GABA<sub>C</sub> Receptors in the Rat Superior Colliculus and Pretectum Participate in Synaptic Neurotransmission

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Boller, Mathias and Matthias Schmidt. GABA<sub>C</sub> receptors in the rat superior colliculus and pretectum participate in synaptic neurotransmission. J Neurophysiol 89: 2035–2045, 2003. First published December 4, 2002; 10.1152/jn.00824.2002. In mammals, GABA<sub>C</sub> receptors seem to be specifically expressed in the retina and the subcortical visual system, with highest extraretinal expression levels in the superior colliculus (SC). Although its presence in the superficial SC has been demonstrated physiologically, a direct involvement of this receptor type in fast synaptic neurotransmission still awaits verification. We addressed the question of a possible synaptic localization of GABA<sub>C</sub> receptors by performing in vitro whole-cell patch-clamp recordings of inhibitory postsynaptic currents (IPSCs) in single neurons of the rat SC and the neighboring pretectal nuclear complex, where GABA<sub>C</sub> receptors are also expressed at significant levels. To increase the likelihood to record IPSCs we induced spontaneous activity by application of the potassium channel blocker 4-aminopyridine (4-AP) and blocked glutamate-mediated excitatory neurotransmission with kynurenic acid. All 4-AP–induced postsynaptic currents were of synaptic origin because they were completely suppressed by lidocaine or by substitution of extracellular calcium with cobalt. In 40% of the SC cells and in 60% of the pretectal neurons, IPSCs in the presence of 4-AP and kynurenic acid were only partly blocked by the selective GABA<sub>A</sub> receptor antagonist bicuculline. Inhibitory currents that were insensitive to bicuculline, however, could be blocked by coapplication of either the specific GABA<sub>C</sub> receptor antagonist 1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid or picrotoxin, an unselective GABA<sub>A</sub> and GABA<sub>C</sub> receptor antagonist. We conclude that GABA<sub>C</sub> receptors are, at least partially, located synthetically in SC and pretectal neurons in the rat, which indicates a direct function of this receptor type for synaptic processing in both structures.

INTRODUCTION

Postsynaptic actions of GABA are mediated by three types of receptors, the ionotropic GABA<sub>A</sub> and GABA<sub>C</sub> receptors and the metabotropic GABA<sub>B</sub> receptor. Among GABA receptor types, GABA<sub>C</sub> receptors exhibit a unique spatial distribution within the CNS. While they were once believed to be specifically expressed in the vertebrate retina (Łukasiewicz 1996), they have now been found in other CNS structures (Boué-Grabot et al. 1998; Enz and Cutting 1999; Wegelius et al. 1998). In addition, effects both resistant to the GABA<sub>A</sub> receptor antagonist bicuculline and independent from GABA<sub>B</sub> receptors have been reported in SC (Arakawa and Okada 1988; Platt and Withington 1998) and dLGN (Zhu and Lo 1999). Together, these results imply a specific role for GABA<sub>C</sub> receptors in subcortical visual information processing.

We have recently shown that selective activation of GABA<sub>C</sub> receptors in vitro differentially affects projection neurons and interneurons in the superficial gray layer of the SC, the stratum griseum superficiale (SGS) (Boller and Schmidt 2001; Schmidt et al. 2001). While GABA<sub>C</sub> receptor activation suppressed the activity of putative GABAergic interneurons, the responses of non-GABAergic projection neurons to afferent stimulation increased. We therefore proposed a disinhibitory function of GABA<sub>C</sub> receptors in rat SGS. However, because we bath applied specific agonists in these experiments, GABA<sub>C</sub> receptor activation was independent from synaptic transmitter release. Furthermore, afferent stimulation evoked GABA<sub>A</sub> but not GABA<sub>C</sub> receptor–mediated inhibitory postsynaptic currents (IPSCs) in neurons that expressed GABA<sub>C</sub> receptors. This leaves the possibility that GABA<sub>C</sub> receptors may be located off synaptic sites, instead of being directly involved in synaptic neurotransmission. In this case they could prevent extrasynaptic accumulation of GABA, by selectively inhibiting GABAergic interneurons. The 10-fold higher affinity to GABA of GABA<sub>C</sub> receptors compared with GABA<sub>A</sub> receptors (Bormann 2000) would be a useful property for such a function. In contrast, if GABA<sub>C</sub> receptors are located at distinct synaptic sites, the activity of local GABAergic SGS interneurons could be specifically regulated by an extrinsic GABAergic input.

