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Differential expression of pre- and postsynaptic $GABA_B$ receptors in rat substantia nigra pars reticulata neurones

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Abstract

Whole-cell recordings were made from substantia nigra pars reticulata in rat midbrain slices to study the functional expression of pre- and postsynaptic $GABA_B$ receptors in GABA output neurones. Baclofen (up to 300 μM) dose-dependently activated a weak current which was insensitive to tetrodotoxin and Ca^{2+}-free solution but blocked by Ba^{2+} and 2-OH-saclofen. The maximum current activated by baclofen (30 μM) was 43.0 ± 4.5 pA ($n = 27$), representing only 23% of that in dopamine neurones. Baclofen (1–30 μM) also reduced the frequency of the $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents while the distribution of their amplitudes was unaffected. This presynaptic effect of baclofen, prominent at a concentration as low as 1 μM, was sensitive to 2-OH-saclofen and occluded by Cd^{2+}, but was unaffected by Ba^{2+}. The results suggest a predominant role of the presynaptic $GABA_B$ receptors in substantia nigra pars reticulata. The relative abundance of pre- and postsynaptic $GABA_B$ receptor subtypes in this brain region may also be important in mediating the anticonvulsant effect of baclofen in rats.

Keywords: Substantia nigra; Baclofen; $GABA_B$ receptor

1. Introduction

The substantia nigra pars reticulata is one of the output centers of the basal ganglia circuit. It contains a high concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Ottersen and Storm-Mathisen, 1984). This observation suggests an important function of GABA-mediated neurotransmission in this area of the brain. In fact, GABAergic projections from striatum to substantia nigra pars reticulata, and from pars reticulata to thalamus, superior colliculus and tegmentum, are important in controlling normal voluntary movement (Chevalier and Deniau, 1990; Parent and Hazrati, 1995). The nigrotectal projection has also been found to play a role in controlling the propagation of epileptic seizures (Gale, 1989; Depaulis et al., 1994).

While the role of $GABA_A$ receptors in substantia nigra pars reticulata has been studied extensively (Martin et al., 1978; Grace and Bunney, 1979; Reisine et al., 1979; Wurpel et al., 1988; Sakamoto and Hikosaka, 1989; Marksteiner et al., 1995), the importance of $GABA_B$ receptors in this area has gained attention slowly. Previous studies do suggest a role of $GABA_B$ receptors in both normal and pathological conditions. For example, it has been shown that intranigral administration of baclofen, the prototypic $GABA_B$ receptor agonist, induces turning behaviour in rats (Waddington, 1977; Kaakkola, 1980). It has also been demonstrated that intranigral injection of baclofen suppresses flurothyl seizures in rats in an age-dependent manner (Sperber et al., 1989). It is effective in young (16 days old) rats but is ineffective in adult rats. This finding shows good parallel with the fact that the density of $GABA_B$ receptor binding sites in substantia nigra is greater in young than adult rats (Garrant et al., 1992). It is therefore tempting to hypothesize that the age-dependency of the effect of baclofen on convulsion may be the consequence of changes in expression of $GABA_B$ receptors. However, $GABA_B$ receptors may exist pre- or postsynaptically. Since activation of the presynaptic receptors is likely to lead to a decrease of GABA release and therefore disinhibition of substantia nigra pars reticulata neurones while activation of the postsynaptic receptors would lead to a direct inhibition of these cells, it is necessary to have a knowledge of the relative abundance of these two types of $GABA_B$ receptors.

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In the present study, we make use of the advantage of the patch-clamp technique in resolving the pre- vs. postsynaptic actions of drugs on neurones. We quantified the postsynaptic response of substantia nigra pars reticulata neurones to baclofen, and compared with that of dopamine neurones in the same area. In addition, we tested the existence of presynaptic $GABA_B$ autoreceptors on the inhibitory nerve terminals by examining the effect of baclofen on the $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents. The results confirm the existence of pre- and postsynaptic $GABA_B$ responses in substantia nigra pars reticulata neurones. Furthermore, the data indicate that postsynaptic $GABA_B$ receptors are only weakly expressed, suggesting the dominance of presynaptic $GABA_B$ receptors in mediating effects of $GABA_B$ receptor agonists and antagonists in substantia nigra pars reticulata of young rats.

