Activation of Presynaptic GABA<sub>B</sub> Receptors Inhibits Evoked IPSCs in Rat Magnocellular Neurons In Vitro

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Mouginot, Didier, Samuel B. Kombian, and Quentin J. Pittman. Activation of presynaptic GABA<sub>B</sub> receptors inhibits evoked IPSCs in rat magnocellular neurons in vitro. J. Neurophysiol. 79: 1508–1517, 1998. Whole cell recordings (nystatin-perforated patch) were carried out on magnocellular neurons of the rat supraoptic nucleus (SON) to study the modulation of inhibitory post-synaptic currents (IPSCs) by γ-aminobutyric acid-B (GABA<sub>B</sub>) receptors. Field stimulation adjacent to the SON in the presence of kynurenic acid, evoked monosynaptic GABAergic IPSCs. Baclofen reversibly reduced the amplitude of the IPSCs in a dose-dependent manner (EC<sub>50</sub>: 0.68 μM) without apparent effect on the holding current (V<sub>h</sub> = −80 mV) or input resistance and altered neither the kinetic properties, nor the reversal potential of IPSCs. Concomitant to IPSC depression, baclofen enhanced the paired-pulse ratio ing this in many brain areas (for review, see Nicoll et al. 1990). In particular, presynaptic GABA<sub>B</sub> receptor antagonist. In testing for involvement of synthetically activated presynaptic GABA<sub>B</sub> receptors, we only recorded paired-pulse facilitation at most ISIs tested (50–300 ms), suggesting that the classical GABA<sub>B</sub> autoreceptors may not normally be activated in our conditions. However, enhancement of local GABA concentration by perfusion of a GABA uptake inhibitor (NO-711) revealed an action of endogenous GABA at these presynaptic GABA<sub>B</sub> receptors. The nonselective K<sup>+</sup> channel blocker Ba<sup>2+</sup> abolished baclofen’s effect and pertussis toxin (PTX) pretreatment (200–500 ng/ml for 18–24 h) was ineffective in blocking the baclofen-induced inhibition, making an involvement of PTX-sensitive G protein unlikely. The present results show that presynaptic GABA<sub>B</sub> receptors that are coupled to PTX-insensitive G-proteins may be activated by endogenous GABA under conditions of reduced GABA uptake, thus regulating the inhibitory synaptic input to SON.

INTRODUCTION

The supraoptic nucleus (SON) of the hypothalamus is composed of two different populations of magnocellular neurons that synthesized either oxytocin (OT) or arginine-vasopressin (AVP) hormones. These neuropeptides are anterogradely transported to the neural lobe of the pituitary and released into the circulation to play an important role in the regulation of physiological functions like hydromineral balance, parturition, and lactation. Release of OT and AVP is directly correlated to the electrical activity of SON neurons (Poulain and Theodosis 1988) and neuropeptides that finely tune the firing rate and pattern of the neurosecretory cells also regulate secretion of OT and AVP. Neurons located in many brain areas project to the SON (Anderson et al. 1990), and thus alteration of the excitability of magnocellular neurons could be achieved by neurotransmitter-mediated changes in intrinsic properties of the postsynaptic cell (for review, see Renaud and Bourque 1991). Among the neurotransmitters, immunocytochemistry has identified both glutamatergic and GABAergic terminals inside the SON (Gies and Theodosis 1994; Theodosis et al. 1986; Van den Pol 1991), and electrophysiological studies performed both in vivo and in vitro reported functional glutamatergic (Day et al. 1990; Gribkoff 1991; Hu and Bourque 1991; Moss et al. 1971) and GABAergic (Bioulac et al. 1978; Mason et al. 1987; Randle and Renaud 1987) synaptic transmission. Modulating this fast synaptic transmission is another way to control neuronal excitability and activation of presynaptic receptors was shown to be a powerful mechanism for achieving this in many brain areas (for review, see Nicoll et al. 1990). In particular, presynaptic γ-aminobutyric acid-B (GABA<sub>B</sub>) receptors were shown to reduce the release of both excitatory and inhibitory neurotransmitters (for review, see Misgeld et al. 1995). In the SON, autoradiographic studies reported the presence of GABA<sub>B</sub> receptors (Bowery et al. 1987; Chu et al. 1990) and a recent study demonstrated the inhibition of excitatory postsynaptic currents (EPSCs) by activation of GABA<sub>B</sub> receptors located on glutamatergic terminals (Kombian et al. 1996). Because γ-aminobutyric acid (GABA)-positive terminals also synapse onto SON magnocellular neurons (Decavel and Van den Pol 1990), in the present study we investigated a possible modulation of GABA release via activation of GABA<sub>B</sub> receptors in an in vitro hypothalamic slice preparation, by using the nystatin-perforated patch technique.

