Presynaptic Interneuron Subtype- and Age-Dependent Modulation of GABAergic Synaptic Transmission by β-Adrenoceptors in Rat Insular Cortex

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1Department of Pharmacology, 2Department of Anesthesiology, and 3Division of Oral and Craniofacial Research, Dental Research Center, Nihon University School of Dentistry, Tokyo; and 4Functional Probe Research Laboratory, Molecular Imaging Research Program, The Institute of Physical and Chemical Research, Kobe, Japan

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Koyanagi Y, Yamamoto K, Oi Y, Koshikawa N, Kobayashi M. Presynaptic interneuron subtype- and age-dependent modulation of GABAergic synaptic transmission by β-adrenoceptors in rat insular cortex. J Neurophysiol 103: 2876–2888, 2010. First published March 24, 2010; doi:10.1152/jn.00972.2009. β-Adrenoceptors play a crucial role in the regulation of taste aversion learning in the insular cortex (IC). However, β-adrenergic effects on inhibitory synaptic transmission mediated by γ-aminobutyric acid (GABA) remain unknown. To elucidate the mechanisms of β-adrenergic modulation of inhibitory synaptic transmission, we performed paired whole cell patch-clamp recordings from layer V GABAergic interneurons and pyramidal cells of rat IC aged from postnatal day 17 (PD17) to PD46 and examined the effects of isoproterenol, a β-adrenoceptor agonist, on unitary inhibitory postsynaptic currents (uIPSCs). Isoproterenol (100 μM) induced facilitating effects on uIPSCs in 33.3% of cell pairs accompanied by decreases in coefficient of variation (CV) of the first uIPSC amplitude and paired-pulse ratio (PPR) of the second to first uIPSC amplitude, whereas 35.9% of pairs showed suppressive effects of isoproterenol on uIPSC amplitude obtained from fast spiking (FS) to pyramidal cell pairs. Facilitatory effects of isoproterenol were frequently observed in FS–pyramidal cell pairs at ≥PD24. On the other hand, isoproterenol suppressed uIPSC amplitude by 52.3 and 39.8% in low-threshold spike (LTS)–pyramidal and late spiking (LS)–pyramidal cell pairs, respectively, with increases in CV and PPR. The isoproterenol-induced suppressive effects were blocked by preapplication of 100 μM propranolol, a β-adrenoceptor antagonist. There was no significant correlation between age and changes of uIPSCs in LTS–LS–pyramidal cell pairs. These results suggest the presence of differential mechanisms in presynaptic GABA release and/or postsynaptic GABA_A receptor-related assemblies among interneuronal subtypes. Age- and interneuron subtype-specific β-adrenergic modulation of IPSCs may contribute to experience-dependent plasticity in the IC.

INTRODUCTION

The insular cortex (IC) plays a critical role in taste aversion learning, a classical conditioning in which a single pairing of a novel taste and subsequent internal malaise prevents animals from repeated ingestion of the taste (Bermúdez-Rattoni 2004; Yamamoto 1994). The IC receives multimodal sensory information, including visceral, thermal sensations, nociception, and gustation from the sensory thalamic nuclei (Allen et al. 1991; Cechetto and Saper 1987; Jasmin et al. 2004; Nakashima et al. 2000; Yamamoto et al. 1988). In addition, limbic afferents from the amygdala and cingulate cortex project to the IC (Allen et al. 1991; Krettek and Price 1977) and thus the IC is considered to integrate the multimodal sensation and limbic information during acquisition of taste aversion learning. Previous studies have shown that formation and extinction of long-term conditioned taste aversion memory depend on β-adrenoceptor activation in the IC—i.e., microinfusion of propranolol, a β-adrenoceptor antagonist, impairs acquisition of conditioned taste aversion memory (Berman et al. 2000) and blocks extinction (Berman and Dudai 2001). These findings suggest that β-adrenergic functions may play an important role in regulation of plasticity in the IC, which contributes to induction of taste aversion learning.

Although the actions of β-adrenoceptors on excitatory synaptic transmission have been well studied in the cerebral cortex, i.e., facilitation of glutamate release from presynaptic terminals (Herrero and Sanchez-Prieto 1996; Huang and Hsu 2006; Kobayashi et al. 2009; Wang et al. 2002), little information is available on β-adrenoceptor-induced modulation of GABAergic synaptic transmission. In the cerebral cortex, 10–20% of neurons are nonsynaptic smooth neurons, which are considered to be GABAergic neurons (Gabbott and Somogyi 1986) and classified into ≥19 types (Peters and Regidor 1981; Szentagotai 1978). Electrophysiological studies have revealed that GABAergic interneurons can be divided into more than four classes according to their firing properties (Kawaguchi and Kubota 1997). These multiple subtypes of GABAergic interneurons may induce heterogeneous modulations on neural functions. In fact, Xiang et al. (1998) reported differential cholinergic actions on cell excitability between fast spiking (FS) and low-threshold spike (LTS) cells. Therefore there is a possibility that the actions of a β-adrenoceptor agonist may differ depending on the targeted interneuron subtypes.