To address the question of a possible synaptic localization of GABA<sub>C</sub> receptors, we performed single-cell patch-clamp recordings in SGS and the pretectum and induced spontaneous activity by bath application of the potassium channel blocker 4-aminopyridine (4-AP). In the presence of the unspecific glutamate receptor antagonist kynurenic acid, we recorded 4-AP–induced inhibitory postsynaptic currents and tested, with specific antagonists, whether these currents are mediated by GABA<sub>A</sub> and/or by GABA<sub>C</sub> receptors. Evidence will be presented that supports the assumption of a synaptic localization of GABA<sub>C</sub> receptors.
of GABA<sub>C</sub> receptors in subpopulations of SC and PNC neurons.

**METHODS**

**Slice preparation**

Midbrain slices were obtained from 23- to 35-day-old Long-Evans hooded rats raised in the institute’s own colony. Younger animals were not used because local GABAergic mechanisms appear adult-like only by the end of the third postnatal week (Boller and Schmidt 2001). Because we did not observe any age-related differences in our results, we regarded all animals as representing the same age group. All experimental procedures were in strict compliance with governmental regulations and in accordance with the Guidelines for the Use of Animals in Neuroscience Research of the Society for Neuroscience. Slice preparation was carried out as described previously (Boller and Schmidt 2001; Schmidt et al. 2001). Briefly, the animals were deeply anesthetized with an intramuscular injection of ketamine hydrochloride (100 mg/kg body wt) and perfused transcardially with ice-cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 123 NaCl, 5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 2 MgSO<sub>4</sub>, 2 CaCl<sub>2</sub>, and 10 glucose, that was continuously gassed with 5% CO<sub>2</sub>-95% O<sub>2</sub>. Coronal slices, 350-μm thick, were collected and stored in an interface chamber containing ACSF for 1 h at 37°C and then at room temperature until recording.

**Patch-clamp recordings**

Whole-cell patch-clamp recordings were performed under visual guidance as described earlier (Boller and Schmidt 2001; Schmidt et al. 2001). Slices were transferred to a submersion-type recording chamber and continuously superfused with oxygenated ACSF at room temperature. Borosilicate-glass micropipettes of 5–10 MΩ impedance were filled with internal solution composed of (in mM) 130 potassium gluconate, 2 sodium gluconate, 20 HEPES, 4 Na<sub>2</sub>ATP, 0.4 NaGTP, and 0.5 EGTA, to which we also added 5 mM QX 314 (Sigma-Aldrich, Deisenhofen, Germany), to which we also added 5 mM QX 314 (Sigma-Aldrich, Deisenhofen, Germany), to block sodium spikes when depolarizing cells to more positive holding potentials, and 0.5% biocytin (Sigma-Aldrich) for intracellular staining of the cells recorded. Whole-cell recording was achieved by using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). After recording, slices were fixed in 4% phosphate-buffered formaldehyde and then reacted with 3,3-diaminobenzidine and intensified with cobalt and nickel to allow a detailed morphological characterization of the cells using a camera lucida apparatus.

**RESULTS**

**Identification of neurons**

Whole cell patch-clamp recordings were performed from 45 neurons in SGS and from 21 neurons in the PNC under voltage-clamp conditions. Filling the cells with biocytin from the patch-clamp pipette allowed us to characterize the dendritic morphology of the recorded neurons after the experiments, which was particularly important for cell type identification of SGS neurons. The dendritic morphology of 28 biocytin-filled SGS cells was fully recovered, which included all major SGS cell types that have been described earlier (Langer and Lund 1974) without an obvious bias toward a particular cell class. We identified 7 narrow-field vertical cells, 4 wide-field cells, 4 horizontal cells, 6 piriform cells, 6 stellate cells, and a marginal cell (Fig. 1).

**Narrow-field vertical cells** were characterized by oval- to spindle-shaped cell bodies with a vertically oriented long axis and a vertically oriented dendritic tree that had a mediolateral extent of <150 μm. Stained axons either run ventrally and left SGS or showed numerous local arborizations (Fig. 1A, left and right cell, respectively). Wide-field cells had round-to-oval cell bodies and dendritic trees that covered more than 100 μm in the mediolateral axis. In comparison to narrow-field vertical cells, the dendritic trees of wide-field cells were more radially oriented. Stained axons always ran ventrally and did not show any local arborizations (Fig. 1B). Horizontal cells had oval to spindle-shaped cell bodies with a horizontally oriented long axis. Three to five primary dendrites formed dendritic trees that were oriented parallel to the dorsal SC surface and that spanned ≤500 μm in the mediolateral axis (Fig. 1C). Piriform cells had small round-to-oval cell bodies and showed only sparsely arborized dendritic tree. No preferred orientation of the dendritic tree could be observed, although dendrites pointing toward the dorsal SC surface usually were infrequent or completely absent (Fig. 1D). Finally, stellate cells had small round cell bodies and densely arborized radially oriented dendritic trees that typically covered ≤100 μm in diameter (Fig. 1E).