METHODS

All experiments were carried out in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the University of Calgary Animal Care Committee.

Slice preparation

Sprague-Dawley rats (180–250 g) were anesthetized with halothane and decapitated. The brain was quickly removed and placed in ice-cold, carbogenated (95% O<sub>2</sub>-5% CO<sub>2</sub>) artificial cerebrospinal fluid (ACSF). Coronal slices (400 μm thick) were cut with a vibratome, from a block of tissue containing the hypothalamus, in cold (4°C) carbogenated ACSF. Hypothalamic slices containing the SON were then taken, hemisected along the midline and incubated in ACSF at room temperature (21°C) for at least 2 h before recording. A slice was transferred into a recording chamber where it was submerged and continuously perfused with prewarmed ACSF (27–29°C) at a rate of 2–3 ml/min. The composition of the ACSF was (in mM) 126 NaCl, 2.5 KCl, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 1.2 MgCl<sub>2</sub>, 2.4 CaCl<sub>2</sub>, 18 NaHCO<sub>3</sub>, and 11 glucose, pH 7.3–7.4.
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FIG. 1. Characterization of inhibitory postsynaptic currents (IPSCs) evoked in magnocellular neurons of supraoptic nucleus (SON). In the presence of kynurenic acid (1 mM), extracellular stimulation of hypothalamic region dorsomedial to SON (at time of vertical transient), evoked monosynaptic, graded IPSCs (A), which were totally abolished with 25 µM bicuculline (B). IPSCs evoked at different holding potentials (C1) showed an averaged reversal potential around −60 mV, as illustrated with current-to-voltage relationship (C2) obtained from 16 magnocellular neurons.

Nystatin-perforated patch recordings

To maintain the integrity of the intracellular contents, whole cell recordings from SON neurons were made with the perforated-patch technique by using nystatin as the perforating antibiotic. Glass micropipettes (tip resistance 5–7 MΩ) were filled with a solution containing (in mM) 120 potassium acetate, 40 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 5 MgCl₂, 10 ethylene glycol-bis(β-aminoethyl ether) - N,N’,N’’,N’’’-tetraacetic acid (EGTA), and 450 µg/ml nystatin. The nystatin solution was prepared in dimethyl sulfoxide (DMSO) and pluronic FC before incorporation in the previous solution.

High-resistance seals (1–5 GΩ) were made by using either an Axopatch 1D, or an Axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, CA) in voltage-clamp mode, and the fast electrode capacitance was then compensated. Adequate access to the cell (access/series resistance of 10–30 MΩ) was attained in 5–20 min after seal formation.

Data acquisition and analysis

Only cells that satisfied the established criteria for magnocellular neurons (Kombian et al. 1996; Tasker and Dudek 1991) were included in this study.

Synaptic currents were evoked in SON neurons with a bipolar stimulating electrode placed in the hypothalamic region dorsomedial to the SON, close to the optic tract, and connected to a stimulus isolation unit. Square pulses (100–200 µs) of an intensity that yielded a response 50–60% of the maximum response (10–20 V) were used for the experiments.

The magnocellular SON neurons were voltage-clamped at −80 or −60 mV and transient hyperpolarizing voltage steps to −100 mV (300–800 ms) were applied to both monitor the input resistance and improve the driving force of the inhibitory currents. The electrical stimulation of the afferent fibers was evoked during the hyperpolarizing pulses with a 150 ms delay or a 50 ms delay for the repetitive stimulation experiments.

The synaptic currents were sampled with the software pClamp 6 (Axon Instruments), filtered at 10 kHz and digitized at 5 kHz via an A/D converter (Digidata 1200A, Axon Instruments) before storage on a computer hard disk for off-line analysis (Clampfit 6, Axon Instruments). As inhibitory postsynaptic current (IPSC) size showed variability, several IPSCs (6–20) were averaged for each data point.

The amplitude of the IPSCs was taken as a measure of the magnitude of synaptic strength. Data were expressed as percentage change from control values as mean percentage ± SE. Cells served as their own controls and only cells that showed significant recovery (>70%) from any drug effect (except NO-711) were included in the analysis. Statistical comparisons were performed by using analysis of variance (ANOVA) and paired or unpaired Student’s t-tests. P < 0.05 was considered significant.

