Inhibition of spontaneous EPSCs and IPSCs by presynaptic $GABA_B$ receptors on rat supraoptic magnocellular neurons

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1. The function of presynaptic GABA receptors in the regulation of transmitter release in supraoptic nucleus (SON) magnocellular neurons was investigated by recording spontaneous postsynaptic currents from rat magnocellular SON neurons in a slice preparation (150 μm thick, 1·8 mm in diameter) using the whole-cell patch-clamp technique.

2. Both the spontaneous EPSCs and IPSCs were TTX resistant. The EPSCs were abolished by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), whereas the IPSCs were abolished by picrotoxin, suggesting that the EPSCs and IPSCs are synaptic inputs from glutamatergic and GABAergic neurons, respectively.

3. The selective $GABA_B$ agonist, baclofen, reduced the frequency of both the EPSCs and IPSCs without affecting the amplitude. The time constant of the decay phase of both the EPSCs and IPSCs remained unchanged after baclofen application.

4. The reduction of the frequency of the synaptic currents by baclofen was dose dependent (10 nM to 100 μM) and the EC_{50} values were 5·8 and 8·5 μM for the EPSCs and IPSCs, respectively.

5. The effect of baclofen (10 μM) was antagonized by the selective $GABA_B$ antagonist, 2-hydroxy-saclofen (2OH-saclofen), at 300 μM.

6. When given alone, 2OH-saclofen (100 μM) increased the frequency of both the EPSCs and IPSCs without affecting their amplitude, suggesting that endogenously released GABA in the slice acts on presynaptic $GABA_B$ receptors.

7. The $GABA_A$ agonist, muscimol, reduced the frequency of EPSCs, and picrotoxin increased the frequency of the EPSCs, suggesting that $GABA_A$ receptors also participate in the presynaptic inhibition of glutamate release.

8. Taken together, these data suggest that $GABA_B$ receptors are present on the presynaptic terminals of both GABA and glutamate neurons in the SON, and that these presynaptic $GABA_B$ receptors play an important role in the regulation of the neuronal activity in SON magnocellular neurons.

The supraoptic nucleus (SON) of the hypothalamus consists of two types of magnocellular neurons that synthesize arginine vasopressin (AVP) or oxytocin (OXT), project into the neurohypophysis, and release these peptides into the systemic circulation. In mammals, AVP and OXT play pivotal roles in the regulation of body fluid and milk ejection, respectively. The release of these peptides is regulated by neuronal activity of the SON that is known to be influenced by body fluid volume, osmolality (Oliet & Bourque, 1993; Inenaga, Cui, Nagatomo, Honda, Ueta & Yamashita, 1997), extracellular Na^+ concentration, blood pressure and other factors (Kannan, Yamashita, Koizumi & Brooks, 1988). These factors are thought to be sensed not only by the central nervous system (CNS), including the SON, but also by peripheral receptors that send signals to the SON through afferent neural pathways (Koizumi & Yamashita, 1978; Kasai, Osaka, Inenaga, Kannan & Yamashita, 1987; Jhamandas & Renaud, 1987). The major neural signals to the SON are those from excitatory glutamatergic inputs and inhibitory GABAergic inputs (Randle & Renaud, 1987; Mason, Poulain & Cobbett, 1987; Decavel & Van den Pol, 1990; Wuarin & Dudek, 1993; Li, Inenaga & Yamashita, 1993). Immunohistochemical studies have shown that presynaptic glutamatergic and GABAergic

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neurons innervate SON neurons (Theodosis, Paut & Tappaz, 1986; Meeker, Swanson & Hayward, 1989; Van den Pol, 1991; Meeker, Swanson, Greenwood & Hayward, 1993), and the binding of GABA and glutamate, as well as $GABA_A$ and glutamate receptor mRNA expression, have been reported in the SON region (Van den Pol, Hermans Borgmeyer, Hofer, Ghosh & Heinemann, 1994). It has also been reported that GABA and glutamate are present in approximately 50 and 30%, respectively, of the total presynaptic neurons in the SON region (Meeker *et al.* 1993).

GABA receptors are classified into three subtypes; ionotropic $GABA_A$ receptors, metabotropic $GABA_B$ receptors and the recently identified ionotropic $GABA_C$ receptors (Sieghart, 1992; Bonanno & Raiteri, 1993; Bormann & Feigenspan, 1995; Smith & Olsen, 1995). The $GABA_A$ receptor has been the most extensively studied receptor subtype and has been shown to mediate fast postsynaptic currents via activation of Cl^- currents (Sieghart, 1992). The $GABA_B$ receptor, found in both presynaptic and postsynaptic sites, has been shown to inhibit neurotransmitter release and postsynaptic potentials induced by $GABA_A$ or glutamate receptor activation (Harrison, 1990; Bonanno & Raiteri, 1992; Jarolimek & Misgeld, 1992; Scanziani, Capogna, Gähwiler & Thompson, 1992; Thompson & Gähwiler, 1992; Bonanno & Raiteri, 1993; Doze, Cohen & Madison, 1995). The cellular mechanisms by which the $GABA_B$ receptor modulates neuronal activity are thought to include the opening of K^+ channels, the closure of Ca^{2+} channels (Newberry & Nicoll, 1984; Holz, Rane & Dunlap, 1986; Scholz & Miller, 1991) and the inhibition of adenylate cyclases (Robinson, Cross, Green, Toczek & Boar, 1989).

In the SON, the $GABA_A$ receptor has been thought to predominate, and the inhibitory effect of $GABA_A$ receptor activation and the properties of fast postsynaptic currents on magnocellular neurons have been well characterized (Wuarin & Dudek, 1993). However, the function of $GABA_B$ receptors in the SON is far less clear. It has been reported that the selective $GABA_B$ receptor agonist, baclofen, has no effect on the membrane potential of SON neurons in brain slice preparations of the guinea-pig (Ogata, 1987), and that no slow K^+ currents indicative of $GABA_B$ receptor-mediated neurotransmission were observed by whole-cell patch-clamp analysis in SON neurons in the brain slice preparations from the rat (Wuarin & Dudek, 1993; Kombian, Zidichoudki & Pittman, 1996). Furthermore, intracerebroventricular injections of baclofen have been reported to inhibit the milk-ejection reflex, but direct injections of baclofen into the SON region had no effect on the reflex (Voisin, Herbison, Chapman & Poulain, 1996). These results have been interpreted to suggest that functional $GABA_B$ receptors do not exist in the SON. On the other hand, if they do exist, the data would suggest that they do not play a major role, and the inhibitory control of SON neurons by presynaptic GABA neurons are exclusively mediated by $GABA_A$ receptors. Recently Kombian *et al.* (1996) have reported the first evidence for functional $GABA_B$ receptors in the rat SON. They found, in the hemisected brain slice preparation, that $GABA_B$ receptors mediate presynaptic inhibition of glutamatergic postsynaptic currents evoked by electrical stimulation of a perinuclear region dorsal to the SON.

In the present study, to investigate the function of $GABA_B$ receptors on the presynaptic terminals of neurons within the SON, the effects of $GABA_B$ receptor agonists and antagonists on both spontaneous excitatory and inhibitory postsynaptic currents of SON neurons were studied using the slice whole-cell patch-clamp technique. Thin (150 μm thick) and small (i.d. 1·8 mm) brain slice preparations (Nagatomo, Inenaga & Yamashita, 1995) that do not contain nuclei other than the SON helped in directly identifying magnocellular neurons under light microscopy and in minimizing the influences from neural pathways outside the SON that may effect synaptic transmission to SON neurons.

METHODS

Slice preparation

Young adult male Wistar rats (150–250 g) were stunned by a blow on the back of the neck and rapidly decapitated. The brain was quickly removed and cooled in a perfusion medium at 4 °C for approximately 1 min. A block containing the hypothalamus was cut from the brain and was glued to the stage of the vibratome-type slicer (DSK-2000; DSK, Kyoto, Japan). After the meninges were carefully removed, coronal slices 150 μm in thickness containing SON were cut as previously described (Nagatomo *et al.* 1995) from the block in the medium at 4 °C. The slices were carefully trimmed with a circular punch (i.d. 1·8 mm, Fig. 1*A*) and pre-incubated in the perfusion medium at room temperature (23–25 °C) for at least 1 h until they were transferred to the recording chamber.

Solution and drugs

The perfusion medium used contained (mM): NaCl, 124; KCl, 5; $MgSO_4$, 1·3; KH_2PO_4, 1·24; $CaCl_2$, 2; $NaHCO_3$, 25·9 and glucose, 10. Baclofen (Sigma), 2-hydroxy-saclofen (2OH-saclofen, RBI, USA), muscimol (Nacalai tesque, Japan), tetrodotoxin (TTX, Sankyo Co, Japan), picrotoxin (Nacalai tesque, Japan), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Sigma) were applied to the slice preparation by perfusing from separate storage bottles. All media used in this experiment were oxygenated with a mixture of 95% O_2–5% CO_2. The pipette solution used in the recording electrodes contained (mM): potassium gluconate, 140; $MgCl_2$, 1; $CaCl_2$, 1; EGTA, 10 and Mg-ATP, 2 (pH 7·3 set with Tris base).

