, an α_1- and a β-adrenoceptor agonist respectively, on properties of eIPSCs and mIPSCs.

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 School of Dentistry. All efforts were made to minimize the number of animals used and their suffering.

Slice preparations

Sprague-Dawley rats of either sex aged from postnatal day 14-35 were used for whole-cell patch clamp recording. Animals were deeply anesthetized with sodium pentobarbital (75 mg/kg, i.p.) and decapitated. Tissue blocks including the visual cortex 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 into 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.

Whole-cell patch clamp recording

After more than 1 h incubation in a submerge-type holding chamber, the slices were transferred to a recording chamber which were perfused continuously with normal ACSF humidified with 95% O_2 / 5% CO_2 at a rate of 1-1.5 ml/min. Whole-cell patch-clamp recordings were obtained from pyramidal cells identified in layer V with Nomarski optics (x 40, Olympus BX51, Tokyo, Japan) and an infrared-sensitive video camera (Hamamatsu Photonics, Hamamatsu, Japan). Electrical signals were recorded by an amplifier (Axoclamp 200B, 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 for voltage-clamp recordings was (in mM): 120 cesium gluconate, 20 biocytin, 10 *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES), 8 NaCl, 5 *N*-(2,6-dimethylphenyl-carbamoylmethyl)triethylammonium bromide (QX-314), 2 magnesium adenosine triphosphate (ATP), 0.3 sodium guanosine triphosphate (GTP), and 0.1 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). Pipette solutions had a pH of 7.3 and osmolarity of 300 mosm. The liquid junction potential for voltage-clamp recordings was 10 mV, and all voltages were corrected accordingly. Thin-wall borosilicate

patch electrodes (2-4 MΩ; 1.5 mm diameter, Harvard apparatus, Holliston, MA) were pulled on a Flaming-Brown micropipette puller (P-97, Sutter Instruments, Novato, CA).

Recordings were obtained from layer V pyramidal cells at 30-31°C. Seal resistance was > 5 GΩ, and only data obtained from electrodes with access resistance of 8-20 MΩ and < 20% change during recordings were included in this study. Series resistance was 70% compensated. eIPSCs were recorded at a holding potential of -70 mV under application of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 30 μM) and D-(-)-2-Amino-5-phosphonopentanoic acid (D-APV, 50 μM). For induction of eIPSCs, a train of ten rectangle pulses (100 μs) was applied at 33.3 Hz through unipolar tungsten electrode placed in the border of layers IV/V. Stimulation intensity was set at 1.2-1.5 times of threshold (4-20 μA). mIPSCs were recorded at 0 mV under application of 1 μM tetrodotoxin (Wako, Tokyo, Japan), 30 μM DNQX, and 50 μM D-APV. To record GABA-induced currents, glass pipettes (the same as patch electrodes) were filled with 200 μM GABA and placed in layer V at a point perpendicular to the soma of recording neurons. The distance between the tip of pipette and soma was approximately 50 μm. GABA was applied by pressure ejection (15 psi, 20 ms) using a pneumatic PicoPump (PV830, World precision Instruments, Sarasota, FL). GABA-induced currents were recorded under the same condition of mIPSCs recording. The interval of puff application of GABA was 0.03 Hz.

The following drugs were added directly to the perfusate: bicuculline methiodide (10-20 μM, Tocris cookson, Bristol, UK), isoproterenol (100 μM, Research Biochemicals International, Natick, MA), phenylephrine (100 μM, Sigma-Aldrich, Saint Louis).

Histology

To visualize biocytin-labeled neurons after whole-cell patch clamp recording, slices were fixed, cryoprotected, and sectioned (60 μm). Sections were processed using the ABC

method (Vector Laboratories, Burlingame, CA) and nickel-intensified diaminobenzidine as the chromogen. The slices were examined microscopically to verify their morphology and location. All chemicals unless specified were purchased from Sigma-Aldrich (St. Louis, MO).

Data analysis

Membrane currents and potentials were low-pass filtered at 1-2 kHz and digitized at 4-8 kHz. eIPSCs and GABA-induced currents were analyzed with Clampfit (pClamp 9, Axon Instruments). mIPSCs were automatically detected by customized software (kindly provided by Dr. J. Huguenard) using the second derivative of the current traces as the trigger (18). Threshold values were set at 3 times the standard deviation of baseline noise amplitude. For every recording, we visually checked at least 10% of the file whether the software accurately detected events. Over 95% of the visually identified events were detected by the software, and false-positives accounted for $< 1\%$ of the events detected by the software. Threshold values for mIPSCs recorded in normal ACSF were 6.8 ± 0.3 pA ($n = 26$). mIPSC frequency was measured from continuous recordings that were at least 2 minutes long.