Calculation of the EC₅₀ for baclofen was done by fitting the experimental data to the following Hill equation that provided the best fit to the data

\[ y = y_0 + \frac{ax^b}{c^x + x^c} \]

where \( y = 50\% \), \( y_0 = 99.05 \), \( a = -111.84 \), \( b = 0.57 \), and \( c = 1.044 \)

Chemical compounds

All drugs were applied by bath perfusion. All substances were prepared as stock solution in distilled water or appropriate solvent and diluted to their final concentration in ACSF just before use. CGP35348 and (-)-baclofen were gifts from Ciba-Geigy. Pluronic FC was from BASF Wyandotte (Michigan). Picrotoxin, bicuculline, kynurenic acid, and nystatin were obtained from Sigma (St. Louis, MO). NO-711 was purchased from RBI (Natick, MA).

RESULTS

Results described in this study were collected from 97 magnocellular neurons of the hypothalamic supraoptic nucleus (SON).
Characterization of evoked IPSCs recorded in magnocellular neurons

Under control condition, extracellular stimulation of the hypothalamic region dorsomedial to the SON and close to the optic tract, elicited a fast postsynaptic inward current in magnocellular neurons maintained at a holding potential of $-80\text{mV}$. In the presence of kynurenic acid (1 mM), a nonspecific blocker of the ionotropic excitatory amino acid receptors, the amplitude of the postsynaptic current was reduced by $56.3\pm3.9\%$ ($n=28$). Under these experimental conditions and when neurons were transiently (300 ms) voltage clamped at $-100\text{mV}$, the inward currents were graded, showing a single peak for different stimulus intensities and were always evoked with a fixed latency, thus indicating monosynaptic inhibitory connections (Fig. 1A).

These currents were almost completely blocked by either 50 $\mu\text{M}$ picrotoxin ($88.3\pm5.4\%$ reduction; $n=6$), or 25 $\mu\text{M}$ bicuculline ($89.5\pm2.8\%$ reduction; $n=7$), two antagonists of the GABA$_A$ response (Fig. 1B), demonstrating that these currents were mediated by the movement of chloride ions through GABA$_A$ receptors present on magnocellular neurons. The current-to-voltage relationship of these inhibitory postsynaptic currents (IPSCs) was determined in 16 cells and showed a reversal potential of $-58\pm1.9\text{mV}$ (Fig. 1C).

Baclofen reduces the amplitude of evoked IPSCs

To record IPSCs in isolation, kynurenic acid (1 mM) was always present in the extracellular solution. Bath application of baclofen (10 $\mu\text{M}$), an agonist for the GABA$_B$ receptor, reduced the amplitude of evoked IPSCs by $95.4\pm4.8\%$ ($n=8$; Fig. 2A, left). The onset of the effect was fast, and the maximal inhibition was achieved in 3–5 min. The baclofen-mediated effect was reversible on washout of the drug (Fig. 2A, right), showing $\sim70\%$ recovery after 10 min.

At a stimulus intensity that yielded $50–60\%$ of the maximum IPSC, baclofen reduced the IPSC amplitude in a dose-dependent manner (Fig. 2B), with an EC$_{50}$ of 0.68 $\mu\text{M}$ calculated from a four variable Hill equation that best fitted the experimental data (see METHODS). Baclofen also depressed the IPSCs over a wide range of stimulus intensities, which is related to the synaptic strength. Input-output responses ranging from subthreshold to maximal IPSC amplitude were generated in five cells. In all of them, bath-applied baclofen (0.3 $\mu\text{M}$) reduced the amplitude of the graded

Taken together, these results demonstrate that electrical stimulation of afferent fibers to the SON in the presence of a glutamate receptor antagonist elicits pharmacologically isolated GABA$_B$ receptor-mediated monosynaptic IPSCs in magnocellular neurons.
IPSCs, with greater inhibition observed at higher stimulus intensities (Fig. 2C). The baclofen-induced inhibition of evoked IPSCs did not desensitize because two consecutive applications of baclofen (1 μM) reduced the amplitude of the IPSCs by 59 ± 7% and 53 ± 8%, respectively (n = 5; P > 0.05; data not shown).

**Baclofen reduces the IPSC amplitude by activation of GABA_B receptors**

We investigated the specificity of the baclofen-induced reduction of the evoked IPSCs by using a competitive GABA_B receptor antagonist, CGP35348, to block the baclofen-induced inhibition. Baclofen (0.3 μM) reduced the amplitude of the evoked IPSCs by 53.3 ± 1.6% in four cells tested (Fig. 3). In the same cells, ACSF containing 500 μM of CGP35348 was perfused for 5–10 min before and during application of baclofen (0.3 μM). Under this condition, baclofen reduced the amplitude of the IPSCs by only 13.5 ± 4.1% (P < 0.05; Fig. 3). On its own, CGP35348 induced a small, but statistically insignificant increase in IPSC amplitude (11.3 ± 5.0%), without any detectable change in the holding current (Fig. 3).