The aim of the present study was to test the hypothesis that β-adrenergic modulation of GABAergic synaptic transmission in the IC depends on presynaptic interneuron subtypes. We performed paired whole cell patch-clamp recordings from GABAergic interneurons and pyramidal cells in layer V of the IC and recorded unitary inhibitory postsynaptic currents (uIPSCs) from postsynaptic pyramidal cells by eliciting action potentials (APs) in presynaptic interneurons. We classified the presynaptic interneurons into three subtypes—FS, LTS, and late spiking (LS) cells—by their electrophysiological properties and examined the effects of isoproterenol, a β-adrenoceptor agonist, on uIPSCs obtained from each of the interneuron–pyramidal cell pairs. We found that uIPSCs recorded from LTS– and LS–pyramidal cell pairs were almost consistently suppressed by isoproterenol, whereas FS–pyramidal cell pairs showed age-dependent and pleiotropic modulation of uIPSCs by isoproterenol.
METHODS

All experiments were performed in accordance with the National Institutes 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

We used a vescicular γ-aminobutyric acid (GABA) transporter (VGAT)–Venus line A transgenic rats of either sex aged from postnatal day 17 (PD17) to PD46, in which Venus, a fluorescent protein (Nagai et al. 2002), is coexpressed with VGAT. VGAT-Venus rats have fluorescent labeling of almost all cortical GABAergic cells (Uematsu et al. 2008). We crossed these transgenic rats with wild-type Wistar rats and used heterozygous transgenic offspring, GABAergic interneurons were easily identified in cortical slices prepared from these rats (Fig. 1A).

Animals were deeply anesthetized with sodium pentobarbital (75 mg/kg, administered intraperitoneally) and decapitated. Tissue blocks including the IC around the intersection of the middle cerebral artery and rhinal fissure, which is known to be activated by gustatory stimulation (Kobayashi et al. 2010), 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 MgSO4, 26 NaHCO3, 2.5 CaCl2, and 10 D-glucose. Coronal slices were cut at 350 μm thickness using a microslicer (Leica slicer 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 Kyoho, Japan). Slices were incubated at 32°C for 40 min in a submersion-type holding chamber, which contained 50% M-ACSF (in mM): 70 potassium gluconate, 70 KCl, 10 NaCl, 1.25 NaH2PO4, 26 NaHCO3, 2.5 CaCl2, 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% O2-5% CO2. Slices were thereafter maintained at room temperature until used for recording.

Cell identification and paired whole cell patch-clamp recordings

The slices were transferred to a recording chamber that was perfused continuously with normal ACSF, humidified with 95% O2-5% CO2 at a rate of 1.0–1.5 ml/min. Paired whole cell patch-clamp recordings were obtained from Venus-positive fluorescent neurons and pyramidal cells identified in layer V by a fluorescence microscope equipped with Nomarski optics (×40, Olympus BX51, Tokyo) and an infrared-sensitive video camera (Hamamatsu Photonics, Hamamatsu, Japan). The distance between Venus-positive and pyramidal 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).

Venus-positive cells were recorded under current-clamp mode. The composition of the pipette solution for current-clamp recordings was (in mM): 70 potassium glutonate, 70 KCl, 10-N-(2-hydroxyethyl)piperezine-N’-2-etanesulmonic acid (HEPES), 15 biocytin, 0.5 EGTA, 2 MgCl2, 2 disodium adenosine triphosphate (ATP), and 0.3 sodium guanosine triphosphate (GTP).

Pyramidal cells were recorded under voltage-clamp mode. The composition of the pipette solution for voltage-clamp recordings was (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-amino-phenoxo)ethane-N,N,N’,N’-tetraacetic acid (BAPTA). The presence of QX-314 and cesium in the pipette solution precluded recording GABA_A-receptor-mediated IPSCs. Both pipette solutions had a pH of 7.3 and osmolality of 300 mOsm. The liquid junction potentials 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 Instrument, Novato, CA).

Recordings were obtained over 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. Recordings were started >10 min after rupture of cell membrane of the second cells. Before uIPSC recordings, voltage responses of presynaptic interneurons were recorded by application of long hyper- and depolarizing current pulse (500–1,000 ms) injections to examine basic electrophysiological properties including input resistance, single spike kinetics, voltage–current (V–I) relationship, and repetitive firing pattern and frequency. During the tests, the pyramidal neurons were voltage-clamped at 0 mV, near the reversal potential of glutamatergic input and their current responses were simultaneously recorded to check synaptic connections from interneurons to pyramidal cells. It took about 10–15 min to complete these tests and then uIPSCs were recorded. uIPSCs were recorded from pyramidal cells (holding potential: 0 mV) by applying depolarizing five train pulses (2 ms duration, 20 Hz) to presynaptic Venus-positive interneurons at 0.05 Hz. We recorded uIPSCs under application of normal ACSF for 7.5–10 min as controls and applied isoproterenol for 7.5–10 min and washed out isoproterenol for 10–15 min.

For blocking GABA_A receptors, 10 μM bicuculline methiodide (Tocris Cookson, Bristol, UK) was applied. Isoproterenol (100 μM; Research Biochemicals International, Natick, MA) and propranolol (100 μM) were added directly to the perfusate. Membrane currents and potentials were low-pass filtered at 5–10 kHz and digitized at 20 kHz.