Data are presented as mean ± standard error of the mean (SEM). Comparisons of the eIPSCs between control and drug application were conducted by a paired *t*-test. The inter-event interval and amplitude of mIPSCs were analyzed by a nonparametric statistical analysis (Kolmogorov-Smirnoff test; K-S test) to assess the significance of shifts in cumulative probability distributions of control and drug applied condition. Paired *t*-test was also used to compare average of mIPSCs amplitude, inter-event interval and 10-90% rise time. The level of $P < 0.05$ was assumed as significant for paired *t*-test, and $P < 0.01$ for K-S test.

Results

Properties of eIPSCs

The present study first examined the effects of α_1- and β-adrenoceptor agonists on GABAergic synaptic transmission by recording eIPSCs in the presence of 40 μM DNQX, a non-NMDA receptor antagonist, and 50 μM D-APV, an NMDA receptor antagonist (holding potential = 0 mV). Repetitive electrical stimulation (100 μs duration, 10 stimuli at 33 Hz) was applied through a unipolar tungsten electrode 50-100 μm toward the pial surface from the recorded cell in layer V. eIPSCs were abolished by bath application of 10 μM bicuculline methiodide, indicating that they were mediated by $GABA_A$ receptors (n = 3, Fig. 1A). In 16/31 cells (51.6%), eIPSCs exhibited paired-pulse depression (PPD), and the other cells (15/31 cells, 48.4%) showed paired-pulse facilitation (PPF). There was no significant correlation between the amplitude of 1st eIPSCs and paired-pulse ratio (PPR) of 2nd eIPSC amplitude to 1st eIPSC amplitude as shown in Fig. 1B (R = -0.17, $P > 0.3$). The effects of phenylephrine and isoproterenol on PPR have little correlation to the property of short-term plasticity, i.e. PPD or PPF. Therefore, both groups of cells were included in the following analyses.

Multiple effects of phenylephrine on eIPSCs

Bath application of 100 μM phenylephrine induced inconsistent effects on the amplitude of the 1st eIPSCs. The 1st eIPSC amplitude was significantly increased in 1 of 12 neurons (8.3%; 7.1% increase in amplitude), decreased it in 4 of 12 neurons (33.3%; 27.0% decrease in amplitude), and little effect on it in the remaining neurons (7 of 12 neurons, 58.3%). Totally, phenylephrine produced little change in average amplitude of the 1st eIPSCs (93.9 ± 6.4 % of control, n = 12). There was little correlation between the amplitude of 1st eIPSCs and the effects of phenylephrine on them (Fig. 2B).

The PPR of synaptic responses changes in association with presynaptic manipulations of transmitter release, but remains unaltered following postsynaptic manipulation of synaptic transmission (19, 20). In the present study, phenylephrine has variable effects on PPR (2nd eIPSC amplitude / 1st eIPSC amptitude) similar to its effect on the 1st eIPSC amplitude, and totally, PPR was 0.80 ± 0.12 in control, and 0.80 ± 0.13 (n = 12) during phenylephrine application. There were significant correlation between the 1st eIPSC amplitude and phenylephrine-induced change of PPR (Fig. 2C): i.e. phenylephrine tended to decrease PPR of eIPSCs with smaller amplitude (Fig. 2Aa), and increase PPR with larger amplitude (Fig. 2Ab).

Isoproterenol suppresses PPR of eIPSCs

Similar to phenylephrine, isoproterenol (100 μM) induced inconsistent effects on the 1st eIPSC amplitude. The 1st eIPSC amplitude was significantly increased in 3 of 19 neurons (15.8%; 21.8% increase in amplitude), decreased it in 2 of 19 neurons (10.5%; 21.5% decrease in amplitude), and little effect on it in the remaining neurons (14 of 19 neurons, 73.7%). On the whole, isoproterenol produced little change in average amplitude of the 1st eIPSCs (102.2 ± 5.0 % of control, n = 19). There was little correlation between the amplitude of 1st eIPSCs and the effects of isoproterenol on them (Fig. 3B). On the other hand, isoproterenol significantly suppressed PPR from 1.14 ± 0.14 in control to 1.02 ± 0.12 (n = 18, $P < 0.02$, paired *t*-test). There was no significant correlation between the 1st eIPSC amplitude and isoproterenol-induced change of PPR (Fig. 3C).