We also recovered the morphology of 15 of the recorded PNC cells. Because, however, a functional classification of PNC neurons based on dendritic morphology seems impossible...
(Schmidt et al. 1996), no attempts will be made here to classify these biocytin-filled PNC cells.

**Influence of 4-AP on SGS neurons**

At our standard holding potential (−60 mV) all tested SGS neurons (n = 41) responded with an excitatory postsynaptic current (EPSC) to SO stimulation. In 23 cells (56%), this EPSC was followed by a recognizable IPSC at least at more positive holding potentials. Sixteen cells (39%) did not show an IPSC in response to SO stimulation even when the holding potential was raised to 0 mV.

Electrical stimulation in SO activates multiple inputs to SGS, both excitatory and inhibitory. However, single shocks may be insufficient to activate all extrinsic inputs, especially those whose axons do not follow the course of optic tract fibers. Furthermore, local circuits that are activated polysynaptically may exist within SGS, which are also not activated by single electric stimuli in SO. Finally, we have not been able to evoke IPSCs that were insensitive to bicuculline with electrical stimulation in SO, although we could activate GABA<sub>C</sub> receptors in SGS neurons by bath-applied agonists (Schmidt et al. 2001). Thus, to activate more effectively the largest possible...
number of inhibitory inputs to individual SGS neurons, we bath applied the potassium channel blocker 4-AP (50 μM) (Aronson 1992). As a result of the spontaneous activity induced by 4-AP, the recorded cell receives strong synaptic input. However, because excitatory input is blocked by the nonselective glutamate receptor antagonist kynurenic acid, only inhibitory postsynaptic current (IPSCs) appear, which differ in peak amplitude, rise time, and decay time.

Coapplication of 4-AP and kynurenic acid induced spontaneous outward currents, at frequencies between 0.3 and 13.1 Hz (mean 4.8 ± 3.2 Hz) in 86% of the SGS cells tested (42/49), including 8 of the 16 SGS cells that did not show IPSCs after electrical SO stimulation (Fig. 2). The remaining SGS cells without an IPSC in response to electrical stimulation also did not exhibit inhibitory currents in the presence of 4-AP. The conclusion that 4-AP–induced spontaneous outward currents were of synaptic origin was supported by the fact that they could be completely suppressed either by application of the sodium channel antagonist lidocaine (200 μM, n = 8), which suppresses the generation of action potentials, or by substituting the calcium in the ACSF by cobalt (n = 6), which leads to a suppression of transmitter release at synaptic sites (Fig. 3).

Pharmacological characterization of 4-AP–induced IPSCs

To characterize the types of receptors involved in mediating the 4-AP–induced outward currents, we first examined the effect of the GABA_A receptor antagonist bicuculline. In 60% (21/35) of the SGS cells that showed 4-AP–induced outward currents, 10 μM bicuculline completely blocked these currents (Fig. 4), indicating that they were mediated by GABA_A receptors. In the remaining cells, the frequency of outward currents was largely reduced by bicuculline (mean reduction 87.3 ± 6.6%), however, a considerable amount of postsynaptic activity still remained (Fig. 5). Thus, in these cells, some of the 4-AP–induced IPSCs seem to be mediated by bicuculline-insensitive receptors. The activity remaining in the presence of bicuculline appeared to be of synaptic origin, because it also could be blocked by application of lidocaine or by extracellular calcium substitution with cobalt.