Recording chamber and microscopic identification

The slice preparation was fixed in the recording chamber as previously described (Nagatomo *et al.* 1995). In short, the slice was placed in a glass-bottomed chamber and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight (Fig. 1*B* and *C*). The volume of the recording chamber was 1·0 ml and the perfusion speed was at 1·4 ml min^{-1}. Magnocellular neurons in the SON, were identified using an upright microscope (Axoscope FS, Carl-Zeiss, Germany) with Nomarski optics under phase contrast (× 400) and could be distinguished easily from other structures by observing their size, shape and the appearance of dendritic processes (Fig. 1*D*).

Recordings and data analysis

The electrodes used in this study were triple pulled with a puller (P-87, Sutter Instrument Co., Novoto, CA, USA) from glass capillary (GD-1·5, Narishige, Japan). They had a final resistance between 6 and 8 MΩ when filled. Electrophysiological recordings were carried out at a room temperature of about 25 °C. Whole-cell recordings were made from microscopically identified SON neurons in the upper surface layers of the slices. After gaining access to the whole cell, the series resistance was in the range of 15–30 MΩ and the cell capacitance was 10–30 pF. Recordings of postsynaptic currents were begun about 5 min after membrane rupture when the currents reached a steady state. Currents and voltages were recorded with an EPC-9 amplifier (HEKA, Germany). Signals were filtered at 3 kHz, digitized at 1 kHz with an analog–digital converter (MacLab/8, ADI, USA), and stored on hard disks using a personal computer (Apple Macintosh, USA). To analyse synaptic currents quantitatively, only the AC components were used for analysis with a software (AxoGraph v.3, Axon Instrument, USA) package.

Values are expressed as means ± S.E.M. except where otherwise noted. Statistical analysis was performed using non-parametric tests (Mann–Whitney *U* test or Wilcoxon signed-rank test) or non-parametric ANOVA tests. The number of neurons tested is represented by '*n*'. $P < 0{\cdot}5$ was considered significant. To minimize the possibility that data are influenced by the rundown of synaptic currents during the course of measurements, the effects of a drug are expressed as a percentage of the mean, measured before and after the drug application.

RESULTS

Spontaneous postsynaptic currents recorded from SON neurons

Spontaneous synaptic currents were recorded from a total of 174 magnocellular neurons in the SON that were identified microscopically in the thin slice preparation (Fig. 1). A representative example of records showing voltage dependency of spontaneous synaptic currents is presented in Fig. 2. When the holding potential of the SON neuron was changed from −20 to −70 mV, both excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) were observed (Fig. 2). At −70 mV, virtually pure EPSCs were recorded and at −20 mV, IPSCs were recorded. Therefore, throughout the remaining experiments holding potentials of −70 and −20 mV were used to analyse spontaneous EPSCs and IPSCs, respectively, unless otherwise noted.

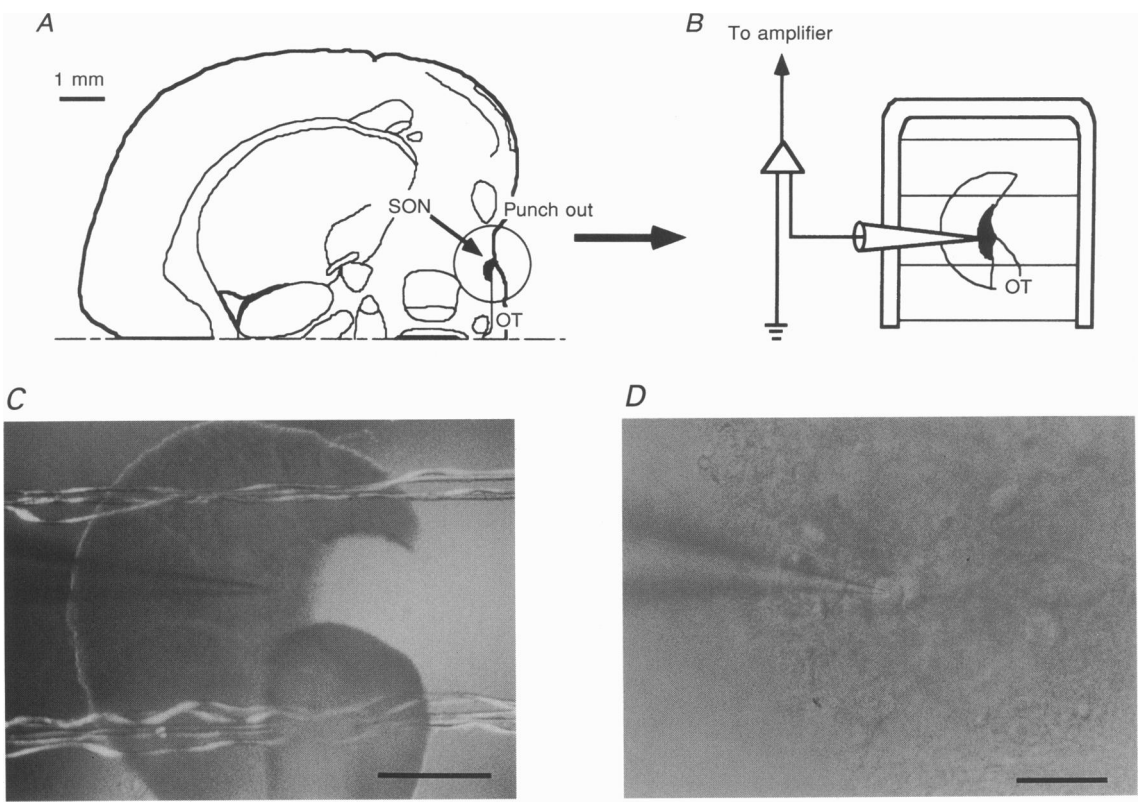

Figure 1. Illustrations and photomicrograhs of the slice preparation of the supraoptic nucleus (SON)

A, diagram of a hemisected coronal slice of rat hypothalamus showing the 'punch-out' SON preparation (Bregma −1·6 mm; OT, optic tract). Note that the punch-out does not contain nuclei other than the SON. *B*, a schematic illustration of a punch-out preparation and recording electrode arrangement. *C*, photomicrograph of the position of recording electrode and the SON; scale bar, 0·5 mm. *D*, a magnocellular neuron with an attached patch-clamp electrode; scale bar, 50 μm.

The mean amplitude of EPSCs recorded from forty-three neurons (at a holding potential (V_h) of −70 mV) ranged between 10·1 and 25·6 pA (15·2 ± 0·6 pA) and the mean frequency of EPSCs was 2·96 ± 0·48 Hz. Mean amplitude of IPSCs recorded from thirty-three neurons (V_h at −20 mV) ranged between 10·1 and 30·1 pA (18·1 ± 0·9 pA) and the mean frequency of IPSCs was 3·00 ± 0·39 Hz.

Unlike EPSCs and IPSCs recorded from SON neurons in brain slice preparations (Wuarin & Dudek, 1993), neither spontaneous EPSCs nor IPSCs were affected by application of TTX (1 μM), a Na^+ channel blocker (Fig. 3*A*a and *Ba*). The amplitude–histogram analysis of these currents (Fig. 3*Ab*, *Ac*, *Bb* and *Bc*) revealed that neither the mean amplitude nor the frequency was affected by TTX (Fig. 3*C*). The frequency and amplitude of spontaneous EPSCs were 105·8 ± 8·1 and 100·5 ± 2·8% of the control, respectively, ($n = 9$) and those of spontaneous IPSCs were 94·4 ± 6·3 and 105·7 ± 3·9% of the control, respectively, ($n = 11$).

Properties of spontaneous EPSCs and IPSCs were further examined by applying the non-NMDA glutamate receptor antagonist, CNQX, and the $GABA_A$ receptor antagonist, picrotoxin, respectively. As shown in Fig. 4, EPSCs and IPSCs were abolished by the application of 10 μM CNQX ($n = 4$) and by the application of 50 μM picrotoxin ($n = 5$), respectively. The IPSCs were also abolished by the application of 10–50 μM bicuculline ($n = 3$). These results indicate that spontaneous EPSCs and IPSCs recorded in the present study were entirely TTX resistant and represent local release of glutamate and GABA, respectively.