Both phenylephrine and isoproterenol increase mIPSC frequency and decrease mIPSC amplitude

To examine the effect of phenylephrine and isoproterenol on inhibitory synaptic transmission, the present study examined mIPSCs from layer V pyramidal neurons under application of 1 μM tetrodotoxin, 40 μM DNQX, and 50 μM D-APV (holding potential = 0 mV). Bath application of bicuculline methiodide (10 μM) diminished mIPSCs, suggesting that mIPSCs were generated by activation of $GABA_A$ receptors (data not shown).

Fig. 4A and B show a typical example of the effects of phenylephrine on mIPSCs. Cumulative amplitude and inter-event interval distribution were obtained by pooling 150 events from each of 9 pyramidal neurons (total events number is 1350) before and during application of phenylephrine. Bath application of 100 μM phenylephrine significantly decreased the inter-event interval of mIPSCs ($P < 0.001$, K-S test). The mean inter-event interval of mIPSCs was 0.70 ± 0.19 s in control, and decreased to 0.50 ± 0.12 s by application of phenylephrine (n = 9, $P < 0.05$, paired *t*-test; Fig. 4D). Phenylephrine had suppressive effect on the mIPSC amplitude (n = 9, $P < 0.001$, K-S test). The mean amplitude of mIPSCs was significantly decreased by phenylephrine (15.4 ± 1.3 pA in control and 13.6 ± 1.0 pA under phenylephrine application, n = 9, $P < 0.01$, paired *t*-test; Fig. 4D). The effect of phenylephrine on the 10-90% rise time was not significant (1.27 ± 0.11 to 1.29 ± 0.11 ms, n = 9, Fig. 4*F*). The half duration of mIPSCs was also unaffected by phenylephrine (15.3 ± 1.2 to 15.1 ± 1.4 ms, n = 9). These results suggest that phenylephrine increases the release of GABA from inhibitory presynaptic terminals while it slightly decreases $GABA_A$ receptor-mediated synaptic currents by activating α_1-adrenoceptor receptors on postsynaptic membrane.

Typical examples of isoproterenol were shown in Fig. 5A and B. Cumulative amplitude and inter-event interval distribution were obtained by pooling 150 events from each

of 8 pyramidal neurons (total events number is 1200) before and during application of isoproterenol. Isoproterenol significantly decreased the inter-event interval of mIPSCs ($P < 0.05$, K-S test). The mean inter-event interval of mIPSCs in control (0.45 ± 0.16 s) was significantly decreased by isoproterenol (0.34 ± 0.11 s, n = 8, $P < 0.05$, paired *t*-test; Fig. 5D). Isoproterenol decreased the amplitude of mIPSCs ($P < 0.01$, K-S test). The mean amplitude of mIPSCs in control was 17.9 ± 2.0 pA, and significantly decreased by isoproterenol to 16.3 ± 1.4 pA (n = 8, $P < 0.05$, paired *t*-test; Fig. 5D). Isoproterenol had little effect on the 10-90% rise time (1.18 ± 0.03 to 1.16 ± 0.30 ms, n = 8; Fig. 5D) and half duration (15.5 ± 1.2 to 15.5 ± 1.4 ms, n = 8). These findings suggest that isoproterenol facilitates GABA release from presynaptic terminals, but decreases $GABA_A$ receptor-mediated synaptic currents.

Differential modulation of mIPSC frequency between phenylephrine and isoproterenol

Both phenylephrine and isoproterenol increased mIPSC frequency as shown in Figs. 4 and 5. However, profiles of changes in frequency by phenylephrine and isoproterenol are different in detail. Population histograms of mIPSC amplitude were obtained from pooling events used to make cumulative probability curves shown in Figs. 4C and 5C. As shown in Fig. 6A, phenylephrine increased mIPSC events with smaller amplitude (6-18 pA), and decrease larger mIPSC events (22-50 pA). Similar to the effects of phenylephrine, isoproterenol increased smaller mIPSC events (6-14 pA; Fig. 6B). Isoproterenol, however, had just marginal effects on larger events.