Data analysis

Clampfit (pCLAMP 9, Axon Instruments) was used for analyses of electrophysiological data. Input resistance of Venus-positive cells was measured from slopes of least-squares regression lines fitted to F–I curves measured at the peak voltage deflection (current pulse amplitude up to ~100 pA). Single APs were evoked by a brief (2 ms) depolarizing current pulse and the amplitudes of the AP and afterdepolarization (ADP) were measured from the AP threshold. By application of depolarizing step current pulses (1 s), the AP threshold was identified as the minimal potential from which the first AP was elicited. The amplitude of afterhyperpolarization (AHP) was measured from the negative peak to the AP threshold. Repetitive firing in response to long (1 s) depolarizing current pulses was evaluated by measuring the slope of least-squares regression line in a plot of the number of spikes versus the amplitude of injected current (F–I curve; up to ~500 pA).

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. We checked the stability of uIPSC amplitude of 20–30 events in control and included the results obtained from pairs that satisfy the following criteria: 1) no significant correlation between amplitude and time and 2) the difference in uIPSC amplitude of first and last 5–10 events was <10%. Average amplitude, failure rate, coefficient of variation (CV), and paired-pulse ratio (PPR) of uIPSCs were calculated from 10–20 consecutive sweeps. Typically the last 10–20 events of each period (control and isoproterenol application) were used for quantification of the effects of isoproterenol on uIPSCs. uIPSC amplitude in the range of synaptic noise was taken as failure. The 20–80% rise time, 80–20% decay time, and onset latency of uIPSCs were measured from average traces, which were obtained from traces aligned to the peak of presynaptic APs. Onset latency was defined as the time interval between the peak of the presynaptic AP and the point at which uIPSCs rose to the threshold that was set at 3SD of baseline noise amplitude.

Data are presented as means ± SE. The intrinsic electrophysiological properties of interneurons and kinetics of uIPSCs were compared...
by one-way ANOVA followed by post hoc Tukey's test. Age dependence of isoproterenol-induced changes of uIPSC amplitude was analyzed using two-tailed multiple $t$-test with Bonferroni correction and Pearson’s correlation coefficient test. In each cell pair, Student’s $t$-test was used to classify the isoproterenol-induced changes of uIPSC amplitude, i.e., facilitation, suppression, or no change. Normalized amplitude of the first to fifth uIPSCs was fitted by a single exponential function (Origin 8, OriginLab, Northampton, MA). Comparisons of the uIPSC amplitude, CV, and PPR between control and isoproterenol application were conducted by paired $t$-tests. Because the distribution of failure rate could not be fitted with normal distribution, nonparametric Wilcoxon test was applied to compare failure rate of control

![Image of layer V IC slice under differential interference contrast infrared video microscopy (a) and fluorescence image of Venus-expressing cells (b) in a postnatal day 19 (PD19) rat. Paired whole cell patch-clamp recording was performed from a Venus-positive cell (arrowheads in a and b) and a pyramidal cell (arrow in a). White dotted lines indicate the outline of patch electrodes. The pial surface is rightward. B: repetitive firing induced by a depolarizing current pulse injection (1 s) to Venus-positive cells in A. This cell was classified as a fast spiking (FS) cell, since it exhibited large and fast afterhyperpolarization and high-frequency spike firing without spike adaptation (bottom). The resting membrane potential was $-70$ mV. C: voltage responses to hyperpolarizing current pulse injections to Venus-positive cells in A. The resting membrane potential was $-71$ mV. D: postsynaptic unitary IPSCs (uIPSCs) recorded from pyramidal cells responding to presynaptic action potentials (APs) induced by short depolarizing current pulse injection (2 ms) to Venus-positive FS cells in A. Five pulses were applied to the presynaptic cell at 20 Hz. The presynaptic FS cell and postsynaptic pyramidal cell were recorded under current- and voltage-clamp condition, respectively. Holding potential of the postsynaptic cell was 0 mV. Bath application of bicuculline (10 $\mu$M) completely blocked uIPSCs. E: time course of 1st uIPSC amplitude before and during 10 $\mu$M bicuculline application obtained from the same FS–pyramidal cell pairs as shown in A–D. F: normalized amplitude of 1st to 5th uIPSCs obtained from 46 FS–pyramidal cell pairs. A smooth curve indicates a single exponential fit. Time constant ($r$) was 74.2 ms.
TABLE 1. *Intrinsic electrophysiological properties of layer V interneurons*

<table>
<thead>
<tr>
<th>Interneuron Subtype</th>
<th>Fast Spiking</th>
<th>Low-Threshold Spike</th>
<th>Late Spiking</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>n</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Resting membrane potential ((V_{m0})), mV</td>
<td>-72.6 ± 3.0</td>
<td>48</td>
<td>-66.1 ± 1.6</td>
</tr>
<tr>
<td>Input resistance ((R_{in})), MΩ</td>
<td>183.9 ± 11.3***</td>
<td>43</td>
<td>512.9 ± 63.6###</td>
</tr>
<tr>
<td>Action potential</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>-52.2 ± 0.9***</td>
<td>44</td>
<td>-59.2 ± 1.2</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>48.0 ± 1.9**</td>
<td>44</td>
<td>61.1 ± 4.0</td>
</tr>
<tr>
<td>Half-duration, ms</td>
<td>0.7 ± 0.0***,##</td>
<td>44</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Afterhyperpolarization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>18.4 ± 0.8*</td>
<td>44</td>
<td>13.6 ± 1.3</td>
</tr>
<tr>
<td>Half-duration, ms</td>
<td>23.4 ± 2.8***,###</td>
<td>44</td>
<td>149.4 ± 29.4###</td>
</tr>
<tr>
<td>Repetitive firing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope, spikes/pA</td>
<td>0.36 ± 0.04**##</td>
<td>40</td>
<td>0.15 ± 0.2</td>
</tr>
</tbody>
</table>

One-way ANOVA followed by post hoc Tukey’s test. *P < 0.05, **P < 0.01, ***P < 0.001 in comparison with low-threshold spike cell. #P < 0.05, ##P < 0.01, ###P < 0.001 in comparison with late spiking cell.

and isoproterenol application. The level of \(P < 0.05\) was adopted to indicate significance.