We have shown earlier that almost half of the SGS neurons express functional GABA_C receptors in addition to GABA_A receptors. Therefore, we investigated the involvement of GABA_C receptors in mediating 4-AP–induced IPSCs. In 50% (18/36) of the SGS cells that showed 4-AP–induced outward currents, 1 mM GABA_C receptor agonist muscimol completely blocked these currents (Fig. 6), indicating that they were mediated by GABA_C receptors. In the remaining cells, the frequency of outward currents was largely reduced by muscimol (mean reduction 89.3 ± 5.9%), however, a considerable amount of postsynaptic activity still remained (Fig. 7). Thus, in these cells, some of the 4-AP–induced IPSCs seem to be mediated by muscimol-insensitive receptors. The activity remaining in the presence of muscimol appeared to be of synaptic origin, because it also could be blocked by application of GABA_A receptor antagonist bicuculline or by extracellular calcium substitution with cobalt.
receptors (Boller and Schmidt 2001; Schmidt et al. 2001). To test whether GABA C receptors contribute to 4-AP–induced IPSCs, we either coapplied the Cl−/H11002 channel blocker picrotoxin, which acts as an antagonist at both GABA A and GABA C receptors (Bormann 2000), or the selective GABA C receptor antagonist TPMPA (Ragozzino et al. 1996) together with bicuculline in 14 SGS cells that still showed IPSCs in the presence of bicuculline. Because TPMPA also acts as an agonist at GABA B receptors, we always added the GABA B receptor antagonist CGP 55845 before we applied TPMPA. When applied alone CGP 55845 had no effect on 4-AP–induced IPSCs, possibly because we included QX-314 into the pipette solution, which has been reported to block GABA B receptor–mediated currents (Otis et al. 1993). In 6 of 8 cells tested with TPMPA, IPSCs that remained during bicuculline were partly suppressed; in the remaining 2 cells and in all 3 cells tested with picrotoxin no IPSCs remained during coapplication of all three GABA receptor antagonists (mean additional reduction induced by TPMPA and picrotoxin, 12.4 ± 7.3%; Fig. 5).

Because GABA A and GABA C receptors differ in their biophysical properties, including conductance, activation, or open time (Bormann 2000), we expected that IPSCs mediated by the two receptor types also have different properties. To test this prediction, we first compared IPSC amplitudes of cells that showed bicuculline-resistant IPSCs with those of cells in which bicuculline blocked all IPSCs. To allow a comparison between cells, we normalized IPSC amplitudes individually to each cell’s maximum during 4-AP and plotted the cumulative dis-

FIG. 4. Current traces recorded from an SC neuron recorded at −30 mV in the presence of 50 μM 4-AP and 2 mM kynurenic acid (A) and after adding the specific GABA A receptor antagonist bicuculline (B). In this cell all 4-AP–induced IPSCs were completely blocked by 10 μM bicuculline, which indicates that inhibitory inputs activated by 4-AP application were exclusively mediated by GABA A receptors.

FIG. 5. Current traces from 2 SC neurons recorded at −30 mV, in which bicuculline-insensitive IPSCs appeared after 4-AP application. In the presence of 50 μM 4-AP and 2 mM kynurenic acid, IPSCs with variable peak amplitudes were evoked (A and D), some of which persisted after adding the specific GABA A receptor antagonist bicuculline (10 μM) and the specific GABA A receptor antagonist (2S)-3-[[11S]-1-(3,4-dichlorophenyl)-ethylamino-2-hydroxypropyl][phenylmethyl]phosphinic acid (CGP 55845; 0.5 μM; B and E). These IPSCs, however, were blocked by adding either the specific GABA C receptor antagonist (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA; 100 μM; C) or the unspecific GABA A and GABA C receptor antagonist picrotoxin (50 μM; D), which indicates that they were mediated by GABA C receptors.
tribution of IPSC amplitudes in the two populations. Although IPSC amplitudes varied considerably, the proportion of IPSCs with smaller amplitudes (<50% of the maximum) was significantly larger \((P < 0.05)\) in cells that exhibited bicuculline-insensitive IPSCs (Fig. 6).

If GABA\(_C\) receptors predominantly mediate small amplitude IPSCs, the IPSC amplitude distribution should change in the presence of bicuculline. We therefore plotted amplitude distributions of IPSCs evoked before and during bicuculline application from cells that showed bicuculline-resistant IPSCs. Although IPSC amplitudes varied widely in both conditions, larger IPSC amplitudes (>70% of the maximum) disappeared in the presence of bicuculline (Fig. 7A).

As another IPSC parameter that might reflect different biophysical properties of GABA\(_A\) and GABA\(_C\) receptors, we analyzed IPSC rise times (from 10 to 90% of the peak amplitude) before and in the presence of bicuculline. In agreement with the data from IPSC amplitudes, bicuculline application specifically abolished IPSC with short rise times (<20 ms), which are expected to be predominantly mediated by GABA\(_A\) receptors, while the distribution of IPSCs with longer rise times remained virtually unchanged (Fig. 7B). Interestingly, bicuculline-insensitive IPSCs appeared not to be significantly different from bicuculline-sensitive IPSCs in other biophysical properties, such as half-width or decay time.