2. Materials and methods

2.1. In vitro slice preparation

With approval of our Animal Research Ethics Committee, Sprague–Dawley rats aged 2 to 3 weeks were used for the preparation of acute brain slices. The animals were deeply anaesthetized (i.p. injection of 40 mg/kg of sodium pentobarbital) and then sacrificed by decapitation. The brains were rapidly removed and immediately placed in ice-cold artificial cerebrospinal fluid of the following composition (in mM): NaCl 125, KCl 2.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, glucose 11, and $NaHCO_3$ 26, which was continuously bubbled with 95% O_2 and 5% CO_2. Thin coronal slices (200–250 μm) containing the substantia nigra were sectioned using a vibrating microtome. The slices were transferred to a small-volume (0.5 ml) perfusion chamber that was mounted on an upright microscope (Zeiss Axioskop). The slice was weighed down by a nylon grid and was superfused with artificial cerebrospinal fluid at a rate of 1.5–2 ml/min. The artificial cerebrospinal fluid was maintained at a temperature of 34 ± 1°C. Neuronal somata and proximal dendrites of neurones were directly visualized by a combination of differential interference contrast optics and contrast-enhanced infrared video microscopy following the method described by Stuart et al. (1993). The dorsal pars compacta of the substantia nigra consisting a high density of large neurones could be easily identified. Cells were selected in the ventral pars reticulata. Medium- to large-sized neurones (>20 μm in diameter) were selected since small neurones were considered as local circuit neurones (Juraska et al., 1977).

2.2. Electrophysiological recording

Whole-cell patch-clamp recordings from substantia nigra pars reticulata neurones were obtained using a patch-clamp amplifier (LM/PCA, List Medical). Whole cell pipettes typically had a resistance of 4–6 MΩ when filled with an internal solution of the following composition (in mM): K-gluconate 120; KCl 10; EGTA 1.0; $MgCl_2$ 1.0; Na_2-ATP 2.0; HEPES 10. The pH was adjusted to 7.3 with KOH. To study the presynaptic action of baclofen on $GABA_A$ receptor-mediated synaptic currents, K-gluconate was replaced with KCl. The inclusion of 130 mM of KCl in the recording pipettes reversed the polarity of $GABA_A$ receptor-mediated currents from outward to inward and