**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 otherwise, were purchased from Sigma-Aldrich (St. Louis, MO).

**Results**

Paired whole cell patch-clamp recordings were performed from Venus-positive GABAergic and Venus-negative pyramidal cells in layer V of the IC (Fig. 1A). Venus-positive presynaptic interneurons were classified into three subtypes on the basis of membrane and firing properties: FS, LTS, and LS cells. The regular spiking interneurons were not included for analysis in this study. The subthreshold and AP properties of FS, LTS, and LS cells are shown in Table 1.

**FS to pyramidal cell synapses**

FS cells were characterized by their unique AHP, i.e., large amplitude with very rapid repolarization (Fig. 1B; Kawaguchi and Kubota 1997; Xiang et al. 1998, 2002). Duration of single APs was shorter than that of other interneuron subtypes (\(P < 0.001\) for LTS cells and \(P < 0.01\) for LS cells, Tukey’s test; Table 1). A long depolarizing current pulse injection (1 s) to FS cells induced repetitive firing at extremely high frequency, which often exceeded 100–150 Hz, and almost no adaptation was observed (Fig. 1B). Slope of the frequency–current (\(F–I\)) curve obtained from FS cells was significantly larger than that of other interneuron subtypes (\(P < 0.01\) for LTS cells and \(P < 0.05\) for LS cells, Tukey’s test; Table 1). The input resistance of FS cells was lower than that of LTS cells (\(P < 0.001\), Tukey’s test; Table 1) and subthreshold responses to hyperpolarizing current pulse injection seldom showed sag (Fig. 1C). Synaptic inputs with high frequency were often observed in FS cells (Fig. 1C). The connection rate from FS to pyramidal cells was 32.4%.

The electrophysiological properties of uIPSCs obtained from FS–pyramidal cell pairs are summarized in Table 2. FS cells evoked uIPSCs in pyramidal cells with a shorter latency than that of LTS/LS cells (\(P < 0.001\), Tukey’s test). uIPSCs evoked by FS cells showed larger amplitude and shorter 20–80% rise time compared with that of LTS cells (\(P < 0.05\) and 0.001, respectively, Tukey’s test), which is consistent with a previous paired recording study in the visual cortex (Xiang et al. 2002). Compared with LS–pyramidal cell pairs, FS–pyramidal cell pairs showed a shorter rise time, decay time, and half-duration (\(P < 0.001\), 0.05, and 0.001, respectively, Tukey’s test).

To examine the properties of short-term dynamics of uIPSCs, five train pulses (2 ms) were applied at 20 Hz to presynaptic FS cells. Most FS–pyramidal cell pairs (38/46 pairs, 82.6%) exhibited short-term depression of uIPSCs (Fig. 1D). The remaining 6/46 pairs (13.0%) showed short-term facilitation and 2/46 pairs (4.3%) showed almost no change. uIPSCs were almost completely diminished by bath application of bicuculline me-
and against age of animals (Fig. 4B). Successive decrement in the normalized amplitude of five trains of uIPSCs was fitted by single exponential functions; the time constant (τ) of the exponential fit was 74.2 ms (Fig. 1F).

Reciprocal effects of isoproterenol on uIPSCs between FS and pyramidal cells

There was variation in the effects of isoproterenol (100 μM) on the first uIPSC amplitude. In 33.3% of FS–pyramidal cell pairs (13/39 pairs), isoproterenol significantly enhanced first uIPSCs in amplitude by 32.0 ± 4.9% (P < 0.001, paired t-test) and the facilitatory effect of isoproterenol on uIPSCs was washable (P < 0.001, paired t-test; Fig. 2, A–C). On the other hand, second to fifth uIPSCs were less affected by isoproterenol (Fig. 2D). To examine which synaptic site (i.e., presynaptic terminal or postsynaptic membrane) is affected by isoproterenol, we measured failure rate; CV, which varies with quantal content and is independent of changes in postsynaptic responses (Bekkers and Stevens 1990); and PPR, which is sensitive to changes in presynaptic release of transmitter (Manabe et al. 1993; Zucker 1989). In the cell pairs exhibiting isoproterenol-induced facilitation of uIPSCs, isoproterenol decreased failure rate of these cell pairs from 10.4 ± 4.6 to 3.2 ± 2.7% (n = 13; P < 0.05, Wilcoxon test; Fig. 2E). CV of the first uIPSC amplitude was significantly decreased from 0.54 ± 0.07 to 0.32 ± 0.05 (n = 13; P < 0.001, paired t-test; Fig. 2E). PPR of the second to first uIPSC amplitude was decreased from 0.86 ± 0.05 to 0.63 ± 0.05 by isoproterenol (n = 13; P < 0.01, paired t-test; Fig. 2E). The decay time constant (τ) of the decrements in normalized amplitude of five train uIPSCs were 67.4 and 38.2 ms in control and isoproterenol application, respectively (Fig. 2D). Taken together, these findings suggest that the facilitative effects of isoproterenol on uIPSCs could be caused by an increase in presynaptic GABA release.