The relationship between cell type, as defined by the dendritic morphology of biocytin-labeled cells, and the effects of the GABA receptor antagonists on 4-AP–induced IPSCs was as follows. Bicuculline completely blocked 4-AP–induced IPSCs in all recovered narrow-field vertical and wide-field cells, in the marginal cell, and in one stellate cell. Of the cells in which bicuculline-insensitive IPSCs were evoked by 4-AP, we were able to classify eight neurons. Four of these exhibited stellate cell morphology and another four were identified as piriform cells. In all these neurons, however, TPMPA was effective in either strongly reducing or completely suppressing IPSCs that remained in the presence of bicuculline. This result supports our earlier proposal that GABA\(_C\) receptors in SGS are predominantly expressed by cells that can be morphologically classified as putative local GABAergic interneurons (Schmidt et al. 2001).

We previously showed that GABA\(_C\) receptor activation by bath application of the GABA receptor agonist muscimol at concentrations below 1 \(\mu\)M has a dual effect on electrically evoked responses depending on the cell type (Schmidt et al. 2001). In putative GABAergic SGS interneurons, EPSC amplitudes are decreased, which indicates that these neurons express GABA\(_C\) receptors. In contrast, in non-GABAergic SGS cells, EPSC amplitudes increase after GABA\(_C\) receptor activation because, first, these cells do not express GABA\(_C\) receptors themselves and, second, inhibitory inputs from local GABAergic interneurons are reduced. This dual effect of muscimol is only found at concentrations in the bath that selectively activate GABA\(_C\) receptors. Higher muscimol concentrations, which also activate GABA\(_A\) receptors, lead to decreased EPSC amplitudes in all SGS cells (Schmidt et al. 2001).

Thus, if GABA\(_C\) receptors in GABAergic interneurons are localized at synaptic sites, these cells should also show 4-AP–induced IPSCs that are insensitive to bicuculline. On the contrary, in SGS projection cells, which do not express GABA\(_C\) receptors, bicuculline should block all 4-AP–induced IPSCs. To test for this distinction, we monitored changes on EPSC amplitudes induced by bath-applied 0.5 \(\mu\)M muscimol in a
small number of neurons (n = 9) before application of 4-AP. Muscimol induced EPSC amplitude increases in five cells, three of which could be morphologically recovered and were identified as narrow-field cells, and in all these cells 4-AP–induced IPSCs were completely blocked by bicuculline. In the remaining cells, including two identified stellate cells, bicuculline-insensitive IPSCs appeared after 4-AP application. Although this sample of cells is small, this result indicates that at least a portion of GABA<sub>C</sub> receptors is localized at synaptic sites in presumed SGS GABAergic interneurons.

Effects of 4-AP on pretectal neurons

Of the PNC cells tested, 86% (18/21) responded with an EPSC to OT stimulation. Electrically evoked IPSCs were observed in 48% of the cells (10/21); the remaining cells responded only with an EPSC in response to OT stimulation, even at holding potentials up to 0 mV.

Coapplication of 4-AP and kynurenic acid induced spontaneous outward currents in 84% of the PNC cells tested (27/32). IPSC frequencies ranged from 1.1 to 9.8 Hz (mean 5.2 ± 2.6 Hz) which was similar to what we had observed in SGS. As we had already observed for a small number of cells in the SC, IPSCs were induced by 4-AP application in five PNC cells that did not respond with IPSCs to electrical OT stimulation.

To verify that GABA<sub>C</sub> receptors also mediate IPSCs in the PNC, we tested whether 4-AP–induced IPSCs were bicuculline sensitive. While IPSCs were completely blocked by bicuculline in 40% of the PNC cells (8/20), IPSCs insensitive to bicuculline were observed in the remaining neurons (Fig. 8). On average, bicuculline reduced the number of IPSCs in these neurons by 90 ± 5.3%; the frequency of bicuculline-insensitive 4-AP–induced IPSCs ranged from 0.1 to 3.5 Hz (mean 1.4 ± 2.5 Hz). Adding either the specific GABA<sub>C</sub> antagonist TPMPA or the GABA<sub>A</sub> and GABA<sub>C</sub> receptor antagonist picROTOXIN (Bormann 2000) strongly or completely suppressed bicuculline-insensitive IPSCs; the mean additional reduction induced by TPMPA or picROTOXIN was 6.7 ± 4.1% (Fig. 8). Again we verified that IPSCs that remained in the presence of bicuculline were of synaptic origin by coapplying 200 μM lidocaine and/or by substituting calcium with cobalt in the ACSF (Fig. 8). As further evidence for the presence of GABA<sub>C</sub> receptor–mediated IPSCs in PNC neurons, and similar to what we had observed in SC cells, the proportion of IPSCs with smaller amplitudes (<60% of the maximum) was significantly larger in cells that showed IPSCs insensitive to bicuculline (P < 0.05). Furthermore, application of bicuculline resulted in a differential loss of IPSCs with large amplitudes and fast rise times (Fig. 9).