Discussion

The principal findings of this study are that: (1) phenylephrine decreased PPR of eIPSCs with smaller amplitude, but decreased PPR with larger amplitude, (2) isoproterenol

consistently decreased PPR of eIPSCs, (3) both phenylephrine and isoproterenol decreased mIPSC amplitude, and (4) that phenylephrine increases the frequency of smaller mIPSC events with a decrease of larger events, while isoproterenol increases the frequency of mIPSCs with smaller amplitude and have little effect on larger events. These findings suggest that modulatory mechanisms of phenylephrine on glutamatergic and GABAergic synaptic transmission are different in several aspects as discussed below.

α_1-Adrenoceptors in presynaptic terminals

Regarding α-adrenergic receptor-mediated effects on IPSCs, it has been reported that adrenaline increases the frequency of $GABA_A$ receptor-mediated spontaneous IPSCs (sIPSCs) through presynaptic α-adrenergic receptors in rat sensorimotor cortex (8). Kawaguchi and Shindou (9) reported an increase of sIPSC frequency by α_1-adrenoceptor activation in rat frontal cortex, and extended them by demonstrating that the α_1-adrenoceptor agonist, 6-fluoronorepinephrine, depolarizes the resting membrane potential of several types of GABAergic interneurons, which is frequently accompanied by spike discharges. These findings indicate that noradrenaline-induced increase of sIPSCs is mediated by its excitatory effect on interneurons.

Although several studies reported that the presynaptic α-adrenoceptors are α_2 type (21), the present study showed an increase in mIPSC frequency by phenylephrine, suggesting the presence of α_1-adrenoceptors in GABAergic presynaptic terminals. There are studies that showed an increase of mIPSC frequency by noradrenaline via α_1-adrenoceptors in the frontal (9) and entorhinal cortices (10). A recent immunoelectron microscopic study supports the hypothesis of the presence of α_1-adrenoceptors in the presynaptic terminals: a small number of immunoreaction products of α_1-adrenoceptors were detected in axons and presynaptic sites in addition to postsynaptic regions such as the somata and dendrites in rat visual cortex.

Taken together with these findings, it is likely that α_1-adrenoceptors exist not only in the postsynaptic sites but also in the presynaptic terminals, and these receptors play a facilitatory role for releasing GABA.

The above view in terms of presynaptic α_1-adrenoceptors poses a question: is the effect of activation of presynaptic α_1-adrenoceptors homologous regardless of the multiple types of GABAergic neurons? GABAergic interneurons in the cerebral cortex can be divided into at least three main categories by their electrophysiological firing properties: fast spiking (FS) cells, late spiking (LS) cells, and low-threshold spiking (LTS) cells (6, 22). FS cells, which show abrupt firing discharge with little adaptation, induced larger unitary IPSCs (uIPSCs) in amplitude (22), and their axons mainly project to soma or proximal dendrites (22). On the other hand, LTS cells, which are characterized by low-threshold spikes or rebound spikes after hyperpolarizing current pulse injections, induced smaller uIPSCs and their axons innervate to distal dendrites (22, 23). The present results of eIPSC recording, i.e. phenylephrine decreased PPR of eIPSCs with smaller amplitude, but increased PPR with larger amplitude (Fig. 2). The PPR of synaptic responses changes in association with presynaptic manipulations of transmitter release, but remains unaltered following postsynaptic manipulation of synaptic transmission (19, 20). Therefore, it is likely that phenylephrine may increase release probability from GABAergic terminals in the distal sites from recording electrode, and decrease release probability of terminals in the proximal dendrites or soma. This hypothesis is supported by the present findings of mIPSC recording, in which phenylephrine increases the frequency of smaller mIPSC events with a decrease of larger events (Fig. 6), and may explain a reason of heterogenous effects of adrenaline on eIPSC amplitude (Fig. 2, (8)). Phenylephrine may decrease the amplitude of eIPSCs that are mainly composed of GABAergic fibers terminating to proximal dendrites or somata, while

eIPSCs evoked by stimulation of fibers projecting to distal dendrites may be less affected or increased by phenylephrine.