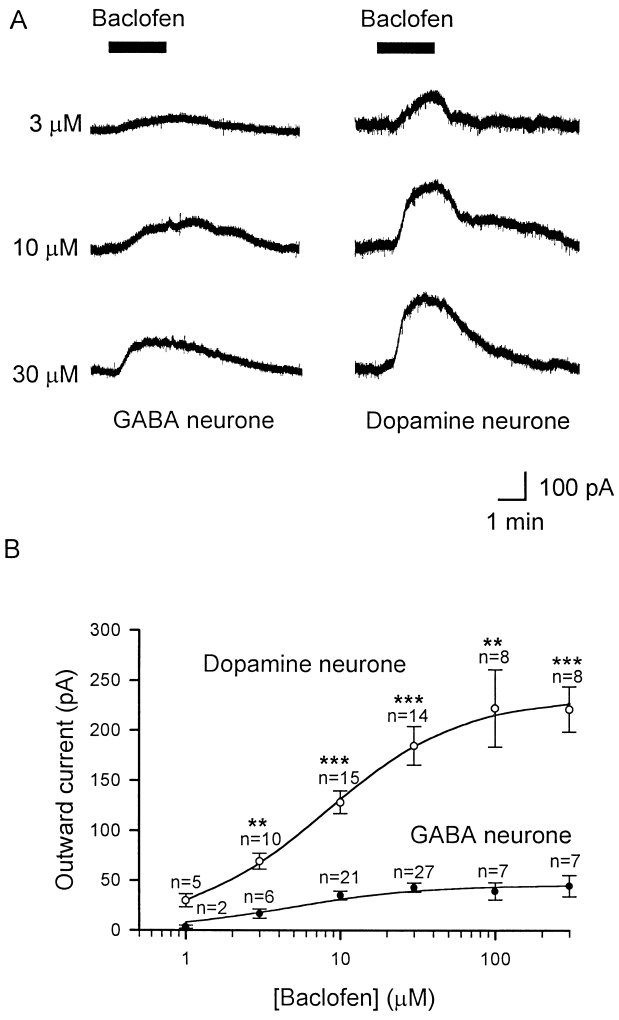

Fig. 1. In voltage-clamp recording, baclofen induced reversible outward currents in both GABA and dopamine neurones. In this example, the two neurones were voltage-clamped at -55 mV. Bath application of baclofen (3, 10 and 30 μM) induced outward currents in a dose-dependent manner. This GABA neuron has an unusually big response to baclofen, which shows more clearly the dose-dependency. However, for the same concentration of baclofen, the outward current was still smaller than that of the typical dopamine neurone. (B) Dose–response curves of baclofen-induced outward currents in both types of neurone. Each neurone was tested with one to three random noncumulative doses of baclofen with washing periods in between. The number in the brackets denotes the sample size. Significant differences in the magnitude of the peak outward currents were found between the two types of neurones for all doses of baclofen greater than 1 μM. ($**$ $P < 0.01$; $***$ $P < 0.001$).

enhanced their detection presumably by increasing the driving force on the Cl ions. To study the postsynaptic effect of baclofen, GTP was freshly dissolved in the internal solution (0.5–1 mM). Monitoring through a television connected to the camera, a pipette was placed on the soma of a pars reticulata neurone and conventional whole-cell recording was made. Normally no series resistance compensation was applied but the cell was rejected if the series resistance increased significantly (>20%) during recording. (±)-2-amino-5-phosphonopentanoic acid (AP5, 20 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) were included to eliminate glutamate-mediated synaptic currents. The current signal was filtered at 3 or 5 kHz and was taped using a DAT recorder (SONY) modified for recording AC and DC signals at a sampling rate of 32 kHz. Pulsed protocols and voltage ramps were generated, and on- and off-line digitization (5 kHz) were made via the CED 1401 plus interface and the Patch and Voltage Clamp software package (Cambridge Electronics Design). A number of neurones were intracellularly labelled by including 1% of biocytin in the pipette solution. The slices were resectioned and tested for tyrosine hydroxylase immunoreactivity as described before (Yung et al., 1991).

2.3. Analysis of synaptic currents

Computer files generated by the CED software containing information of synaptic currents were analysed by a program developed in our laboratory. This program uses a simple detection algorithm similar to that described in detail by Vincent and Marty (1993). Essentially, a synaptic event is detected when the difference between the average current values of two brief periods of time, separated by a gap, exceeds a threshold. The duration of the two time periods and the gap are adjustable. Once a synaptic current is detected, information on the time of occurrence, peak amplitude and kinetics are generated automatically. Other functions of the program include fitting of the decay of a synaptic current with a mono-exponential function, generation of histogram and cumulative probability distributions, and statistical comparison of two cumulative probability distributions using the Kolmogorov–Smirnov test. All detected events with the computed parameters are allowed visual inspection before acceptance.

2.4. Drugs and statistics

GTP was purchased from Sigma. All other drugs used were obtained from Research Biochemicals International. (±)-Baclofen was used for all experiments and will be referred to as baclofen. Dose–response curves were fitted to a sigmoidal function by SigmaPlot (Jandel Scientific) and the EC_{50} estimated from the equation. Numerical data are expressed as mean ± S.E.M. Kolmogorov–Smirnov test was used to compare two distributions of synaptic current interevent intervals or amplitudes using a probabil-