In contrast, the same percentage of FS–pyramidal cell pairs (35.9%, 14/39 pairs) showed uIPSC suppression by isoproterenol (30.6 ± 2.4%), which was not washable (Fig. 3A–C). As shown in Fig. 3E, a decrease in uIPSC amplitude by isoproterenol was often accompanied by an increase in PPR (0.70 ± 0.05 to 0.82 ± 0.08; P < 0.05, paired t-test), although both failure rate and CV of the first uIPSC amplitude were not significantly different between control and isoproterenol application (Wilcoxon test and paired t-test, respectively). In these cell pairs, second to fifth uIPSCs were also decreased by isoproterenol (Fig. 3D). The values of decay time constant of the amplitude of five train uIPSCs were 53.4 ms in control and 88.9 ms in isoproterenol application (Fig. 3D). These results suggest that a decrease in uIPSCs by isoproterenol may be ascribed to both presynaptic and postsynaptic mechanisms.

The other cell pairs (30.8%, 12/39 pairs) were not affected significantly by isoproterenol.

Relationship between effects of isoproterenol and uIPSC amplitude or age

To explore the mechanism of variation of isoproterenol-induced modulation on uIPSCs from FS to pyramidal cells, the normalized amplitude of uIPSCs during isoproterenol application was plotted against uIPSC amplitude in controls (Fig. 4A) and against age of animals (Fig. 4B).

There was no linear relationship between normalized amplitude of uIPSCs during isoproterenol application and uIPSC amplitude in control, although the pairs with larger uIPSC amplitude tended to be less affected by isoproterenol (Fig. 4A). On the other hand, plots of isoproterenol-induced effects on uIPSC amplitude indicated age-dependent modulation by isoproterenol (Fig. 4B). There was a significant correlation between the age and isoproterenol-induced increase of uIPSC amplitude during PD18–PD29 (P < 0.05, Pearson’s correlation coefficient test). Some FS–pyramidal cell pairs showed isoproterenol-induced uIPSC depression throughout all aged tested, whereas facilitation by isoproterenol was often observed in animals of ≥PD24, with the significant increase occurring in PD24–PD29 (Fig. 4C, P < 0.05, two-tailed multiple t-test with Bonferroni correction, three comparisons in four groups).

LTS to pyramidal cell synapses

LTS cells were characterized by low-threshold spikes (Table 1) and rebound APs after hyperpolarizing current pulse injections (500 ms; Fig. 5, A and B). Subthreshold responses to hyperpolarizing current pulse injection often showed sag (Kawaguchi and Kubota 1997; Xiang et al. 2002).

Connection from LTS interneurons to pyramidal cells was 13.4%. The electrophysiological properties of uIPSCs obtained from LTS–pyramidal cell pairs are summarized in Table 2. Repetitive depolarizing current pulse injection (five pulses at 20 Hz) to presynaptic LTS cells induced short-term depression of uIPSCs recorded from pyramidal cells in 92.3% of LTS–pyramidal cell pairs (12/13 pairs), as shown in Fig. 5C. One of 13 LTS–pyramidal cell pairs (7.7%) exhibited slight short-term facilitation.

Bath application of 100 μM isoproterenol invariably decreased uIPSC amplitude obtained from LTS–pyramidal cell pairs (Fig. 5, C–E) and the decrement of uIPSCs could not be recovered by about 10 min washout of isoproterenol. The first uIPSCs were significantly suppressed by 52.3 ± 5.0% in amplitude (P < 0.01, paired t-test). Preapplication of propranolol (100 μM), a β-adrenoceptor antagonist, prevented this suppressive effect of isoproterenol on uIPSCs obtained from LTS–pyramidal cell pairs (n = 8; Fig. 7A). The values of time constant of the exponential fit to successive decrements in normalized amplitude of uIPSCs were 25.1 ms in control and 34.4 ms in isoproterenol application, respectively (Fig. 5D). Isoproterenol-induced suppression of uIPSCs was accompanied by an increase in failure rate (n = 13; P < 0.05, Wilcoxon test), CV of the first uIPSC amplitude (n = 13; P < 0.01, paired t-test), and PPR (n = 13; P < 0.05, paired t-test), as shown in Fig. 5F. These results suggest that a decrease of release probability from presynaptic terminals is likely to be involved in β-adrenergic suppression of uIPSCs obtained from LTS–pyramidal cell pairs. In contrast to FS–pyramidal cell pairs, there was no significant relationship between suppression rate of uIPSC amplitude and age of animals (PD17–PD40; data not shown).