Presynaptic facilitation of GABA release by β-adrenoceptor agonist

A whole-cell patch clamp study has revealed that an activation of presynaptic β-adrenoceptors facilitates glutamate release from presynaptic terminals in rat prefrontal cortex (13), and the previous study in rat visual cortex corroborates their report (7). These results are in agreemFent with the anatomical evidence of presynaptic localization of β-adrenoceptors (24) and studies that show glutamate release by β-adrenoceptor activation in cerebrocortical synaptosome preparations (11,12). However, little information is available on the β-adrenoceptor-mediated GABA release in the cerebral cortex. The present results of PPR reduction and an increase in mIPSC frequency by isoproterenol suggest that GABA release is facilitated by activation of β-adrenoceptors. In comparison to the effects of phenylephrine, isoproterenol tended to decrease the PPR across a broader range of eIPSC amplitude (Fig. 3C). Taken the decrease in mIPSC amplitude into account, less effect on large amplitude of mIPSCs (> 20 pA) by isoproterenol may be due to an increase in release probability of the terminals on proximal dendrites and somata.

Functional implication

The present study suggests that both α_1- and β-adrenoceptors in presynaptic terminals contribute to facilitate GABA release from, at least, a subpopulation of GABAergic interneuron terminals. On the other hand, it was suggested that activation of α_1-adrenoceptors had little effect on glutamate release though β-adrenoceptor activation facilitates glutamate release. These findings suggest the differential modulation mechanisms of neurotransmitter release in the cerebral cortex. Indeed, several important proteins

involved in the neurotransmitter release machinery such as synapsin, synaptophysin, and synaptosomal-associated protein (SNAP)-25 are differentially expressed in glutamatergic and GABAergic axon terminals in rat cerebral cortex (25). There might be a case that α_1- and β-adrenoceptors have differential mechanisms of phosphorylation of these proteins.

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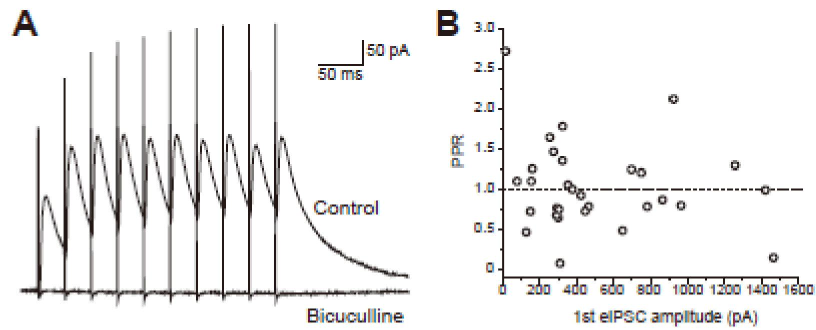

Fig. 1 Properties of eIPSCs recorded from layer V pyramidal neurons. ***A.*** Ten consecutive IPSCs were induced by repetitive stimulation at 33 Hz under application of 40 μM DNQX and 50 μM D-APV. Bath application of bicuculline methiodide (10 μM) diminished eIPSCs completely. Averaged traces of 10 consecutive eEPSCs are shown. ***B.*** PPR of the amplitudes of 1st and 2nd eIPSCs are plotted against the 1st eIPSC amplitude. There is no correlation between PPR and 1st eIPSC amplitude.