The effects of the selective $GABA_B$ agonist, baclofen, on spontaneous EPSCs and IPSCs

Figure 5 shows examples of the effects of the selective $GABA_B$ agonist, baclofen, on EPSCs (Fig. 5*A*) and IPSCs (Fig. 5*B*). Baclofen (10 μM) reversibly inhibited both EPSCs ($n = 27$) and IPSCs ($n = 25$). The pattern of inhibition was a decrease in the number of events without a reduction of amplitude of either the EPSCs or IPSCs. This is evident in the amplitude–histogram analysis of the currents before, during and after application of baclofen (Fig. 6). The analysis showed that in response to baclofen, the frequency of EPSCs and IPSCs fell from 0·85 to 0·33 Hz (Fig. 6*A*) and 2·08 to 0·70 Hz (Fig. 6*B*), respectively. By contrast, the

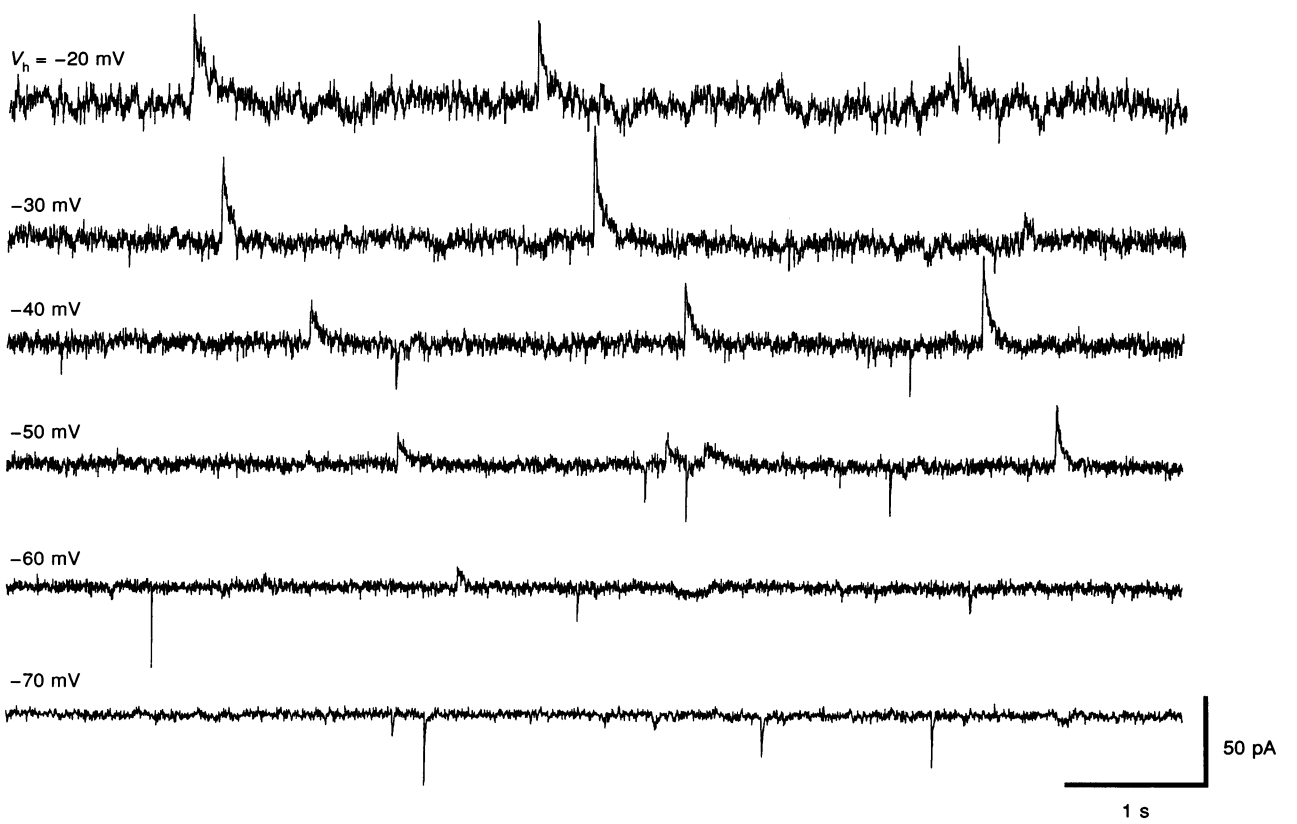

Figure 2. The voltage dependency of spontaneous EPSCs and IPSCs

Representative examples of voltage dependency of spontaneous postsynaptic currents recorded from a single SON neuron. The synaptic currents were recorded by changing the holding potential (V_h) from −20 to −70 mV as indicated. The upward and downward deflections represent inhibitory (IPSCs) and excitatory (EPSCs) postsynaptic currents, respectively. Note that IPSCs were observed at −20 mV and EPSCs were at −70 mV.

mean amplitude was not significantly affected (EPSC amplitude, 19·8 ± 0·7 *vs.* 20·1 ± 1·3 pA; IPSC amplitude, 24·8 ± 0·7 *vs.* 24·1 ± 0·7 pA). The nature of the inhibitory effect of baclofen was further investigated by measuring the time constant of the decay phase of the spontaneous EPSCs and IPSCs by single exponential curve fittings. The decay phase of both EPSCs and IPSCs was generally well fitted by a single exponential (Fig. 6*Ca* and *Da*). The time constant obtained for EPSCs was 2·8 ± 0·2 ms ($n = 5$), which was approximately shorter by one order of magnitude than that for IPSCs (33·2 ± 4·0 ms; n = 6). However, the time constants for EPSCs and IPSCs obtained during baclofen application were not significantly different ($P > 0·05$) from the pre-application values (Fig. 6*Cb* and *Db*).

Baclofen-induced inhibition of EPSCs and IPSCs was dose dependent (Fig. 7*A* and *B*). Inhibition of postsynaptic currents usually became evident from 100 nM, and when

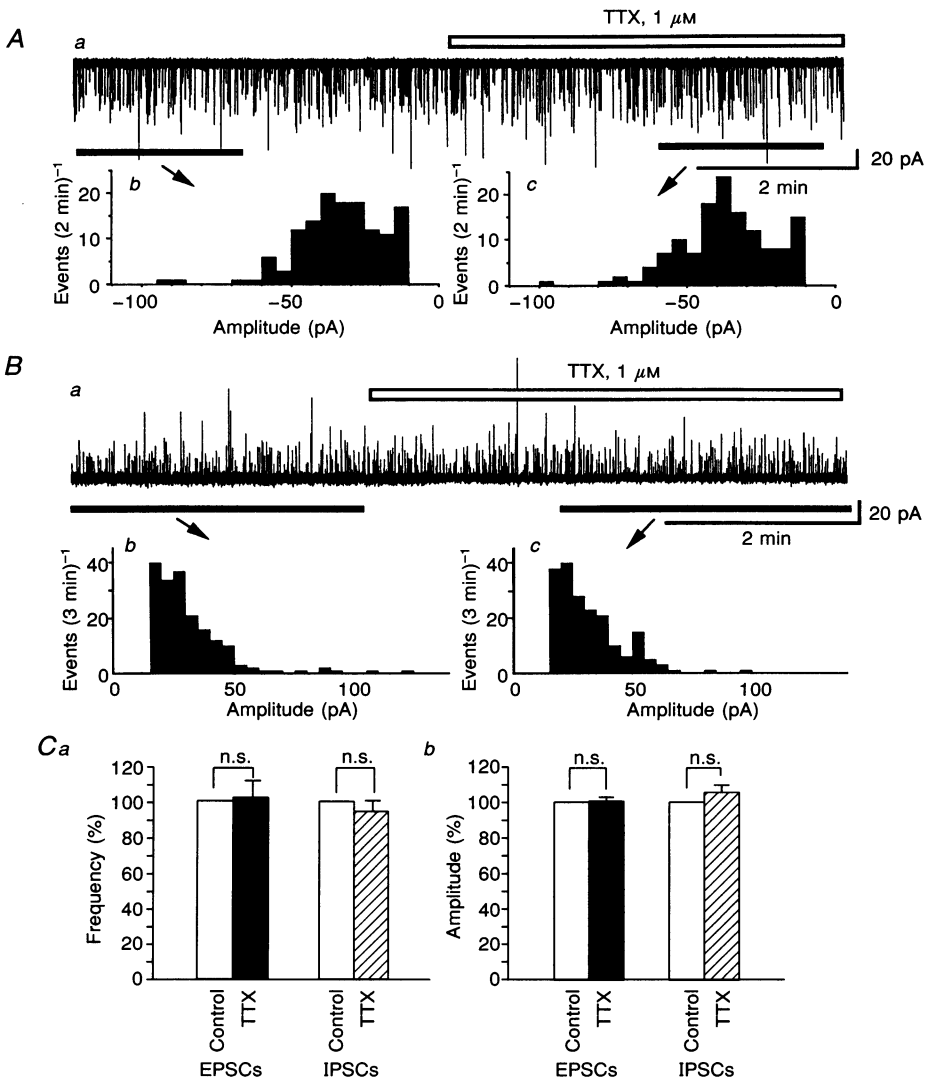

Figure 3. Effects of TTX on spontaneous EPSCs and IPSCs

Aa, the effect of bath application of TTX (1 μM) on spontaneous EPSCs. The holding potential was −70 mV. *Ab* and *Ac* show the amplitude–histogram analysis of the effects of TTX on EPSCs. The synaptic currents above the filled horozontal bars were used for the amplitude–histogram analysis. TTX had no significant effect on the distribution pattern, the frequency and the mean amplitude of spontaneous EPSCs (*Ab*, 1·13 Hz and 33·3 ± 1·3 pA; *Ac*, 1·12 Hz and 36·8 ± 1·4 pA). *Ba*, the effect of bath application of TTX (1 μM) on spontaneous IPSCs. The holding potential was −20 mV. *Bb* and *Bc* show the amplitude–histogram analysis of the effect of TTX on IPSCs. Similarly to EPSCs, TTX had no significant effect on spontaneous IPSCs (*Bb*, 1·02 Hz and 31·3 ± 1·9 pA; *Bc*, 1·07 Hz and 31·4 ± 1·2 pA). *C*, the summary of the effects of TTX on the frequency (*Ca*) and the amplitude (*Cb*) of EPSCs ($n = 9$) and IPSCs ($n = 11$). n.s., not significantly different ($P > 0·05$) from the control value. For the statistical analysis the Wilcoxon signed-rank test was used.