**FIG. 8.** Current traces from 2 pretectal nuclear complex (PNC) neurons recorded at −40 mV (A–C) and −20 mV (D–F) in which bicuculline-insensitive IPSCs appeared after 4-AP application. In the presence of 50 μM 4-AP and 2 mM kynurenic acid, IPSCs with variable peak amplitudes were evoked (A and D), some of which persisted after adding the specific GABA<sub>C</sub> receptor antagonist bicuculline (10 μM) and the specific GABA<sub>A</sub> receptor antagonist CGP 55845 (0.5 μM; B and E). Furthermore, excitatory postsynaptic current also appeared in both cells, despite the presence of kynurenic acid, and their number slightly increased during GABA<sub>A</sub> and GABA<sub>C</sub> receptor blockade. The IPSCs that persisted, however, were blocked by adding either the Na<sup>+</sup> channel blocker lidocaine (200 μM; C) or TPMPA (100 μM; F), which indicates that they were of synaptic origin and that they were mediated by GABA<sub>C</sub> receptors, respectively.
bicuculline-insensitive IPSCs (n = 10 to 90% of the peak amplitude), evoked by 4-AP in PNC cells that showed GABAC receptors for the following reasons. First, application of the selective GABA C receptor antagonist bicuculline completely blocked 4-AP-induced IPSCs in 60% of the SGS neurons and the average had smaller peak amplitudes and longer rise times.

DISCUSSION

We have investigated in vitro a possible synaptic localization of GABA C receptors in rat SGS and PNC neurons. In the majority of cells, bath application of 4-AP, in the presence of the glutamate receptor antagonist kynurenic acid, induced IPSCs of variable amplitudes and rise times. Coapplication of the specific GABA A receptor antagonist bicuculline completely blocked 4-AP-induced IPSCs in 60% of the SGS neurons and in 40% of PNC cells. In the remaining cells, distinct IPSCs could still be observed in the presence of bicuculline, although their number was greatly reduced. We propose that IPSCs that persisted in the presence of bicuculline were mediated by GABA C receptors for the following reasons. First, application of the selective GABA C receptor antagonist TPMPA (Ragossino et al. 1996) or the GABA A and GABA C receptor antagonist picrotoxin (Bormann 2000) strongly reduced or completely blocked bicuculline-resistant IPSCs. Second, bicuculline application reduced the number of IPSCs with large amplitudes and short rise times, which is in agreement with the larger conductance of GABA A compared with GABA C receptors. Finally, all IPSCs, including those insensitive to bicuculline, were of synaptic origin since they disappeared when we blocked the generation of action potentials by lidocaine or when we blocked synaptic transmitter release by a substitution of extracellular calcium with cobalt.

IPSCs that persisted in the presence of bicuculline, CGP 52432, and TPMPA most likely reflect the fact that TPMPA, in contrast to picrotoxin, not always completely antagonizes GABA C receptor activation. Similar observations have been reported from other preparations, e.g., retinal bipolar cells (McCall et al. 2002). Such IPSCs most likely were not GABA A receptor–mediated because bicuculline in the concentrations used in our study was able to completely block all IPSCs in other cells.

The morphological reconstruction of recorded SGS neurons revealed that most of the cells in which we observed bicuculline-resistant IPSCs evoked by 4-AP had the dendritic morphology of putative GABAergic interneurons that have been proposed previously to express GABA C receptors (Schmidt et al. 2001). Although none of the cells with bicuculline-insensitive IPSCs induced by 4-AP showed horizontal cell morphology, we still assume that horizontal cells as one class of GABAergic SGS interneurons express GABA C receptors.

While we are aware of the fact that incomplete filling might have lead to misclassification of individual cells, cell type–specific characteristics of the dendritic morphology, as for example vertical or horizontal orientation of primary dendrites or number and density of dendritic arborizations, usually allow unambiguous classification of well-filled cells. Misclassification may have occurred with incompletely filled cells that we could have mistakenly regarded as piriform cells, which are characterized by only sparsely branched dendritic trees. However, because piriform cells in our sample were not overrepresented, we believe that we could reliably classify the majority of our biocytin-filled cells.

Assuming proper morphological classification, our results strongly support the hypothesis that GABAergic interneurons in the rat SGS receive a GABAergic input that activates GABA C receptors at synaptic sites. Similarly, we propose that more than half of cells in the rat PNC also receive a GABAergic input that activates GABA C receptors at synaptic sites. Of course, the proposal of a synaptic localization of GABA C receptors does not exclude the possibility that GABA C receptors located off synaptic sites in addition may be activated by extrasynaptic GABA.