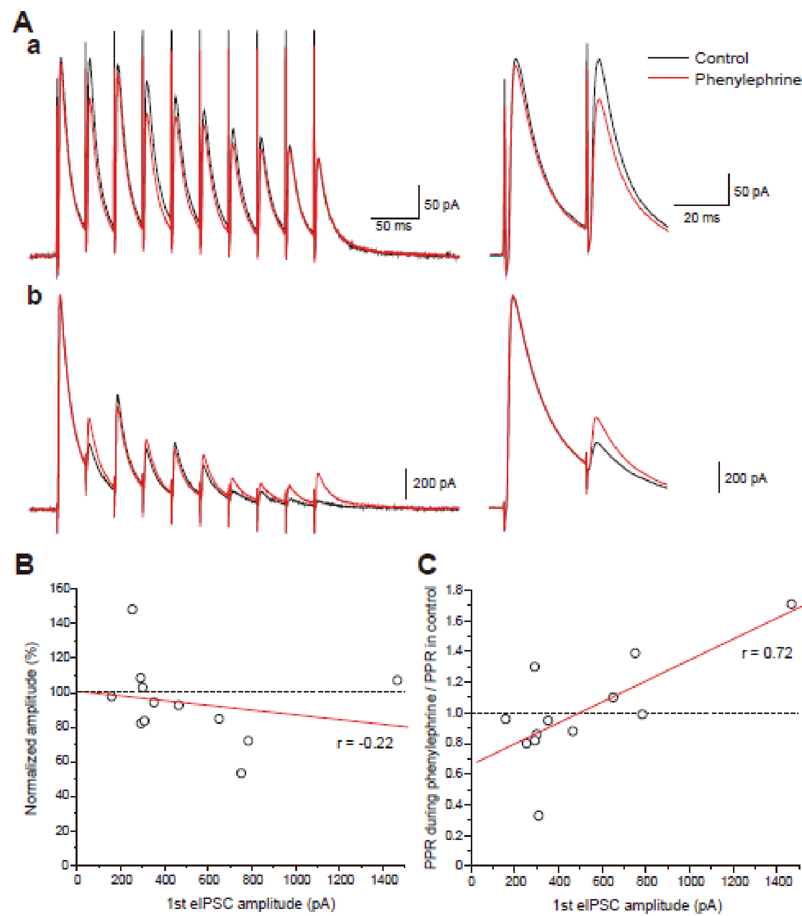

Fig. 2 Effects of phenylephrine on eIPSCs. ***A.*** Examples of the effects of 100 μM phenylephrine on 10 consecutive IPSCs with smaller amplitude of the 1st eIPSC (***a***) and larger amplitude of the 1st eIPSC (***b***) under application of 40 μM DNQX and 50 μM D-APV. Ten traces were averaged. Note that phenylephrine decreased the amplitude of most of 2nd to 10th eIPSCs in ***a***, and increased the amplitude of 2nd to 10th eIPSCs in ***b***. Right panels of ***a*** and ***b*** show time-expanded traces of 1st and 2nd eIPSCs. Black and red lines indicate traces obtained from control and phenylephrine conditions, respectively. ***B.*** Normalized amplitude of 1st eIPSC amplitude under 100 μM phenylephrine application was plotted against the 1st eIPSC amplitude in control. No correlation between them was seen (n = 12). ***C.*** Ratio of PPRs of the first and 2nd eIPSCs during phenylephrine application devided by PPRs in control was plotted against the 1st eIPSC amplitude in control (n = 12). There is a significant positive correlation between them (r = 0.72, $P < 0.01$).