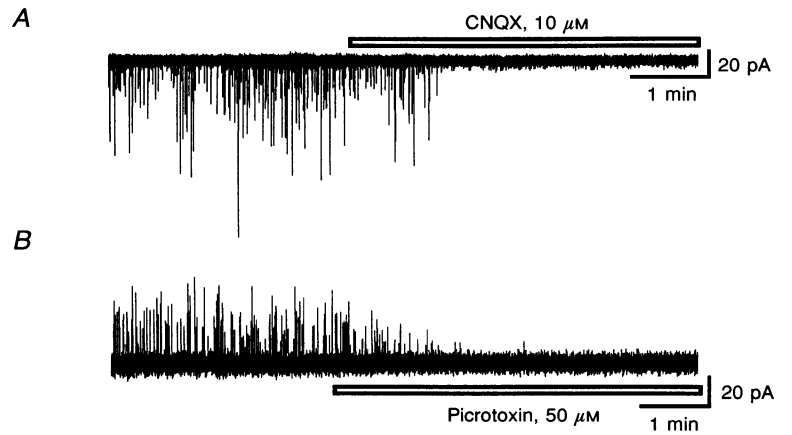

Figure 4. Effects of CNQX on spontaneous EPSCs and those of picrotoxin on spontaneous IPSCs
A, spontaneous EPSCs were abolished during the application of non-NMDA glutamate receptor antagonist, CNQX (10 μM). The holding potential was −70 mV. *B*, spontaneous IPSCs were suppressed by the $GABA_A$ receptor antagonist, picrotoxin (50 μM). The holding potential was −20 mV.

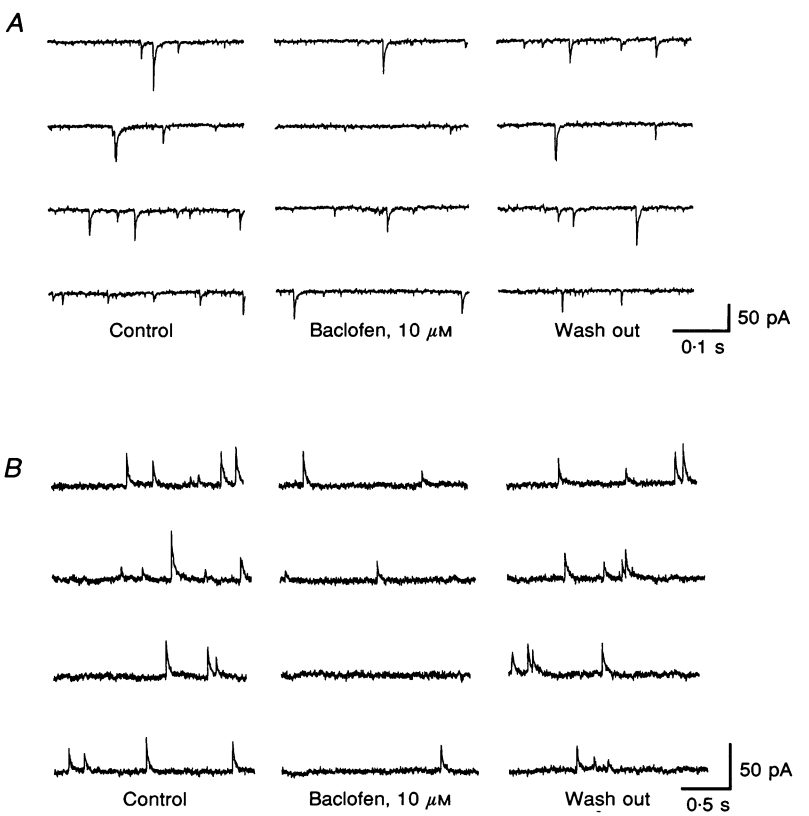

Figure 5. Inhibition of spontaneous EPSCs and IPSCs by baclofen
A, a representative example of inhibition of spontaneous EPSCs by baclofen. Continuous traces of EPSCs recorded before, during and after application of baclofen (10 μM) are shown. The holding potential was −70 mV. *B*, a typical example of inhibition of spontaneous IPSCs by baclofen. Continuous traces of IPSCs recorded before, during and after application of baclofen (10 μM) are shown. The holding potential was −20 mV.

the concentration of baclofen was increased, the onset of inhibition became more rapid and the recovery was delayed. Figure 7*C* and *E* show the dose–response relationships of baclofen-induced inhibition of the frequency of EPSCs and IPSCs. Significant ($P < 0{\cdot}05$) inhibition was observed with baclofen from 1 μM (both EPSCs and IPSCs) and the EC_{50} values for inhibition of EPSCs and IPSCs were estimated to be 5·8 and 8·5 μM, respectively. By contrast, the amplitude of EPSCs and IPSCs remained unchanged during baclofen application (Fig. 7*C* and *D*). It should be noted that even with baclofen at the highest concentration tested (100 μM), no discernible slow outward or inward currents were observed in raw traces (before AC filter) during baclofen application.

The effects of TTX on the inhibition by baclofen (10 μM) of EPSCs and IPSCs were examined. The frequency of EPSCs and IPSCs was reduced by baclofen to 68·7 ± 3·2% ($n = 5$) and 67·3 ± 5·4% ($n = 5$), respectively, in the presence of TTX (1 μM), and to 58·5 ± 9·9 ($n = 5$) and 59·1 ± 10·1% ($n = 5$), respectively, in the absence of TTX. There was no statistical difference ($P > 0{\cdot}05$) between the values obtained