Our data show that baclofen-induced inhibition of evoked IPSCs was achieved by activation of GABA_B receptors.

**Baclofen has no apparent postsynaptic effects on SON neurons**

The baclofen-induced reduction in the amplitude of evoked IPSCs may be the result of an action of baclofen at the postsynaptic level. To test this hypothesis, different sets of experiments were performed. First, bath application of a high concentration of baclofen (5 μM) neither modified the holding current, nor changed the input resistance of recorded magnocellular neurons (800 ± 213 MΩ in control vs. 779 ± 228 MΩ in the presence of baclofen; n = 5, P > 0.05; Fig. 4A), making it unlikely that a postsynaptic action of baclofen modulates IPSCs. Second, a possible interaction between γ-aminobutyric acid-A (GABA_A) and GABA_B receptors was investigated by evaluating the biophysical properties of the evoked IPSCs under control condition and in the presence of baclofen.

In control, the activation time constant (time to peak) and the decay of the evoked IPSCs could be both fitted to a monoeponential function, giving time constants of 2.6 ± 0.4 ms and 13.5 ± 0.8 ms, respectively (n = 11). In the presence of baclofen (0.3 μM), the time to peak was 2.7 ± 0.5 ms and the decay time constant was of 14 ± 1.1 ms, values that were not statistically different from those obtained in control condition (P > 0.05; Fig. 4B). Finally, the reversal potential of the evoked IPSCs (E_rev IPSC), was determined under control condition and in the presence of baclofen. In control the E_rev IPSC was −57 ± 1.6 mV compared with −57 ± 1.5 mV and −57 ± 3.6 mV in the presence of baclofen 1 and 5 μM, respectively (n = 5; P > 0.05), indicating baclofen had no effect on E_rev IPSC (Fig. 4C).

Taken together, these results demonstrate that baclofen inhibits the amplitude of evoked IPSCs without affecting either the kinetic properties or the reversal potential of the IPSCs.

**Baclofen-mediated inhibition of IPSCs involves activation of presynaptic GABA_B receptors**

The previous observations showing that baclofen has no apparent postsynaptic effects on the magnocellular neurons suggested that the inhibition of the evoked IPSCs was probably mediated by presynaptic GABA_B receptors. At excitatory synapses studied in this nucleus, baclofen was shown to increase the paired-pulse ratio (PPR) of two consecutive responses [interstimulus interval (ISI) 50 ms], indicating a change in glutamate release (Kombian et al. 1996). Therefore, to test for a presynaptic locus of action for baclofen, we tested whether or not modulating the postsynaptic locus of action. Figure 5A shows that baclofen reduced the amplitude of evoked IPSCs by only 59 ± 2.5% (P < 0.05) and 53 ± 7.5% (P < 0.05) in the presence of baclofen 1 and 5 μM, respectively (n = 5). The presence of CGP35348 (1 μM) antagonized the baclofen-induced inhibition (4A), making it unlikely that a postsynaptic action of baclofen modulates IPSCs. Second, a possible interaction between γ-aminobutyric acid-A (GABA_A) and GABA_B receptors was investigated by evaluating the biophysical properties of the evoked IPSCs under control condition and in the presence of baclofen.

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Taken together, these results demonstrate that baclofen inhibits the amplitude of evoked IPSCs without affecting either the kinetic properties or the reversal potential of the IPSCs.
we tested the effect of CGP35348 (500 μM) on the baclofen-induced change in PPF. In the presence of this GABA_B receptor antagonist, baclofen (0.3 μM) only slightly reduced the amplitude of the first IPSC (13.5 ± 4.1%; P > 0.05 compared with control), without enhancing PPF (0.5 ± 2.5%; P > 0.05 compared with control, Fig. 5).

Taken together, these results are consistent with baclofen reducing the amplitude of evoked IPSCs via presynaptic GABA_B receptor activation.

Presynaptic GABA_B receptors are activated by endogenously released GABA in the presence of a GABA uptake inhibitor

To determine whether or not GABA released from the inhibitory terminals acts retrogradely to inhibit its own release via activation of the previously characterized presynaptic GABA_B receptors, the paired-pulse profile with 50–60% of maximal IPSC amplitude was tested at different ISIs (ranging from 50 to 500 ms). For ISI of 100 ms, significant increase in the amplitude of the second IPSC was recorded (PPR 1.4 ± 0.05, n = 12), as previously observed at ISI of 50 ms (PPR 1.6 ± 0.07, n = 12). For ISI of 200 and 500 ms, PPR was near unity (1.1 ± 0.1, n = 10 and 1.0 ± 0.1, n = 6, respectively; Fig. 6, A–C). To test whether or not the observed facilitation of the second IPSC depended on the synaptic strength and therefore on a putative heterogeneity in the inhibitory fibers recruited, we applied a graded stimulus intensity at an ISI of 50 ms. In five neurons tested, weak to moderate stimulus intensities (10–60% of the intensity giving the maximal IPSC amplitude) always showed an increase of the second IPSC size. For maximal stimulus intensities, PPF was reduced, but no PPD was recorded (Fig. 6B).