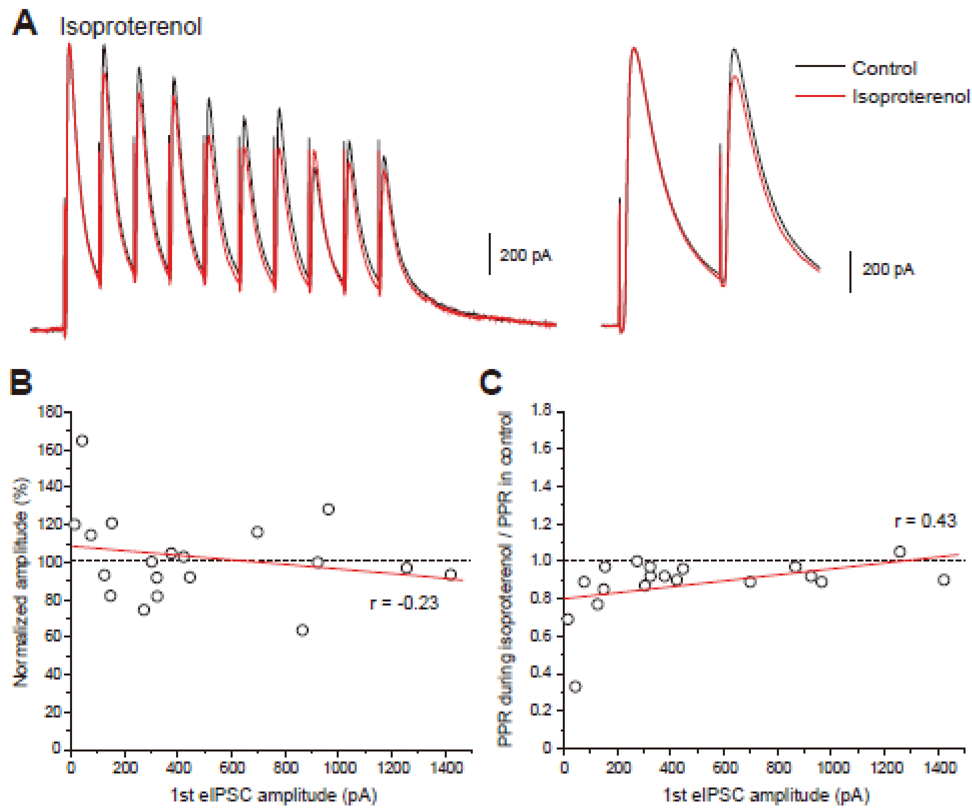

Fig. 3 Effects of isoproterenol on eIPSCs. ***A.*** An example of the effects of 100 μM isoproterenol on 10 consecutive IPSCs under application of 40 μM DNQX and 50 μM D-APV. Ten traces were averaged. Note that isoproterenol decreased the amplitude of 2nd to 10th eIPSCs. Right panel shows time-expanded traces of 1st and 2nd eIPSCs. Black and red lines indicate traces obtained from control and isoproterenol conditions, respectively. ***B.*** Normalized 1st eIPSC amplitude under 100 μM isoproterenol application was plotted against the 1st eIPSC amplitude in control. No correlation between them was seen (n = 20). ***C.*** Ratio of PPRs of the first and 2nd eIPSCs during isoproterenol application devided by PPRs in control was plotted against the 1st eIPSC amplitude in control (n = 20). There is a slight but insignificant correlation between them (r = 0.43, $P > 0.06$).

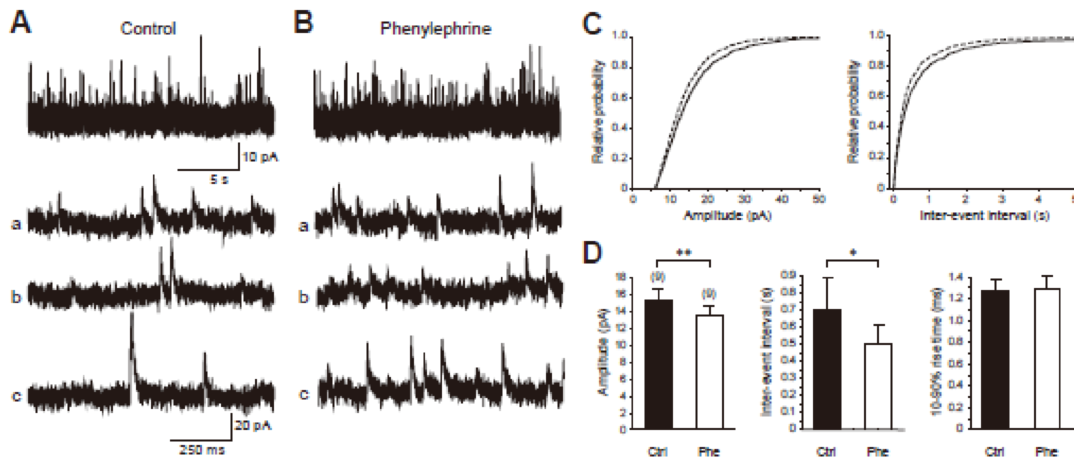

Fig. 4 The effects of phenylephrine on mIPSCs recorded from layer V pyramidal neurons. ***A,B.*** Consecutive mIPSCs before (***A***) and during bath application of 100 μM phenylephrine (***B***). Top traces represent 20-s recordings, and lower panels of ***a-c*** showed time-expanded segments. ***C.*** Cumulative probability plots of pooled data from 9 neurons. Phenylephrine decreased the amplitude ($P < 0.001$, K-S test) and inter-event interval of mIPSCs ($P < 0.0001$, K-S test). ***D.*** The effects of phenylephrine on the mean amplitude, inter-event interval, and 10-90% rise time of mIPSCs. Phenylephrine significantly decreased the mean of the amplitude ($P < 0.01$, paired *t*-test) and the inter-event interval of mIPSCs ($P < 0.05$, paired *t*-test).