LS to pyramidal cell synapses

LS cells were characterized by a slowly developing ramp depolarization to spike threshold (Fig. 6A; Kawaguchi and
FIG. 2. Facilitative effects of isoproterenol on uIPSCs obtained from FS–pyramidal cell pairs. A: an example of the facilitative effects of 100 μM isoproterenol on 5 consecutive uIPSCs. Averages of 10–15 traces in control and during isoproterenol application are shown. Note that isoproterenol increases the amplitude of uIPSCs (arrows). Depolarized potentials just after APs (arrowheads) were formed by GABAergic inputs via autapse. B: time course of the increase of 1st uIPSC amplitude (open circles) and the decrease of paired-pulse ratio (PPR, 2nd/1st uIPSC amplitude; filled circles) by isoproterenol in the same pair as in A. C: time course of the averaged 1st uIPSC amplitude before, during, and after bath application of 100 μM isoproterenol obtained from FS–pyramidal cell pairs (n = 13) that showed isoproterenol-induced facilitation of uIPSCs. D: normalized amplitude of 1st to 5th uIPSCs obtained from 13 FS–pyramidal cell pairs that showed uIPSC facilitation by isoproterenol. Filled and open circles indicate normalized amplitude of uIPSCs in control and during isoproterenol application, respectively. Solid and dotted curves indicate single exponential fits to control (τ = 67.4 ms) and isoproterenol (τ = 38.2 ms), respectively. E: failure rate of 1st uIPSCs, coefficient of variation (CV) of the 1st uIPSC amplitude and PPR in control and during isoproterenol application that elicited an enhancement of 1st uIPSC amplitude (n = 13). Thick horizontal bars indicate mean values. All of these parameters were significantly decreased by isoproterenol. †P < 0.05, Wilcoxon test. **P < 0.01, ***P < 0.001, paired t-test.
FIG. 3. Suppressive effects of isoproterenol on uIPSCs obtained from FS–pyramidal cell pairs. A: an example of the suppressive effects of 100 μM isoproterenol on 5 consecutive uIPSCs. Averages of 10 –15 traces in control and during isoproterenol application (arrows) are shown. Note suppression of uIPSC amplitude by isoproterenol. B: time course of the decrease of 1st uIPSC amplitude (open circles) and the increase of PPR (2nd/1st uIPSC amplitude; filled circles) by isoproterenol in the same pairs as in A. C: time course of the averaged 1st uIPSC amplitude before, during, and after bath application of 100 μM isoproterenol obtained from FS–pyramidal cell pairs (n = 14) that showed isoproterenol-induced suppression of uIPSCs. D: normalized amplitude of 1st to 5th uIPSCs obtained from 14 FS–pyramidal cell pairs that showed uIPSC suppression by isoproterenol. Filled and open circles indicate normalized amplitude of uIPSCs in control and during isoproterenol application, respectively. Solid and dotted curves indicate single exponential fits to control (τ = 53.4 ms) and isoproterenol (τ = 88.9 ms), respectively. E: failure rate of 1st uIPSCs, CV of the 1st uIPSC amplitude and PPR in control and during isoproterenol application that elicited a suppression of 1st uIPSC amplitude (n = 14). Thick horizontal bars indicate the mean values. *P < 0.05, **P < 0.01, ***P < 0.001, paired t-test.
These results suggest that isoproterenol-induced suppression of uIPSCs obtained from LS–pyramidal cell pairs may be, at least partially, due to a decrease of release probability from presynaptic terminals. Similar to LTS–pyramidal cell pairs, however, isoproterenol suppressed uIPSCs by 10.2 ± 0.3% of control. The principal findings of this study are: 1) isoproterenol almost consistently decreased the amplitude of uIPSCs obtained from LTS– and LS–pyramidal cell pairs, which were accompanied by increases in failure rate, CV, and PPR; and 2) facilitatory effects of isoproterenol on uIPSCs were observed in FS–pyramidal cell pairs aged ≥PD24, whereas younger animals showed a tendency toward suppression of uIPSCs by isoproterenol. Thus the present study suggests that β-adrenergic modulation of uIPSCs depends on presynaptic interneuron subtype and age of animals. It is therefore possible that each interneuron subtype has its specific machineries involved in GABA release from presynaptic terminals and/or postsynaptic GABA_A receptors.

Pleiotropic β-adrenergic modulation of uIPSC obtained from FS–pyramidal cell pairs

About one third of FS–pyramidal cell pairs showed an enhancement of uIPSCs by isoproterenol. These β-adrenergic effects were accompanied by a decrease in CV and PPR, suggesting modulation of presynaptic GABA release mechanisms. As has been shown in pyramidal–pyramidal cell pairs, a β-adrenergic modulation mechanism, i.e., cAMP-dependent protein kinase (PKA) and its downstream signaling pathways, including p42/p44 mitogen-activated protein kinase (MAPK), facilitate glutamate release from presynaptic terminals (Huang and Hsu 2006). The FS–pyramidal cell pairs may share a common β-adrenergic modulation mechanism.

In contrast to the FS–pyramidal cell pairs showing isoproterenol-induced facilitation of uIPSCs, some of FS–pyramidal cell pairs showed the opposing β-adrenergic effect, i.e., suppression of uIPSCs. In the FS–pyramidal cell pairs with isoproterenol-induced uIPSC suppression, isoproterenol had little effect on failure rate and CV, suggesting postsynaptic regulation of GABA_A receptors by isoproterenol. In these FS–pyramidal cell pairs, however, isoproterenol suppressed uIPSC amplitude with an increase in PPR and the decay time constant of five train uIPSCs tended to be larger in isoproterenol application compared with that in control.