Activity induced by 4-AP

Bath application of 4-AP has been used in neocortex (e.g., Aram et al. 1991; Avoli et al. 1994) and hippocampus (e.g., Sinha and Saggau 2001; Traub et al. 2001) to study in vitro local inhibitory mechanisms and underlying circuitry. In both structures, 4-AP induces both hyperpolarizing and depolarizing potentials or currents. Because at least some of the 4-AP–induced postsynaptic responses were too large to be generated by single-neuron firing, 4-AP is believed to induce synchronous firing of groups of neurons. In our experiments in rat SGS, bath application of 4-AP also induced IPSCs with large amplitudes, also suggesting that synchronous interneuron activity may have occurred. Because we always coapplied kynurenic acid with 4-AP, no EPSCs were observed in most cells, thereby preventing cells from being impaired by excitotoxicity. However, distinct EPSCs appeared after adding bicuculline to the ACSF in some cases (cf. Figs. 3 and 7), obviously because excitatory inputs become more effective when inhibition is blocked. Furthermore, the complete block of 4-AP–induced postsynaptic currents that we observed either after

FIG. 9. Frequency distribution of IPSC amplitudes (A) and rise times, from 10 to 90% of the peak amplitude (B), evoked by 4-AP in PNC cells that showed bicuculline-insensitive IPSCs (n = 7). Most of the large amplitude (70–100% of maximum) and short rise time (<15 ms) IPSCs that were evoked by 4-AP (gray bars) disappeared in the presence of bicuculline and CGP 53845. IPSCs that were present during GABA A and GABA C receptor blockade, which therefore are most likely mediated by GABA C receptors (black bars), on average had smaller peak amplitudes and longer rise times.
adding the Na\(^+\)-channel blocker lidocaine, which obstructs the generation of action potentials, or after substitution of extracellular Ca\(^{2+}\) by Co\(^{2+}\), which prevents transmitter release, indicates that all 4-AP effects observed were of synaptic origin. This excludes the possibility that the recorded currents resulted from a direct influence of 4-AP on the recorded neurons.

**Pharmacology of 4-AP–induced inhibition**

In the rat SGS, all three known GABA receptor types seem to mediate local inhibition (Binns 1999). Specific functions have been assigned to GABA\(_A\) and GABA\(_B\) receptors, since surround inhibition and response habituation are selectively affected by bicuculline and the GABA\(_B\) receptor antagonist CGP 35348, respectively (Binns 1999; Binns and Salt 1997). The functional role of GABA\(_C\) receptors in SGS seems to mediate a disinhibitory influence on SGS projections cells (Pasternack et al. 1999; Schmidt et al. 2001). Much less is known about the expression of different GABA receptor types in PCN neurons apart from the presence of GABA\(_A\) receptors (Schmidt et al. 1994).

In a previous study on SGS neurons, we activated GABA\(_C\) receptors by bath application of either GABA or muscimol at concentrations too low to also activate GABA\(_A\) receptors. The specific activation of GABA\(_C\) receptors was verified by the ability of the selective GABA\(_C\) receptor antagonist TPMPA to block agonist-induced effects. Bath application of agonists, however, activates receptors even if they are not synaptically localized. Therefore, in the present study, we induced spontaneous transmitter release by 4-AP application and examined evoked IPSCs to verify a synaptic localization of GABA\(_A\) and GABA\(_C\) receptors. As expected from the ubiquitous distribution of GABA\(_A\) receptors, most of the IPSCs induced by 4-AP were blocked by bicuculline. However, in 40% of the SGS neurons and in 60% of the PNC cells, bicuculline-resistant IPSCs were observed. We propose that these currents were mediated by synaptically located GABA\(_C\) receptors because they could be completely or largely blocked by addition of either TPMPA or the GABA\(_A\) and GABA\(_C\) receptor antagonist picrotoxin. Furthermore, bicuculline-resistant IPSCs had smaller amplitudes and longer rise times than those that were blocked by bicuculline. These differences could reflect different biophysical properties because GABA\(_C\) receptors have a smaller conductance and are activated more slowly than GABA\(_A\) receptors (Bormann 2000). Thus a considerable number of cells in SGS and PNC seem to receive a GABAergic input that activates synaptically located GABA\(_C\) receptors.