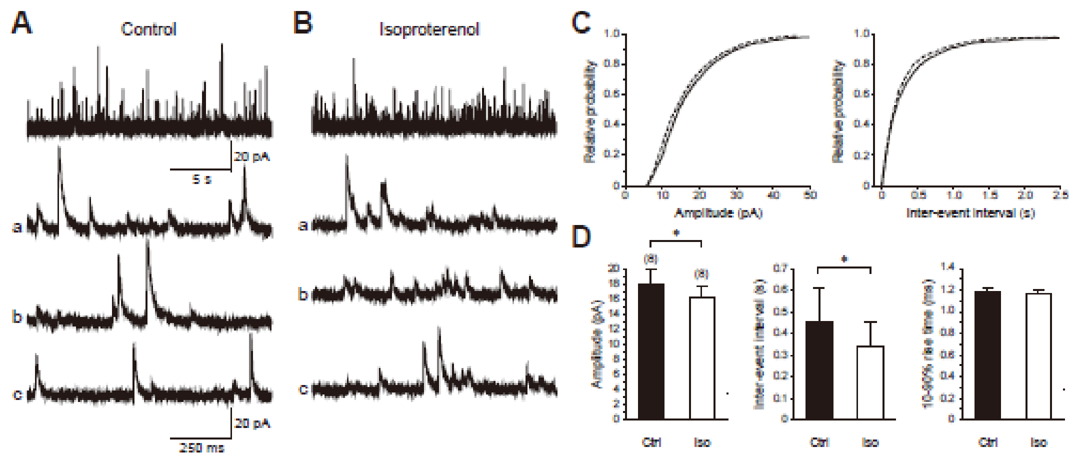

Fig. 5 The effects of isoproterenol on mIPSCs recorded from layer V pyramidal neurons. ***A,B.*** Consecutive mIPSCs before (***A***) and during bath application of 100 μM isoproterenol (***B***). Top traces represent 20-s recordings, and lower panels of ***a-c*** show time-expanded segments. ***C.*** Cumulative probability plots of pooled data from 8 neurons. Isoproterenol decreased the amplitude ($P < 0.001$, K-S test) and inter-event interval of mIPSCs ($P < 0.05$, K-S test). ***D.*** The effects of isoproterenol on the mean amplitude, inter-event interval, and 10-90% rise time of mIPSCs. Isoproterenol decreased the mean of mIPSC amplitude and inter-event interval ($P < 0.05$, paired *t*-test).

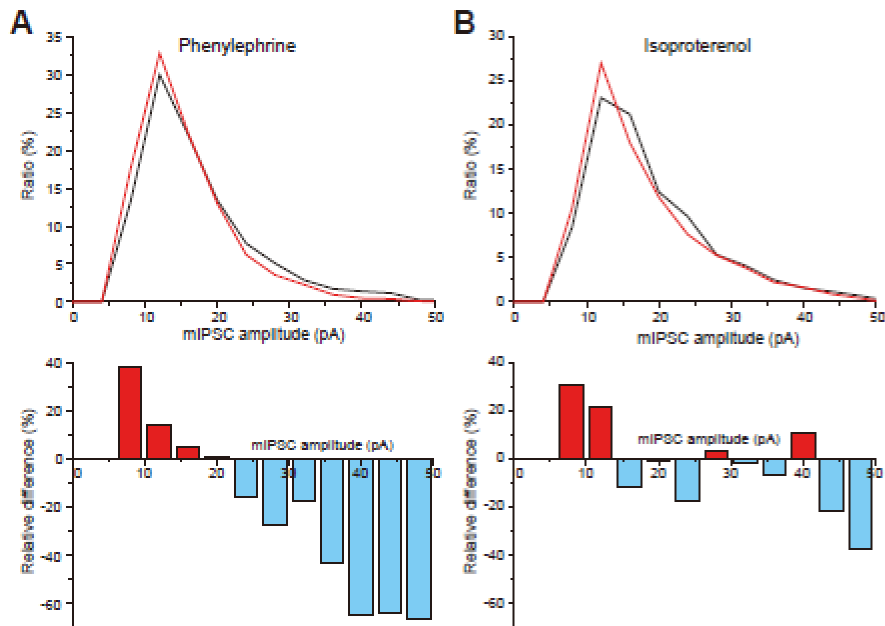

Fig. 6 Population histograms of mIPSC amplitude before and during application of phenylephrine (***A***) and isoproterenol (***B***). The same pooled events in Figs. 4C and 5C were used to obtained the histograms. The upper and lower panels show ratio of each events per total events, and relative difference between control and adrenergic agonists divided by control value, respectively. Phenylephrine increased mIPSC events with smaller amplitude and decrease larger mIPSC events, whereas isoproterenol increased smaller mIPSC events and had just slight effects on larger events.