not it would alter the PPR between two IPSCs evoked at ISI of 50 ms (PPR: amplitude of the IPSC 2/amplitude of the IPSC 1) whereby a PPR greater than unity indicates paired-pulse facilitation (PPF) and PPR less than unity indicates paired-pulse depression (PPD). Because of the variability in the amplitude of evoked IPSCs, stimulus intensities giving 50–60% of the maximal IPSC amplitude were used to test the PPR paradigm. This level of synaptic transmission allowed changes in both IPSC amplitudes to occur without saturating the second response. In 12 magnocellular neurons tested, all displayed an increase in the second IPSC amplitude (PPR 1.6 ± 0.07) and bath application of baclofen (0.3 μM) reduced the amplitude of the first IPSC by 49.6 ± 3.2%. Concurrent with this decrease, the PPR was enhanced to 2.1 ± 0.13, a facilitation of 32 ± 5.8% (P < 0.05 compared with control; Fig. 5). In four of these neurons,
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35348 (500 μM), NO-711 decreased the amplitude of the evoked IPSCs by only 12.0 ± 5% (P < 0.05, compared with NO-711 alone; unpaired test, Figs. 7, C and D). In addition, in the presence of CGP35348, NO-711 enhanced PPF by only 10 ± 7.5%, a value that is significantly different from the depression induced by NO-711 alone (P < 0.05, n = 6; unpaired test, Fig. 8, B and C). These results show that presynaptic GABA_B receptors could be activated in the presence of a GABA uptake inhibitor to reduce inhibitory synaptic transmission.

Additional pharmacology of the presynaptic GABA_B receptors involved in IPSC reduction

Our experiments provided evidence that baclofen affects the inhibitory synaptic transmission in the SON via a presynaptic decrease in evoked GABA release. A proposed mechanism of action of baclofen has been for it to modulate ionic conduc-

Release of GABA during the paired-stimulation protocol may not reach a sufficient concentration to activate the presynaptic GABA_B receptors. To enhance the concentration of GABA in the synaptic cleft, we evoked IPSCs in the presence of NO-711, an inhibitor of both glial and neuronal GABA uptake (Suzdak et al. 1992). Bath application of NO-711 (10 and 50 μM) reduced the amplitude of the evoked IPSC by 41 ± 5% (n = 9; P < 0.05 compared with control, Fig. 7, A and D). NO-711 did not trigger an inward current at −80 mV holding potential, neither did it change the input resistance of SON cells (789 ± 145 MΩ in control vs. 719 ± 146 MΩ in the presence of NO-711; n = 9; P > 0.05; Fig. 7B). In addition, no change in the decay of the evoked IPSCs was detected (Fig. 7A, right). The depression of the evoked IPSC was, however, accompanied by an enhancement in PPF of 69 ± 18% (n = 9; P < 0.05 compared with control; Fig. 8, A and C), an effect similar to baclofen. Under our recording conditions, the effects of NO-711 (10 and 50 μM) did not recover after 10–30 min washing out of the drug.

In six magnocellular neurons, we investigated the effect of the GABA_B receptor antagonist CGP 35438 on the NO-711-induced inhibition of the IPSCs. In the presence of CGP

FIG. 6. Predominantly paired-pulse facilitation (PPF) of IPSCs is recorded in magnocellular neurons. A: a pair of IPSCs (average of 15 sweeps) was evoked in quick succession at different interstimulus intervals (50–200 ms) and paired-pulse ratio determined (IPSC2/IPSC1). B: example of a pair of averaged IPSCs (5–10 sweeps) evoked at a fixed ISI of 50 ms but with weak (5 V), moderate (10 V), and maximal (40 V) stimulus intensities. C: dependence of PPF on ISI. Number above each bar represents number of cells tested.