FIG. 4. A and B: relationship between isoproterenol-induced change of uIPSC amplitude and uIPSC amplitude or age of animals. There was little correlation between normalized change of uIPSCs by isoproterenol and uIPSC amplitude (A), whereas a significant correlation was observed between normalized uIPSC amplitude and age of animals ranging from PD18 to PD29 (P < 0.05, Pearson’s correlation coefficient test, r = 0.47; B). C: the average of isoproterenol-induced change in uIPSC amplitude. *P < 0.05; n.s., not significant (2-tailed multiple t-test with Bonferroni correction, 3 comparisons in 4 groups).

DISCUSSION

The intrinsic electrophysiological properties of LS cells, including the resting membrane potential, input resistance, spike threshold, spike, and AHP amplitude and duration, were in a position between those of FS and LTS cells (Table 1).

Connection rate from LS interneurons to pyramidal cells was 19.2%. The electrophysiological properties of uIPSCs obtained from LS–pyramidal cell pairs are summarized in Table 2. Compared with FS cells, LS cells evoked slower kinetics of uIPSCs in postsynaptic pyramidal cells: longer 20–80% rise time, 80–20% decay time, and half-duration of uIPSCs; these values were not significantly different from those of LTS cells. Repetitive depolarizing current pulse injection (five pulses at 20 Hz) to presynaptic LS cells induced short-term depression of uIPSCs recorded from pyramidal cells in 81.8% of LS–pyramidal cell pairs (9/11 pairs), as shown in Fig. 6C. Two of 11 LS–pyramidal cell pairs (18.2%) exhibited slight short-term facilitation.

Bath application of 100 μM isoproterenol invariably decreased the first uIPSC amplitude by 39.8 ± 9.4% obtained from LS–pyramidal cell pairs (Fig. 6, C–E). Similar to the case of LTS–pyramidal cell pairs, the decrement of uIPSCs could not be recovered by about 10 min washout of isoproterenol. Propranolol (100 μM) prevented this suppressive effect of isoproterenol on uIPSCs obtained from LS–pyramidal cell pairs (n = 10; Fig. 7B). The values of time constant of the exponential fit to successive decrements in normalized amplitude of uIPSCs were 40.0 ms in control and 56.6 ms in isoproterenol application (Fig. 6D). Isoproterenol-induced suppression of uIPSCs was accompanied by an increase in failure rate (n = 10; P < 0.05, Wilcoxon test), CV of the first uIPSC amplitude (n = 10; P < 0.05, paired t-test), and PPR (n = 10; P < 0.05, paired t-test), as shown in Fig. 6F. These results suggest that β-adrenergic suppression of uIPSCs obtained from LS–pyramidal cell pairs may be, at least partially, due to a decrease of release probability from presynaptic terminals. Similar to LTS–pyramidal cell pairs, there was no significant relationship between suppression rate of uIPSC amplitude by isoproterenol and age of animals (PD17–PD45).

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Therefore we cannot exclude the possibility that a part of suppression by β-adrenoceptors in FS–pyramidal cell pairs is mediated by presynaptic mechanism.

GABAergic terminals of FS cells express P/Q-type (Zaitsev et al. 2007) or N-type voltage-gated calcium channels (VGCCs; Ali and Nelson 2006). Currie and Fox (1997) showed that G protein-
FIG. 6. Effects of isoproterenol on uIPSCs from Venus-positive late spiking (LS) cells to pyramidal cells. A: voltage responses of Venus-positive cells around spike threshold in response to depolarizing current pulse injections. Note a slow ramp depolarizing potential (arrowhead) before spike initiation. B: repetitive firing induced by a depolarizing current pulse injection (1 s) to Venus-positive cells in A. C: isoproterenol (100 µM) suppressed 5 consecutive uIPSCs. Averages of 10 traces in control and during isoproterenol application are shown. D: normalized amplitude of 1st to 5th uIPSCs obtained from LS–pyramidal cell pairs in control and during isoproterenol application (n = 10). Nine pairs with short-term depression and one pair with short-term facilitation were included. Filled and open circles indicate normalized amplitude of uIPSCs in control and during isoproterenol application, respectively. Solid and dotted curves indicate single exponential fits to control (τ = 40.0 ms) and isoproterenol (τ = 56.6 ms), respectively. E: time course of the averaged 1st uIPSC amplitude before, during, and after bath application of 100 µM isoproterenol obtained from LS–pyramidal cell pairs (n = 10). Note suppression of uIPSCs by isoproterenol. F: failure rate of 1st uIPSCs, CV of the 1st uIPSC amplitude, and PPR in control and during isoproterenol application (n = 10). Thick horizontal bars indicate mean values. All of these parameters were significantly increased by isoproterenol. The values on the left of voltage traces in A–C indicate the resting membrane potential. †P < 0.05, Wilcoxon test; *P < 0.05, paired t-test.
mediated inhibition of N-type calcium channels is larger than that of P/Q-type calcium channels, providing a possibility that the controversial effects of isoproterenol described earlier could be explained by different expression of VGCC subtypes in FS presynaptic terminals. There are at least two subtypes of FS interneurons in the cerebral cortex: 1) basket cells, which project their axons to the soma and proximal dendrites of postsynaptic cells (Tamás et al. 1997); and 2) chandelier cells, which terminate onto axon initial segments (DeFelipe 1999). If basket and chandelier cells express different VGCC subtypes at their terminals, such anatomical variations of FS interneurons could explain the discrepancy of isoproterenol-induced changes in uIPSCs. Another possible explanation for isoproterenol-induced controversial effects on uIPSCs is that the difference in target molecules may contribute to contradictory β-adrenergic effects between these cell pairs. Indeed, several release machinery proteins involved in neurotransmitter release, i.e., synapsin, synaptophysin, and synaptosomal-associated protein (SNAP)-25, are differentially expressed in glutamatergic and GABAergic axon terminals in rat cerebral cortex (Bragina et al. 2007).