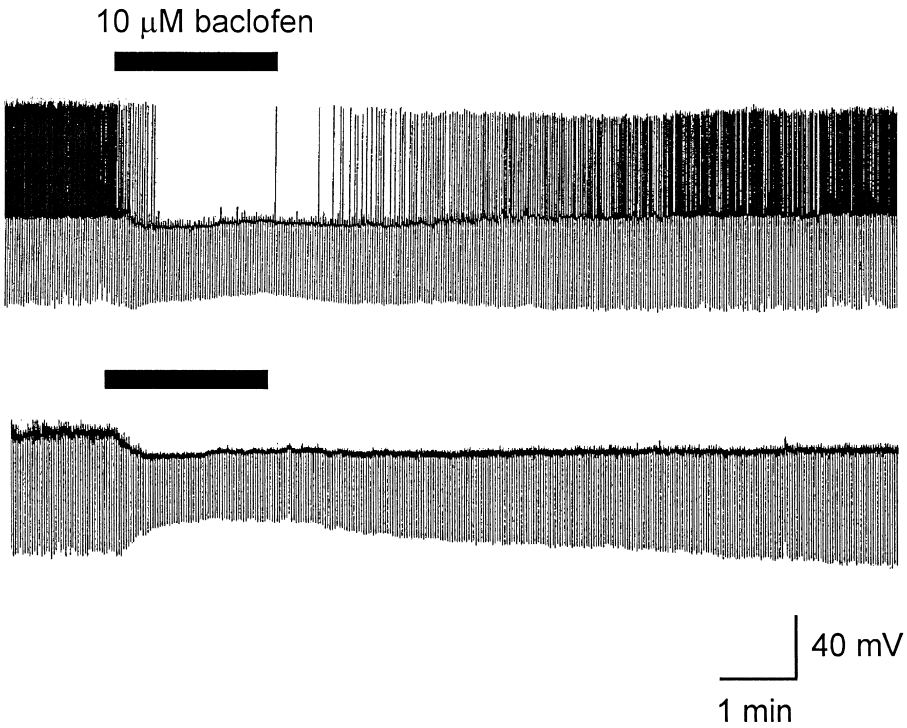

Fig. 2. Responses of a GABA neurone (upper trace) and a dopamine neurone (lower trace) to bath application of 10 μM of baclofen. The membrane potentials of both neurones were hyperpolarized and the firing of the GABA neurone was inhibited. The hyperpolarizations in both neurones were accompanied by a decrease in the input resistance as indicated by the reduced voltage responses to 0.2 nA of hyperpolarizing current injections (150 ms in every 2 s). These responses were typical in that the magnitude of hyperpolarization and decrease in input resistance of the dopamine neurone were bigger than that of the GABA neurone. Both neurones were current-clamped at −55 mV.

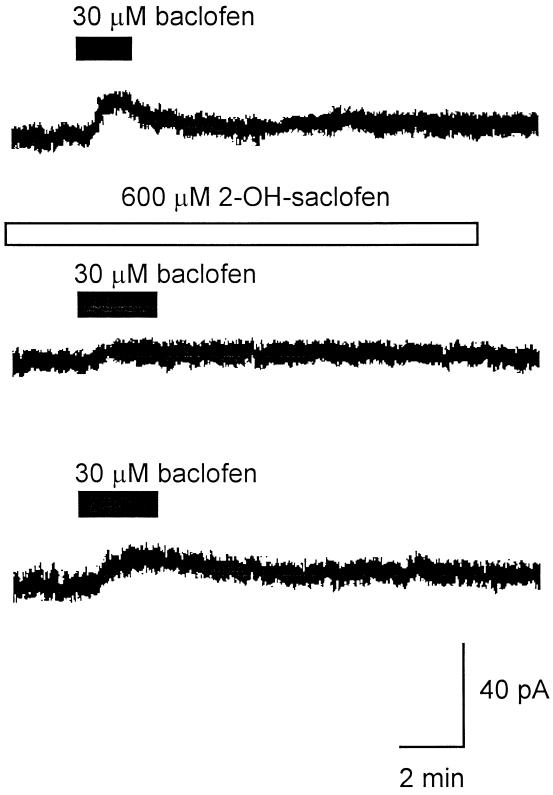

Fig. 3. In this substantia nigra pars reticulata GABA neurone, the response to a saturating concentration (30 μM) of baclofen (A) was reduced by 600 μM of 2-OH-saclofen (B) which was pre-incubated for 5 min. (C) The effect of 2-OH-saclofen was washed out. These results suggest the involvement of $GABA_B$ receptor in mediating the action of baclofen. The neurone was held at -55 mV.

ity (P) threshold of 0.01. Otherwise Student's t-test was used using a P value of 0.05.