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FIG. 7. Enhancement of extracellular GABA reduces amplitude of evoked IPSCs. A: bath application of a GABA uptake inhibitor, NO-711 (50 μM), decreased evoked IPSC amplitude (left, average of 12 sweeps) without changing decay of IPSCs (right, average of 12 sweeps). Note that IPSC amplitude in presence of NO-711 (——) was scaled to control IPSC (—) for comparison. B: a chart record showing that NO-711 has no effect either on holding current of an SON cell held at −80 mV or on input resistance of cell, as indicated by averaged membrane currents (4 sweeps) represented at an expanded time scale. C: superimposed traces obtained from the same cell showing that pretreatment with CGP35348 (500 μM) blocked NO-711-induced IPSC depression. D: effect of NO-711 and CGP35348 + NO-711 tested on a different population of cells. * Statistical significance at P < 0.05 compared with NO-711 (unpaired t-test).
for the same period of time (18 h), baclofen (1 μM) which is not statistically different from control (P > 0.05; Fig. 9A), compared with control condition, bath application of barium (1 mM), a nonselective K⁺ channel blocker, slightly increased the amplitude of evoked IPSCs by 9.7 ± 2.6% (n = 7, data not shown). In its presence, baclofen (0.3 μM) only weakly reduced the amplitude of the IPSCs by 8.9 ± 1.3% (n = 7), compared with the baclofen-induced IPSC reduction of 41.8 ± 8.5% recorded under control conditions (P < 0.05; Fig. 9A).

Furthermore we investigated the possible coupling of the presynaptic GABA<sub>B</sub> receptors to ionic conductances via a pertussis toxin (PTX) sensitive G-protein. Slices were hemi-sectioned along the midline and one-half was incubated, at room temperature, in ACSF containing PTX (200 ng/ml) over 18 h. The other hemislices were incubated in regular ACSF for the same period of time. In control slices, baclofen (1 μM) reduced the IPSC amplitude by 48.1 ± 5.3% (n = 10, Fig. 9B1). In PTX treated slices, baclofen-induced inhibition of IPSC amplitude was still 49.2 ± 3.4% (n = 11, Fig. 9B2), which is not statistically different from control (P > 0.05). When the concentration of PTX was raised to 500 ng/ml for the same period of time (18 h), baclofen (1 μM) was still able to reduce the IPSC amplitude by 52.8 ± 1.3% (n = 4; P > 0.05; data not shown).

The lack of effect of the PTX pretreatment on the baclofen-induced effect may be because of the lack of activity of the toxin itself. To test this possibility, we used lateral parabrachial nucleus (LPBN) slices (Saleh et al. 1996). We previously observed that LPBN neurons possessed postsynaptic GABA<sub>B</sub> receptors that are coupled to a PTX sensitive G-protein, which induces an outward current in these neurons when voltage-clamped at −65 mV (S. B. Kombian, unpublished observation). Slices containing the LPBN were therefore concurrently incubated with SON slices in ACSF containing PTX (500 ng/ml) for 18 h. Under these experimental conditions, baclofen (10 μM) no longer triggered the outward current in LPBN cells (n = 4, data not shown), while still depressing EPSCs that are PTX insensitive.

**DISCUSSION**

Field stimulation of the hypothalamic region dorsomedial to the SON elicited pharmacologically isolated IPSCs. These synaptic currents resulted from the activation of GABA<sub>A</sub> receptors, as shown by their almost complete abolition by either picrotoxin or bicuculline. On the basis of the fact that permeability of chloride ions through the nystatin-generated pores is not negligible (for review, see Akaike and Harata 1994), the reversal potential of the IPSCs (approximately −60 mV) was slightly different from the theoretical E<sub>N</sub> calculated for our experimental conditions using the Nernst equation (−67 mV). This small discrepancy may be explained by a leak of bicarbonate ions through the GABA<sub>A</sub> receptor complex, as a result of using a bicarbonate-buffered ACSF (Staley et al. 1995).

**GABA<sub>A</sub>-mediated IPSCs evoked in magnocellular neurons are inhibited by presynaptic GABA<sub>B</sub> receptors**

Baclofen, a GABA<sub>B</sub> receptor agonist, dose-dependently decreased the IPSC amplitude in a reversible manner. This depression was prevented by a competitive GABA<sub>B</sub> receptor antagonist, CGP35348, further demonstrating the involvement of GABA<sub>B</sub> receptors in the baclofen-induced inhibition of inhibitory synaptic transmission.