The suppressive effects of isoproterenol could not be washed out in LTS−, LS−, and a part of FS−pyramidal cell pairs. Isoproterenol-induced activation of second messenger cascade including cAMP and PKA (Kobayashi 2007) could not easily be interrupted by short-term wash, although the facilitatory effect of isoproterenol on uIPSC in a part of FS−pyramidal cell pairs was partially recovered. As previously reported, IPSCs obtained from interneuron−pyramidal cell pairs often show rundown (Bennett et al. 1998). In the present study, we excluded the results of uIPSCs that accompanied by rundown during control recordings. However, there is a possibility that rundown in a later recording period might prevent a recovery from the suppressive effect of isoproterenol.

In contrast to the β-adrenergic facilitation of glutamatergic synaptic transmission (Huang and Hsu 2006; Kobayashi et al. 2009), uIPSCs recorded from pyramidal cells by eliciting APs in LTS/LS interneurons showed opposing actions of β-adrenoceptor activation. These effects were accompanied by an increase in CV and PPR, suggesting presynaptic modulation by isoproterenol. Therefore there seems to be a different mechanism of β-adrenergic modulation of transmitter release between pyramidal−pyramidal and LTS−/LS−pyramidal cell pairs. Several possibilities could explain the discrepancy of β-adrenergic effects on synaptic transmission. Although it is still unknown what VGCCs express in presynaptic terminals of LTS and LS interneurons, it is possible that different expression of VGCCs might play a role in different regulation by β-adrenoceptors. Another possibility is that a difference in target molecules may contribute to contradictory β-adrenergic effects between these cell pairs. Indeed, several release machinery proteins involved in neurotransmitter release, i.e., synapsin, synaptophysin, and synaptosomal-associated protein (SNAP)-25, are differentially expressed in glutamatergic and GABAergic axon terminals in rat cerebral cortex (Bragina et al. 2007).

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Functional implications

Previous studies have reported that activation of β-adrenoceptors modulates not only presynaptic functions but also postsynaptic properties of pyramidal cells in the cerebral cortex. Isoproterenol effectively enhances repetitive firing frequency and decreases spike adaptation (Dodt et al. 1991; Foehring et al. 1989; Kobayashi et al. 2008a; Nowicky et al. 1992). Taken together with enhancement of evoked EPSCs (Huang and Hsu 2006; Kobayashi et al. 2009), β-adrenergic activation heightens responses of pyramidal cells to excitatory synaptic inputs. The present findings of suppression of uIPSCs evoked by LTS/LS or a part of FS cell activation support this idea. Most FS cells are considered to be basket cells, which project their axons densely to proximal dendrites and somata of pyramidal cells (Tamás et al. 1997) and evoke relatively large amplitude IPSCs (Kobayashi et al. 2008b; Xiang et al. 2002). Therefore it is likely that β-adrenoceptors occasionally suppress neural activities of pyramidal cells by an enhancement of uIPSCs evoked by a subpopulation of FS cells. Recent studies have reported that subpopulations of pyramidal cells receive more potent excitatory inputs than other cells and may play a leading role in processing sensory information in local circuits.
of the cerebral cortex (Lefort et al. 2009). If this can be generalized to some FS cells, these FS cells might control sensory processing by inhibiting key pyramidal cells; noradrenaline may regulate this modulation via β-adrenoceptors.

The adrenergic system is likely to modulate acquisition and maintenance of taste aversion learning as previously reported (Berman and Dudai 2001; Berman et al. 2000; Miranda et al. 2008), whereas N-methyl-d-aspartate (NMDA) receptors are also considered to play a crucial role for induction of taste aversion learning (Berman et al. 2000). These findings raise the question: how do β-adrenoceptors communicate with NMDA receptors in the IC? β-Adrenoceptor-mediated disinhibition of some pyramidal cells may cause membrane potential depolarization and activate NMDA receptors in cooperation with facilitation of glutamate release to pyramidal cells (Huang and Hsu 2006; Kobayashi et al. 2009). Those FS cells that show suppressive effects of isoproterenol on uIPSCs may contribute to controlling a specific local circuit to refine gustatory information processing in the IC.

The age-dependent facilitation of uIPSCs from FS to pyramidal cells by isoproterenol may contribute to experience-dependent refinement of cortical circuits. Indeed, in the visual cortex noradrenaline, especially β-adrenoceptors, regulates ocular dominance plasticity, which is age dependent and involves processes of activity-dependent synaptic modification (Kasamatsu and Pettigrew 1976; Wiesel 1982); activation of β-adrenoceptors with low-frequency stimulation induces LTP of synaptic transmission in the visual cortex (Kasamatsu and Pettigrew 1976; Wiesel 1982); activation of β-adrenoceptors increases firing frequency through protein kinase C in pyramidal neurons of rat cerebral cortex. Neurosci Lett 430: 175–180, 2008a.

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D i s c l o s u r e s

No conflicts of interest are declared by the authors.

R e f e r e n c e s


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