It has to be emphasized that, in all cells in which 4-AP induced IPSCs, bicuculline strongly reduced the number of IPSCs. This indicates that, first, all cells that receive inhibitory input express GABA\(_A\) receptors, and, second, all cells in which bicuculline-resistant IPSCs were observed, and which therefore are thought to express GABA\(_C\) receptors, also receive inhibitory input via GABA\(_A\) receptors. This is different from our earlier observation that many SGS interneurons, in particular GABAergic priform and stellate cells, did not exhibit IPSCs when we delivered single electric stimuli to SO, in contrast to projection neurons in which IPSCs were always elicited with the same stimulus (Schmidt et al. 2001). However, in the present studies we blocked glutamate receptors with kynurenic acid and therefore prevented the transmission of excitatory inputs. Thus GABAergic input to many GABAergic interneurons might be masked by strong excitatory input that is normally activated by electric SO stimulation.

**Possible function of GABA\(_C\) receptors in SC and PNC**

We showed earlier that local inhibition in the rat SGS to a large extent depends on GABA\(_A\) receptor function. Thus IPSCs evoked by electrical stimulation of afferents in SO were completely blocked by bicuculline (Boller and Schmidt 2001; Schmidt et al. 2001). Nevertheless, activation of GABA\(_C\) receptors by bath application of either GABA or muscimol resulted in a direct inhibition of about half of the recorded SGS cells, as the amplitudes of EPSCs evoked by SO stimulation decreased. Most of these cells were morphologically characterized as local GABAergic interneurons. In the remaining cells, morphologically identified as SGS projection neurons, GABA\(_C\) receptor activation lead to increased excitatory responses and/or decreased inhibitory responses. Based on these results, we proposed that local GABAergic interneurons in SGS express GABA\(_C\) receptors, while projection neurons do not express them (Schmidt et al. 2001). As we further propose that GABA\(_C\) receptors in SGS interneurons are synaptically located, at least partially, a GABAergic input to these cells must exist. What might be the source of this GABAergic input? We think that this source is not intrinsic to the SGS circuitry because, first, all known GABAergic cell types in SGS are activated by SO stimulation, including all classes of GABAergic interneurons, because electrical stimuli delivered to SO elicit EPSCs in all SGS cells. Second, all IPSCs that are elicited by SO stimulation can be blocked by bicuculline. Thus all inhibitory input from local GABAergic interneurons to other SGS cells is mediated by GABA\(_A\) receptors. If, however, a local input from GABAergic interneurons in SGS mediated by GABA\(_C\) receptors would exist, bicuculline-insensitive IPSCs should appear after SO stimulation in those SGS cells that receive this input (Fig. 10).

On the other hand, if our proposal of an extrinsic GABAergic input to SGS interneurons that selectively contacts postsynaptic GABA\(_C\) receptors is correct, the course of its fibers does not seem to follow other afferent systems, which reach SGS by...
way of SO. Otherwise we should be able to activate this input by SO stimulation in a similar way as we activate the excitatory input of mostly retinal and cortical origin that leads to the EPSCs observed in SGS cells. It seems reasonable to propose that the putative extrinsic GABAergic input that terminates on GABA_C receptor-containing synapses can modulate the amount of inhibition in SGS. Because SGS receives multiple GABAergic input, from both visual and nonvisual sources (Appell and Behan 1990; Binns 1999; Nunes Carodozo et al. 1994), the precise origin of this specific GABAergic input remains to be determined. It may be speculated that the source of this GABAergic input is located not too distant from the SC because it may be more likely that input fibers from distant sources follow optic tract fibers in SO.

Our knowledge about the intrinsic circuitry in the PNC is less detailed than that for SGS. PNC nuclei also contain many GABAergic cells (Giolli et al. 1985; Nabors and Mize 1991; Van der Want et al. 1992), whose innervation may differ from that in SGS and the PNC may have different neuronal populations with different functional significance and projection targets (Schmidt et al. 1995). Because it may be more likely that input fibers from distant sources follow optic tract fibers in SO.

Whether the GABA_C receptor at those synapses, where it is expressed, is the only GABA receptor type present also remains to be studied. It is possible that GABA_C and GABA_A receptors coexist at single synaptic sites. Such an arrangement would increase the dynamic range in which such a synapse operates. Low-level GABA release could activate GABA_C receptors, leading to a moderate postsynaptic inhibition. Stronger GABA release could coactivate GABA_A receptors, which would largely increase the inhibitory influence on the postsynaptic cell and induce a nonlinear response characteristic.

Finaly, apart from a synaptic localization in SGS and the PNC, it is also possible that GABA_A receptors, partially or entirely, are located extrasynaptically and are activated by GABA spillover from synchronously activated GABA_A receptors at nearby GABAergic synapses. Because they are selectively expressed by GABAergic interneurons in SGS, the function of extrasynaptic GABA_C receptors could be to regulate GABA release by an inhibition of local interneurons, thereby preventing accumulation of GABA in the extracellular space.

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REFERENCES