A number of postsynaptic mechanisms could account for the reduction in the IPSC amplitude observed in neurosecretory cells in response to baclofen. First, baclofen could activate postsynaptic GABA<sub>B</sub> receptors, which in turn could modulate the GABA<sub>A</sub> receptor-mediated response. However, this possibility is unlikely to account for the observed reduction in the IPSC amplitude because baclofen neither changed the holding current, nor the input resistance of rat magnocellular cells, a result supported by previous findings (Kombian et al. 1996; Wuarin and Dudek 1993). Second, GABA<sub>B</sub> receptors could modify the function of postsynaptic GABA<sub>A</sub> receptors, so that the postsynaptic response to a given amount of GABA is decreased. In cerebellar granule cells, baclofen was shown to inhibit muscimol-induced <sup>36</sup>Cl<sup>-</sup> uptake (Hahner et al. 1991), suggesting a change in chloride channel function. Although activation of GABA<sub>B</sub> receptors may modify the function of GABA<sub>A</sub> receptors in certain neurons, our data on IPSC kinetics and reversal potential demonstrate that there is no evident interaction between postsynaptic GABA<sub>A</sub> and GABA<sub>B</sub> receptors in SON neurosecretory cells, as is the case in hippocampal (Harrison et al. 1988) and neocortical neurons (Howe et al. 1987).
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Location of the presynaptic GABA<sub>B</sub> receptors: possible involvement of a polysynaptic network

Despite the fact that a prominent GABAergic input to the SON was described (for review, see Armstrong 1995; Renaud and Bourque 1991), the source of this innervation remains unclear. Although an electrophysiological study combined with excitotoxic lesions indicated monosynaptic connections from GABAergic neurons located in the median preoptic area (Renaud et al. 1990), axonal transport and immunohistochemical methods concluded that direct GABAergic projections from brain areas to the SON are a minor component of this innervation. The proposition of GABAergic interneurons located in the perinuclear zone (PNZ) and projecting to the neurosecretory cells appears well accepted (Jhamandas et al. 1989; Roland and Sawchenko 1993). Considering this anatomic situation and the finding that polysynaptic IPSPs are depressed to a greater extent by baclofen than monosynaptic IPSPs (Mott et al. 1993; Thompson and Gähwiler 1992), the baclofen-induced depression of IPSCs evoked by electrical stimulation of the zone dorsomedial to the SON could be secondary to the depression of the excitatory inputs to the GABAergic interneurons. However, this hypothesis is unlikely to explain the baclofen-induced decrease of IPSC amplitude in SON slices because all the experiments were carried out in the presence of kynurenic acid, a broad spectrum blocker of ionotropic excitatory amino acid receptors. Nevertheless, we cannot eliminate the possibility that our electrical stimulation activated excitatory inputs to interneurons that do not utilize glutamate as a transmitter.

The pharmacologically isolated IPSCs showed a constant latency, a single peak over a range of stimulation intensities and exhibited a monophasic decay. These results indicate that these IPSCs result from monosynaptic activation of many synapses, presumably formed by projections from extrahypothalamic inhibitory fibers and local axons from the inhibitory interneurons in the PNZ. Therefore the GABA<sub>B</sub> receptors responsible for the disinhibition of the magnocellular cells could be found on the somata of the inhibitory interneurons and/or on the inhibitory terminals of the GABAergic afferents.

Location of the presynaptic GABA<sub>B</sub> receptors on inhibitory interneurons

The GABA<sub>B</sub> receptors could be found on the somata of the inhibitory interneurons located in the PNZ. A baclofen-induced hyperpolarization and conductance changes could reduce the number of these interneurons reaching the spike threshold, thereby affecting the release of GABA (Misgeld et al. 1989). The fact that the baclofen-induced decrease in IPSC amplitude was abolished in the presence of barium could suggest that the GABA<sub>B</sub> receptors involved in the inhibitory effect are linked to a K<sup>+</sup> conductance, like the one present on CA3 pyramidal cells (Gähwiler and Brown 1985) or neurons of the locus coeruleus (Osmanovic and Shefner 1988). These postsynaptic GABA<sub>B</sub> receptors are usually coupled to K<sup>+</sup> channels via a PTX sensitive G-protein (Andrade et al. 1986; Colmers and Pittman 1988; Dutar and Nicoll 1988; Thalmann 1988). However, our data demonstrate that PTX pretreatment of slices does not affect the baclofen-induced depression of IPSCs under the same conditions where it blocked the postsynaptic GABA<sub>B</sub> response in neurons of the LPBN (S. B. Kombian, unpublished observation).

The block of K<sup>+</sup> conductances and subsequent enhanced Ca<sup>2+</sup> entry could also mask a baclofen-induced inhibition of Ca<sup>2+</sup> conductances activated during depolarization of the cell. The reduction in somatic Ca<sup>2+</sup> current amplitude, however, seems unlikely, based on the PTX insensitivity of baclofen’s action. Indeed, in CNS neurons, the GABA<sub>B</sub> re-
Receptor-induced inhibition of somatic voltage-activated Ca\(^{2+}\) currents was shown to be mediated via a PTX-sensitive G protein (Mintz and Bean 1993; Pfrieger et al. 1994; Scholz and Miller 1991). These results do not, however, rule out a PTX-insensitive action of baclofen on Ca\(^{2+}\) currents at the presynaptic terminal, such as was demonstrated in hippocampal pyramidal cells for the presynaptic inhibition of EPSPs by neuropeptide Y (NPY) (Colmers et al. 1988).