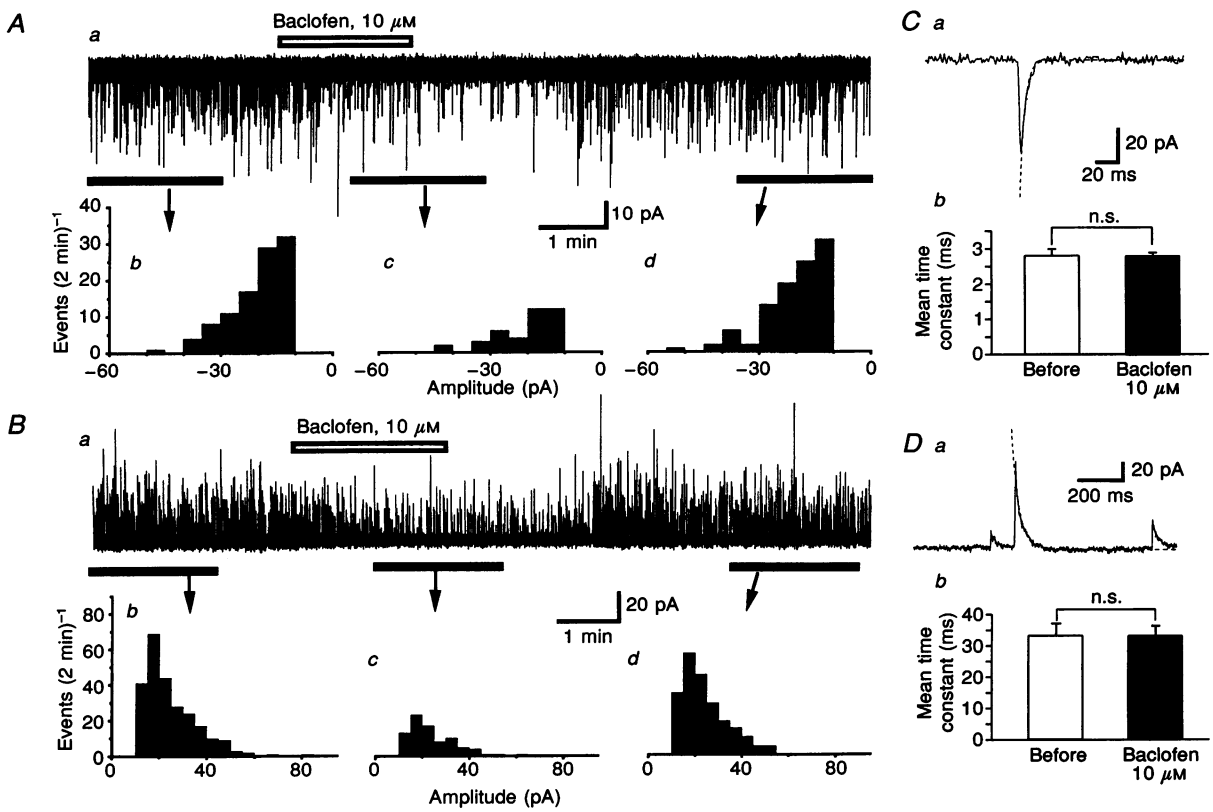

Figure 6. Analysis of baclofen-induced inhibition of postsynaptic currents

Aa, an example of the time course of the effects of baclofen (10 μM) on spontaneous EPSCs. Baclofen was added by bath application for 2 min, the time indicated by the open horizontal bar. The holding potential was −70 mV. *Ab–Ad* show the amplitude–histogram analysis of the effect of baclofen shown in *Aa*. EPSCs were recorded and analysed during the time indicated by the filled horizontal bars. Note that bath application of baclofen decreased the frequency of the events (*Ab*, 0·85 Hz; *Ac*, 0·33 Hz; *Ad*, 0·83 Hz), whereas the distribution pattern of the synaptic currents and the mean amplitude were unaffected (*Ab*, 19·8 ± 0·7 pA; *Ac*, 20·1 ± 1·3 pA; *Ad*, 20·3 ± 0·8 pA). *Ba*, an example of the time course of the effects of baclofen (10 μM) on spontaneous IPSCs. Baclofen was added for 2·5 min, the time indicated by the open horizontal bar. The holding potential was −20 mV. *Bb–Bd* show the amplitude–histogram analysis of the effect of baclofen shown in *Ba*. IPSCs recorded during the time indicated by the filled horizontal bars were analysed. The frequency of the events was decreased (*Bb*, 2·08 Hz; *Bc*, 0·70 Hz; *Bd*, 1·94 Hz), but the mean amplitude was not affected (*Bb*, 24·8 ± 0·7 pA; *Bc*, 24·1 ± 1·1 pA; *Bd*, 24·7 ± 0·7 pA). *C* and *D*, show the analysis of the time constant of decay phase. *Ca* and *Da* show examples of a trace of a spontaneous EPSC and IPSC, and their single exponential curve fits. *Cb*, the time constant for the decay phase of EPSCs before and during baclofen were calculated by single exponential curve fitting and compared ($n = 5$). *Db*, the time constants for the decay phase of IPSCs before and during baclofen were calculated by single exponential curve fitting and compared ($n = 6$). There was no significant difference ($P > 0{\cdot}05$) in either the EPSCs or IPSCs. For the statistical analysis, the Wilcoxon signed-rank test was used.

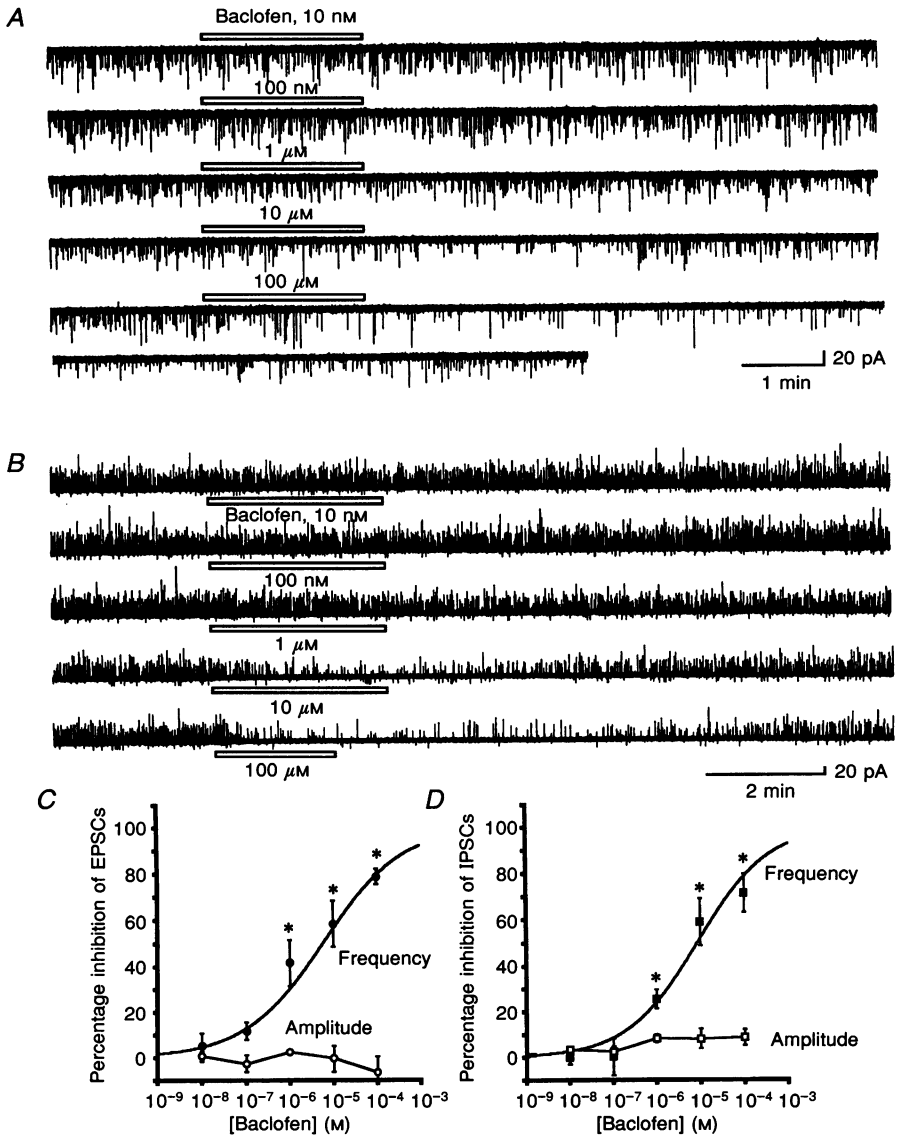

Figure 7. Dose-dependent inhibition of spontaneous EPSCs and IPSCs

A, EPSCs recorded from a single SON neuron. The holding potential was −70 mV. Baclofen at concentrations from 10 nM to 100 μM was added at the time indicated by the open horizontal bars. *B*, IPSCs recorded from a single SON neuron. The holding potential was −20 mV, and baclofen was added over the time and at the concentration indicated by the horizontal bars. *C* and *D* show the dose–response relationship for baclofen-induced inhibition of the frequency and amplitude. The rate of frequency inhibition is expressed as a percentage of the mean frequency measured during baclofen application to the control value measured before and after baclofen application. The synaptic currents for 2 min (starting from 1 min after the beginning of baclofen application when the inhibitory effects became stable) were used for the analysis of the effects of baclofen. The pre- and post-control values were calculated from 2 min of synaptic currents measured just before baclofen application and those measured 5 min after withdrawal of baclofen, respectively. When 100 μM baclofen was added, the post-control value was calculated from synaptic currents measured 10 min after withdrawal of baclofen because of the delayed recovery. The dose–response curves were calculated by the least-squares method after the pseudo-Hill plot of the inhibition rate of postsynaptic currents in response to baclofen by assuming the maximum inhibition of 100% (EPSCs in *C*, $n = 5$; IPSCs in *D*, $n = 5$). From these curves, the EC_{50} values for EPSCs and IPSCs were estimated to be 5·8 μM and 8·5 μM, respectively. Dose–response relationships for the effect of baclofen on the amplitude of spontaneous EPSCs and IPSCs are also shown. The rate of amplitude inhibition is expressed as a percentage of the mean amplitude measured during baclofen application to the control value. The vertical bars indicate S.E.M.; * significantly different from the control value ($P < 0·05$). Non-parametric ANOVA test was used for the statistical analysis.

in the presence and absence of TTX. Further analysis shown below was performed in the absence of TTX.

The effects of the selective $GABA_B$ antagonist, 2OH-saclofen

The selective $GABA_B$ antagonist, 2OH-saclofen, completely reversed the inhibition of EPSCs ($n = 5$) and IPSCs ($n = 5$) induced by baclofen (10 μM) when the antagonist was given at concentrations 30 times higher (300 μM) than that of baclofen (Fig. 8). When 2OH-saclofen (100 μM) was added alone, the frequency of both EPSCs and IPSCs was significantly increased in number (Fig. 9). The frequency and amplitude of EPSCs during 2OH-saclofen application were 168·0 ± 19·3 and 94·2 ± 4·2% of the control values ($n = 5$), respectively, and those of IPSCs were 156·6 ± 21·7 and 100·9 ± 2·7% of the control values ($n = 5$), respectively (Fig. 9*Ca* and *Cb*).

The effects of $GABA_A$ agonist and antagonist on EPSCs and IPSCs

To elucidate the contribution of $GABA_A$ receptors in the presynaptic regulation of EPSCs, the effects of the selective $GABA_A$ agonist, muscimol, on EPSCs was examined. In these experiments, the holding potential was set to −68 mV to minimize the postsynaptic effects of muscimol. Muscimol (1 μM and 10 μM) reversibly inhibited EPSCs in a dose-dependent manner. The amplitude–histogram analysis showed that the inhibition was due mainly to a reduction in the frequency of the EPSCs (Fig. 10*A* and *B*). The frequency of EPSCs was significantly reduced to 69·7 ± 5·8% ($n = 5$) of the control in response to 1 μM muscimol and 32·4 ± 9·7% of the control in response to 10 μM muscimol ($n = 5$, Fig. 10*Da*). In response to muscimol, the amplitude of EPSCs tended to decrease, but the significance ($P < 0·05$) was confirmed only with 10 μM (Fig. 10*Db*).