In light of the above arguments, it is most likely that the GABA\(_B\) receptors are located on the terminals of the GABAergic input to the SON. In support of this, baclofen enhanced PPF of two consecutive IPSCs, a finding consistent with previous data demonstrating that the probability of release of neurotransmitter significantly influences synaptic responses to repetitive activation (Khzipov et al. 1995; Lupica et al. 1992; Manabe et al. 1993; Wilcox and Dichter 1994). Furthermore, a preliminary report on SON slices indicates that baclofen decreased miniature IPSC frequency (Ibrahim et al. 1996).

**Activation of presynaptic GABA\(_B\) receptors in vitro**

In electrophysiological studies, a double shock depression paradigm for pharmacologically isolated IPSP/IPSCs, which is blocked or reduced by GABA\(_B\) receptor antagonists (Davies et al. 1990; Deisz and Prince 1989) suggests the presence of GABA\(_B\) receptors on the terminal themselves (autoreceptors). Under control condition, independent of the synaptic strength, no paired-pulse depression (PPD) of evoked IPSCs (ISIs ranging from 50 to 500 ms) was seen in magnocellular neurons. The apparent lack of frequency-dependent depression of evoked IPSCs result from the already existing occupation of the presynaptic GABA\(_B\) receptors by GABA, which is tonically released. The presence of the GABA\(_B\) receptor antagonist (CGP35348) increased the amplitude of the IPSCs as would be expected for an occupation of autoreceptors during basal GABA release. However, this increase in IPSC amplitude was not statistically significant, indicating that presynaptic GABA\(_B\) receptors may or may not be adequately accessible to endogenously released GABA, whether or not this is because of the location of these receptors, or to a weak GABAergic tone. Another alternative could be the presence of a potent GABA uptake mechanism that prevents the build up of a sufficient concentration of GABA in the synaptic cleft to activate these receptors. This possibility seems to be supported by the finding that the selective GABA uptake inhibitor NO-711 (previously, NNC-711) (Suzdak et al. 1992), both decreased the IPSC amplitude and enhanced facilitation of two consecutive IPSCs, thus mimicking baclofen effect. Both actions of NO-711 were abolished in the presence of CGP35348, indicating the involvement of GABA\(_B\) receptors. The observation that NO-711 did not prolong the decay of the IPSCs suggests that the duration of IPSCs evoked at the inhibitory synapses of SON cells is not determined by GABA uptake, as observed at other CNS inhibitory synapses (Morishita and Sastry 1995; Oh and Dichter 1994). More likely, the effect of NO-711 would be to increase the delay for clearance of synaptically released GABA, allowing endogenous GABA to activate the presynaptic GABA\(_B\) receptors. The possibility that NO-711 directly activated presynaptic GABA\(_B\) receptors seems unlikely. Indeed, perfusion of another GABA uptake inhibitor of neuronal and glial cells, nipecotic acid, produced a similar decrease in IPSC size (D. Mouginot, unpublished observation) and the probability that structurally different chemicals would activate GABA\(_B\) receptors is rather weak. Furthermore, tiagabine, an analogue of NO-711, does not activate postsynaptic GABA\(_B\) receptors in hippocampal slice cultures (Thompson and Gähwiler 1992).

**Physiological relevance**

Our data reveal functional presynaptic GABA\(_B\) receptors in the SON, suggesting that GABA release may be regulated locally. The GABA\(_B\) receptor-mediated action could then lead to disinhibition of magnocellular neurons and may regulate their electrical activity. As the present experiment in perfused slices did not reveal an action of endogenous GABA on these receptors in the absence of a GABA uptake inhibitor, it is possible that in vivo the situation would be different. Microdialysis experiments may be useful in revealing such action.

The absence of an efficient tonic GABAergic tone on presynaptic GABA\(_B\) receptors in control condition contrasts with our previous finding made on excitatory terminals in the SON (Kombian et al. 1996). The fact that a GABA\(_B\) receptor antagonist enhanced the size of EPSCs, but not IPSCs raises several hypotheses: (1) different location of the GABA\(_B\) receptors on the excitatory and inhibitory terminals (within the synapse area vs. extrasynaptic location), (2) different subtypes of GABA\(_B\) receptors on these terminals (all sensitive to baclofen), and (3) the affinity of the GABA uptake mechanism in the vicinity of the synapse may be different for the two terminal populations.

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