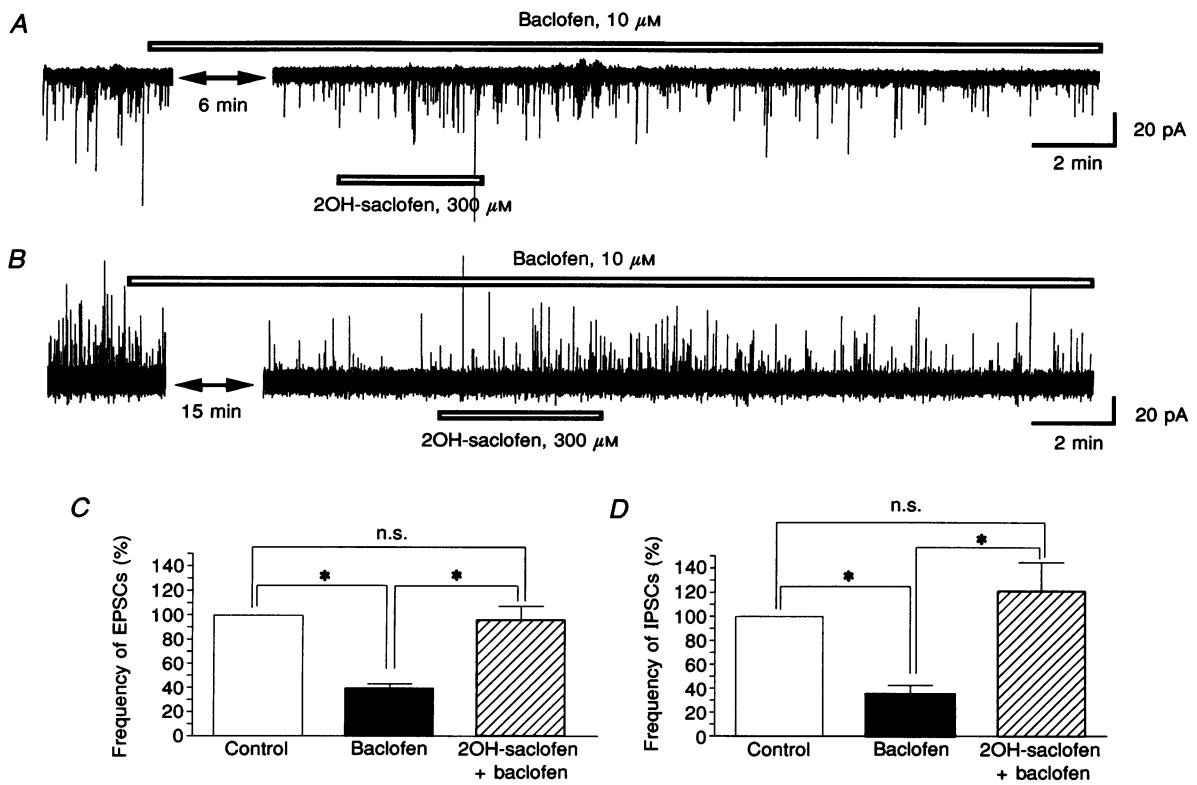

Figure 8. Saclofen reversed the baclofen-induced inhibition of spontaneous EPSCs and IPSCs

Examples of the reversal of baclofen (10 μM)-induced inhibition of spontaneous EPSCs, *A* and IPSCs, *B* by the application of $GABA_B$ antagonist, 2OH-saclofen (300 μM). The holding potential was −70 and −20 mV for EPSCs and IPSCs, respectively. The inhibition of the frequency of postsynaptic currents in response to baclofen and its reversal by 2OH-saclofen are summarized in *C* (EPSCs; $n = 5$) and *D* (IPSCs; $n = 5$). The responses are expressed as a percentage of the mean ± S.E.M. of the frequencies measured during the drug applications to the control value (mean of pre- and post-control values). The synaptic currents for 2 min (just before application of 2OH-saclofen) were used for the analysis of the effects of baclofen and the synaptic currents for 2 min (starting from 1 min before withdrawal of 2OH-saclofen) were used for the analysis of the effects of 2OH-saclofen. The pre- and post-control values were calculated from 2 min of synaptic currents measured just before baclofen application and those measured 5 min after withdrawal of baclofen, respectively. * and n.s. indicate significant ($P < 0·05$) and insignificant differences, respectively, from the control value. Non-parametric ANOVA test was used for the statistical analysis.

Picrotoxin, at a concentration of 50 μM, eliminated spontaneous IPSCs (Fig. 4), but increased EPSCs (Fig. 10*Ca*). The amplitude–histogram analysis revealed that the change was due to an increase in the frequency of EPSCs (Fig. 10*Cb*, *c* and *d*). The frequency and mean amplitude of EPSCs were 175·0 ± 19·9 and 90·8 ± 5·8% of the control, respectively ($n = 5$). The significance was confirmed only with the effect on frequency (Fig. 10*E*). The competitive $GABA_A$ receptor antagonist, bicuculline (20 μM), also increased the frequency (156·1 ± 7·6%, $n = 5$) of EPSCs without significantly affecting the amplitude (100·3 ± 5·4%, Fig. 10*E*).

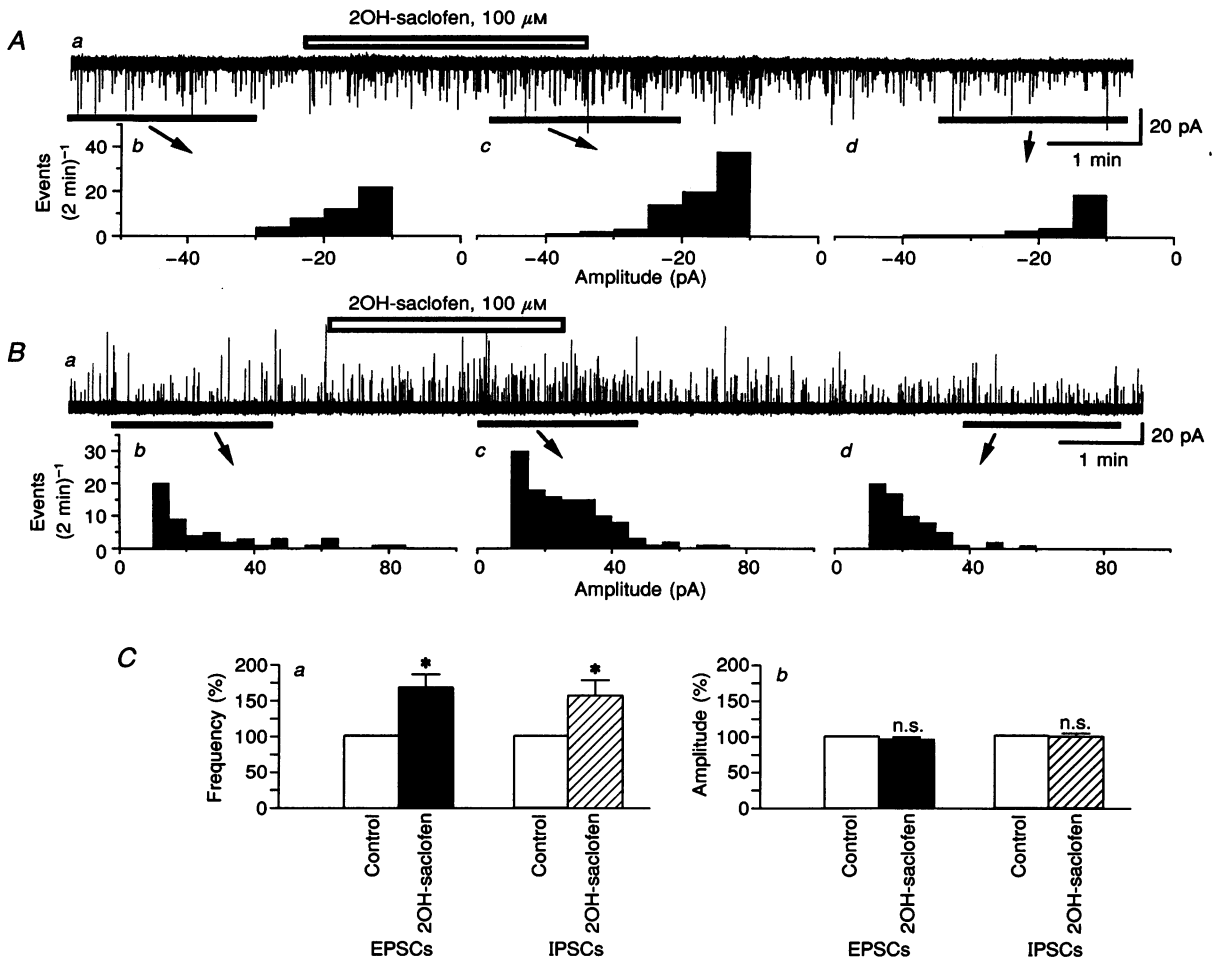

Figure 9. Potentiating effects of 2OH-saclofen (100 μM) on spontaneous EPSCs and IPSCs

The holding potential was −70 and −20 mV for EPSCs and IPSCs, respectively. *Ab–Ad* show the amplitude–histogram analysis of the effect of 2OH-saclofen on EPSCs shown in *Aa*. EPSCs recorded during the time indicated by the filled horizontal bars were analysed. The frequency of EPSCs was increased during 2OH-saclofen (*Ab*, 0·38 Hz; *Ac*, 0·67 Hz; *Ad*, 0·25 Hz), but the mean amplitude remained unchanged (*Ab*, 16·8 ± 0·3 pA; *Ac*, 16·8 ± 0·6 pA; *Ad*, 16·5 ± 1·1 pA). *Bb–Bd* show the amplitude–histogram analysis of the effect of 2OH-saclofen on IPSCs shown in *Ba*. IPSCs recorded during the time indicated by the filled horizontal bars were analysed. The frequency of IPSCs was increased during 2OH-saclofen (*Bb*, 0·45 Hz; *Bc*, 1·03 Hz; *Bd*, 0·526 Hz) but the mean amplitude was not affected (*Bb*, 26·1 ± 1·5 pA; *Bc*, 25·6 ± 1·2 pA; *Bd*, 20·9 ± 1·2 pA). *C*, the effects of 2OH-saclofen on the frequency (*Ca*) and amplitude (*Cb*) of EPSCs ($n = 5$) and IPSCs ($n = 5$) are summarized. The effects are expressed as a percentage of the mean ± S.E.M. of the frequencies and the amplitudes to the control. The synaptic currents for 2 min (starting from 1 min before withdrawal of 2OH-saclofen) were used for the analysis of the effects of 2OH-saclofen. The pre- and post-control values were calculated from 2 min of synaptic currents measured just before 2OH-saclofen application and those measured 5 min after withdrawal of 2OH-saclofen, respectively. * and n.s. indicate significant ($P < 0·05$) and insignificant differences, respectively, from the control value. The Wilcoxon signed-rank test was used for the statistical analysis.

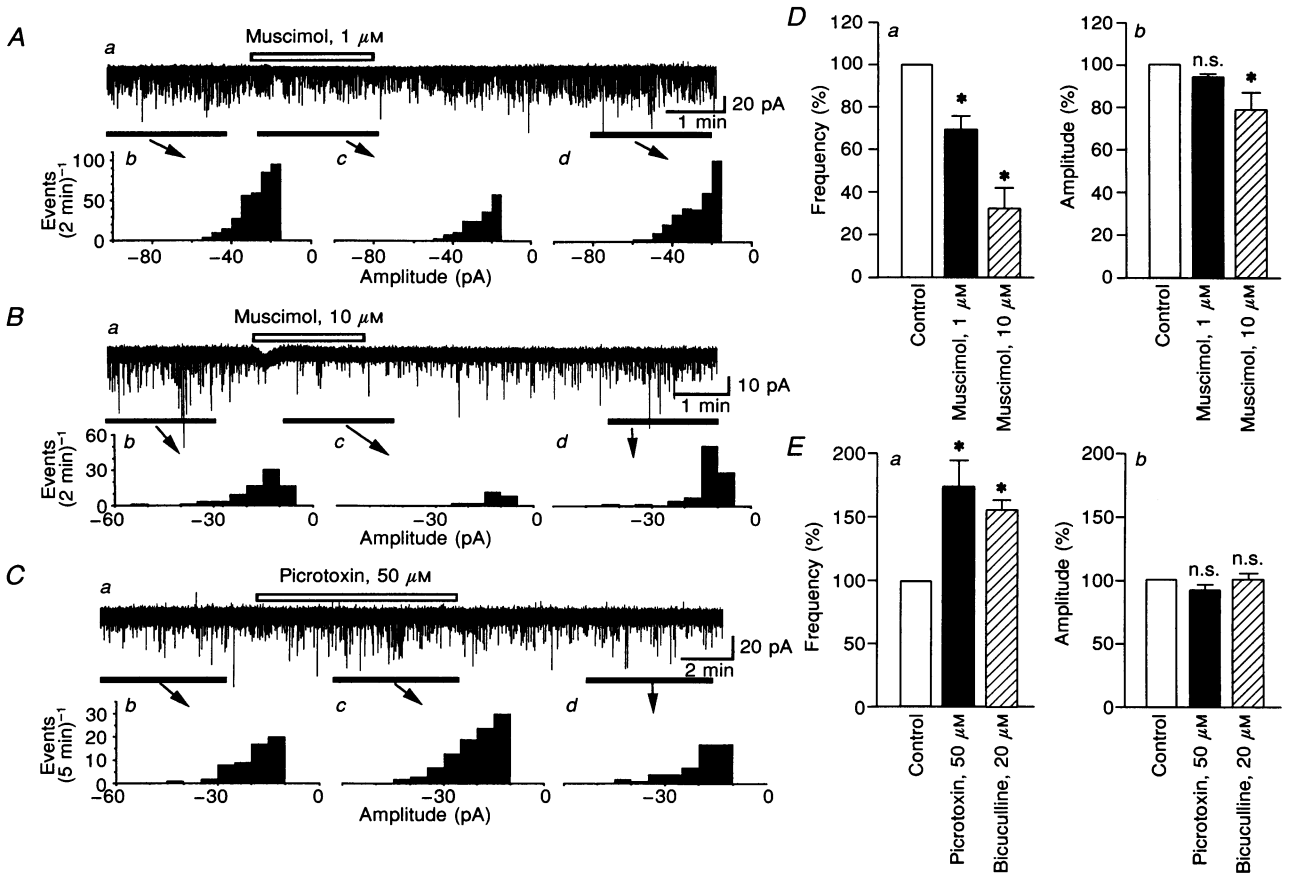

Figure 10. Effects of the $GABA_A$ agonist and antagonist on spontaneous EPSCs

Inhibitory effects of the $GABA_A$ agonist, muscimol, on EPSCs. *Aa*, muscimol at 1 μM inhibited EPSCs. The holding potential was −68 mV. *Ab–Ad* show the amplitude–histogram analysis of the effect of muscimol on EPSCs shown in *Aa*. EPSCs recorded during the time indicated by the filled horizontal bars were analysed. The frequency of EPSCs was decreased during muscimol application (*Ab*, 2·97 Hz; *Ac*, 1·41 Hz; *Ad*, 2·68 Hz) but the mean amplitude was not affected (*Ab*, 27·0 ± 0·5 pA; *Ac*, 25·6 ± 0·7 pA; *Ad*, 27·8 ± 0·6 pA). *Ba*, muscimol at 10 μM inhibited EPSCs. The holding potential was −68 mV. *Bb–Bd* shows the amplitude–histogram analysis of the effect of muscimol on EPSCs shown in *Ba*. The frequency of EPSCs was 0·68 Hz (*Bb*), 0·20 Hz (*Bc*) and 0·76 Hz (*Bd*), and the mean amplitude was 15·7 ± 0·8 pA (*Bb*), 12·3 ± 0·8 pA (*Bc*) and 12·6 ± 0·5 pA (*Bd*). *Ca* shows that picrotoxin (50 μM) potentiated the frequency of spontaneous EPSCs. The holding potential was −70 mV. *Cb–Cd* show the amplitude–histogram analysis of the effect of picrotoxin on EPSCs shown in *Ca*. EPSCs recorded during the time indicated by the filled horizontal bars were analysed. The frequency of EPSCs was increased during application of the $GABA_A$ antagonist, picrotoxin (*Cb*, 0·19 Hz; *Cc*, 0·32 Hz; *Cd*, 0·17 Hz), but the mean amplitude was not affected (*Cb*, 19·1 ± 0·9 pA; *Cc*, 20·4 ± 0·7 pA; *Cd*, 20·3 ± 1·1 pA). *D*, summary of the effects of muscimol on the frequency (*Da*) and amplitude (*Db*) of EPSCs ($n = 5$). The synaptic currents for 2 min (starting from 30 s after application of muscimol) were used for the analysis of the effects of muscimol. The pre- and post-control values were calculated from 2 min of synaptic currents measured just before muscimol application and those measured 5 min after withdrawal of muscimol, respectively. *E*, summary of the effects of picrotoxin ($n = 5$) and bicuculline ($n = 5$) on the frequency (*Ea*) and amplitude (*Eb*) of EPSCs, respectively. The effects are expressed as a percentage of the mean ± S.E.M. of the frequencies and the amplitudes to the control. The synaptic currents for 2 min (starting from 1 min before withdrawal of picrotoxin or bicuculline) were used for the analysis of the effects of the $GABA_A$ antagonists. The pre- and post-control values were calculated from 2 min of synaptic currents measured just before application of the $GABA_A$ antagonists and those measured 8 min after withdrawal the $GABA_A$ antagonists, respectively. * and n.s. indicate significant ($P < 0·05$) and insignificant differences, respectively, from the control value. The non-parametric ANOVA test was used for the statistical analysis.

DISCUSSION

The present study has demonstrated that the selective $GABA_B$ agonist, baclofen, consistently inhibited both spontaneous EPSCs and IPSCs from SON magnocellular neurons. As the preparation used in this study contained only the SON and perinuclear region, these results suggest that $GABA_B$ receptors, which are located in the presynaptic terminals of GABA and glutamate neurons innervating the SON, exert inhibitory actions on spontaneous transmitter release.

The effect of baclofen on both EPSCs and IPSCs was dose dependent and reversible. Moreover, the selective $GABA_B$ antagonist, 2OH-saclofen, antagonized the effects of baclofen suggesting that baclofen effects were mediated through presynaptic $GABA_B$ receptors as reported in other CNS preparations (Harrison, 1990; Otis, Staley & Mody, 1991; Bonanno & Raiteri, 1992; Jarolimek & Misgeld, 1992; Scanziani *et al.* 1992; Doze *et al.* 1995; Jiang, Allen & North, 1995). The amplitude–histogram analysis revealed that the effects of baclofen were mainly due to the reduction of frequency of synaptic currents, whereas the amplitude of the synaptic currents remained unchanged. Moreover, the inhibitory effects of baclofen on the synaptic currents were unaffected by the presence of TTX. These results, taken together with the observation that the time constant of the decay phase of the synaptic currents was unchanged during baclofen application, suggests that the site of action of baclofen was presynaptic. These results are consistent with those reported by Kombian *et al.* (1996) in that $GABA_B$ receptor activation inhibits the evoked EPSCs at the level of the presynaptic terminals of glutamate neurons in the SON. The present study extends these earlier findings by demonstrating $GABA_B$ receptor-mediated inhibition of spontaneous EPSCs, and that $GABA_B$ receptors mediate the inhibition of spontaneous IPSCs in the SON. The latter result is consistent with the more recent results reported by the same group (Mouginot, Kombian & Pittman, 1996) that $GABA_B$ receptor activation leads to inhibition of evoked IPSCs via a presynaptic mechanism in the SON. The results showing that $GABA_B$ receptor activation potently inhibits both GABA and glutamate neurons suggest that $GABA_B$ receptors play a major role in the regulation of neuronal activity of the SON, especially as it has been reported that GABA and glutamate neurons account for more than two-thirds of the total presynaptic neurons in the SON area (Meeker *et al.* 1993).

The patterns of inhibition of spontaneous EPSCs and IPSCs by baclofen observed in this study were similar in the minimum effective concentrations, the EC_{50} values, and the time course of the onset and recovery. This suggests that similar mechanisms may be involved in the inhibition of these two synaptic currents. However, the EC_{50} obtained for baclofen-induced inhibition of evoked EPSCs (Kombian *et al.* 1996) was approximately 20 times smaller than that of spontaneous EPSCs obtained in the present study. As the methods for perfusion and drug application used in the study of Kombian *et al.* (1996) and in the present study were similar, the difference in the EC_{50} values suggest that the inhibition by baclofen of spontaneous and evoked EPSCs may be mediated through different mechanisms. This suggestion is supported by the finding that both spontaneous and evoked EPSCs in cultured midbrain neurons were inhibited by baclofen, but a larger inhibition was observed with evoked EPSCs (Jarolimek & Misgeld, 1992). The difference between the mechanisms of inhibition by baclofen on spontaneous and evoked postsynaptic currents seems to be greater for inhibition of IPSCs, since it was observed in hippocampal neurons, that baclofen inhibited evoked IPSCs, but not spontaneous IPSCs, whereas it inhibited both spontaneous and evoked EPSCs (Scanziani *et al.* 1992; Doze *et al.* 1995). Although it has been reported that presynaptic inhibition by $GABA_B$ receptors are due to depression of voltage-dependent Ca^{2+} channels (Pfrieger, Gottmann & Lux, 1994; Dittman & Regehr, 1996), spontaneous (TTX resistant) postsynaptic currents recorded from the hippocampus were insensitive to Ca^{2+} channel blockers (Scanziani *et al.* 1992). Moreover, inhibition of spontaneous postsynaptic currents in midbrain neurons by baclofen was observed even in the presence of 100 μM Cd^{2+} (Jarolimek & Misgeld, 1992), which would be expected to block most of the voltage-dependent Ca^{2+} channels. These results suggest that $GABA_B$ receptor activation could inhibit transmitter release by mechanisms downstream from voltage-dependent Ca^{2+} entry. The suggestion is supported by the result that baclofen inhibited ionomycin-induced synaptic currents in the hippocampus (Capogna, Gähwiler & Thompson, 1996). Such mechanisms could explain the decreased sensitivity of spontaneous EPSCs and IPSCs to baclofen, if the mechanisms were relatively resistant to the inhibition by $GABA_B$ receptors.

The finding that 2OH-saclofen increased the frequency of spontaneous EPSCs and IPSCs without altering the amplitude of the synaptic currents suggests that endogenously released GABA is acting on $GABA_B$ receptors in presynaptic terminals of GABA and glutamate neurons in the SON. That muscimol also decreased the frequency of the spontaneous EPSCs and that picrotoxin and bicuculline potentiated the frequency of the spontaneous EPSCs suggests that glutamate neurons also possess presynaptic $GABA_A$ receptors, and that GABA neurons form synaptic contact on glutamate neurons. Moreover, endogenously released GABA may act on the presynaptic $GABA_A$ receptors, a finding reported in other CNS preparations (Vautrin, Schaffner & Barker, 1994). Our results further show a clear contrast between the results obtained in the terminals of SON neurons (the neurohypophysis) in that $GABA_A$, but no $GABA_B$ receptors are present (Zhang & Jackson, 1995). Although the presence of $GABA_A$ receptors in the presynaptic sites of GABA neurons in the SON is still not clear, the present results suggest that both presynaptic $GABA_A$ and $GABA_B$ receptors function in the regulation of SON neurons.

As the present study is the first report of the properties of spontaneous EPSCs and IPSCs recorded from the thin 'punch-out' slice preparation of the SON, it is important to note the differences between spontaneous postsynaptic currents recorded in the present study and spontaneous postsynaptic currents in previous studies in the SON which used large brain slice preparations (Wuarin & Dudek, 1993). The most obvious difference between these postsynaptic currents is the susceptibility to TTX. Both spontaneous EPSCs and IPSCs recorded from large slices containing the SON and other brain regions showed a marked reduction of both amplitude and frequency in response to TTX (Wuarin & Dudek, 1993). In the present study, both spontaneous EPSCs and IPSCs recorded from the small, thin slice preparation were TTX resistant. Therefore, these results rule out the possibility that $GABA_B$ receptors in regions other than those of presynaptic terminals in the SON, might affect EPSCs and IPSCs through some neural circuit. There are also similarities between both synaptic currents recorded in the large brain slices and the thin 'punch-out' slices. These include the susceptibility of IPSCs to a $GABA_A$ receptor antagonist, and that of EPSCs to a non-NMDA glutamate receptor antagonist. Taken together these observations suggest that the spontaneous synaptic currents recorded in the present study are of a similar nature to the TTX-resistant postsynaptic currents recorded from the large slice preparations (Wuarin & Dudek, 1993).

The marked difference in the amount of TTX-sensitive postsynaptic currents observed between our preparations and the larger brain slices preparations would suggest more $GABA_B$ receptors may be occupied and activated by the endogenous ligand GABA, *in vivo*. This may be the reason that other studies have failed to observe the effect of baclofen in SON neurons (Ogata, 1987; Voisin *et al.* 1996). Alternatively, the difference in not finding functional $GABA_B$ receptors may be related to species or sex difference as guinea-pig SON was used in one study (Ogata, 1987) and the other study used lactating female rats (Voisin *et al.* 1996). The negative finding of $GABA_B$ receptors in SON by Wuarin & Dudek (1993) was based on the fact that slow outward currents indicative of $GABA_B$ receptor activation were not seen in their voltage-clamp experiments. In fact, this is consistent with the present results that showed no slow inward or outward currents upon application of baclofen. These findings are also supported by the observation that baclofen did not change postsynaptic ionic currents other than EPSCs (Kombian *et al.* 1996). Although, $GABA_B$ receptor activation is known to activate K^+ channels in other CNS preparations (Jarolimek & Misgeld, 1992; Thompson & Gähwiler, 1992; Jiang *et al.* 1995), it seems that in the SON, postsynaptic $GABA_B$ receptors are either not present, or not coupled with K^+ channels.

In conclusion, the results of this study provide evidence to suggest that $GABA_B$ receptors are present in the presynaptic terminals of both GABA and glutamate neurons that innervate SON magnocellular neurons of the rat. These data also suggest the presynaptic $GABA_B$ receptors may play an important role in the regulation of presynaptic GABA and glutamate neurons in the SON, and thereby in the control of AVP and OXT release.

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Acknowledgements

We are grateful to Professor John Ciriello (Western Ontario University, Canada) for the helpful comments on the manuscript. This work was supported by Grant-in-Aid for Scientific Research (*A*) and (*B*) from the Ministry of Education, Science, Sports and Culture, Japan, to H.Y. (07507004) and to I.S. (07670071 and 09470020), and Special Grant by the Ministry of Labor for 'Occupational Health Studies' to N.K.

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Received 31 March 1997; accepted 19 June 1997.