3. Results

3.1. Identification of GABA and dopamine neurones

It is known that substantia nigra pars reticulata contains mainly GABA neurones but also some dopamine neurones (e.g., Yung et al., 1991). The latter are generally believed to be neurones displaced from the dorsal pars compacta. In the present study, the distinction between dopamine and non-dopamine, putative GABA neurones was based on the well-documented electrophysiological characteristics of dopamine cells (Grace and Onn, 1989; Yung et al., 1991; Häusser et al., 1995). Briefly, dopamine neurones were characterized by a prominent sag of the membrane potential during a large (>30 mV) hyperpolarization (average steady-state to peak ratio $= 0.52 \pm 0.03$, $n = 7$), broad action potentials (>3 ms) and low firing rate at rest (mean $= 2.7 \pm 0.8$ Hz, $n = 7$). In fact, 12 out of 31 (38.7%) dopamine neurones were silent. In contrast, presumed GABA neurones displayed a modest degree of sag (average steady-state to peak ratio $= 0.88 \pm 0.02$, $n = 16$), relatively brief action potential (<2.5 ms) and substantially higher firing frequency (8.2 ± 1.0 Hz, $n = 6$). In agreement with previous reports (Grace and Onn, 1989; Yung et al., 1991), the former type of neurones always showed positive reaction to immunostaining with antibodies against tyrosine hydroxylase and could be retrogradely labelled by microinjection of fluorescent microspheres into the striatum. Presumed GABA neurones were consistently negative in these tests. These data are not shown. The results described in the present report were obtained from 91 putative GABA output neurones and from 36 dopamine neurones.

3.2. Postsynaptic $GABA_B$ receptors in pars reticulata neurones

Quantification of the postsynaptic effect of baclofen was made in voltage-clamp recording, in which a small outward current was activated in pars reticulata neurones clamped at -55 mV (Fig. 1A). The response was reversible within minutes when baclofen-free artificial cerebrospinal fluid was reperfused. Since the expression of currents mediated by postsynaptic $GABA_B$ receptors has been shown to be G-protein-dependent (Thompson and Gähwiler, 1992; Sodickson and Bean, 1996), GTP was always included in the recording pipette. To demonstrate

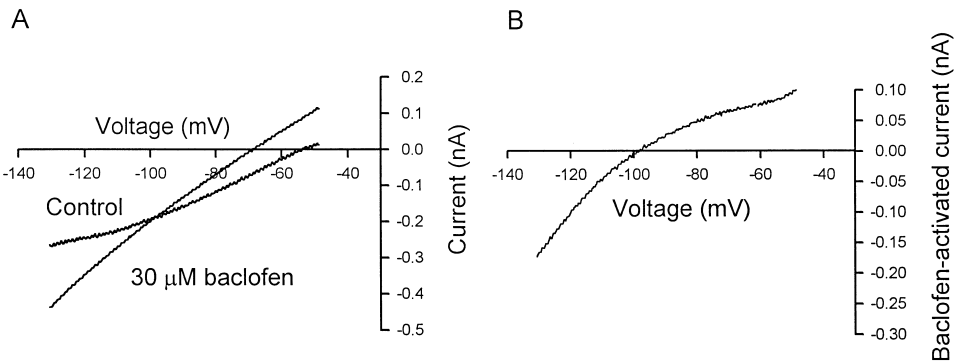

Fig. 4. (A) Current–voltage relationship in the absence and presence of baclofen was determined by giving a voltage ramp (from -140 mV to -40 mV, lasting 100 ms) to the neurone. The resulting currents were each averaged from 20 traces. (B) The baclofen-induced current was obtained by subtraction of the traces shown in (A). In this cell, the reversal potential was -98 mV.

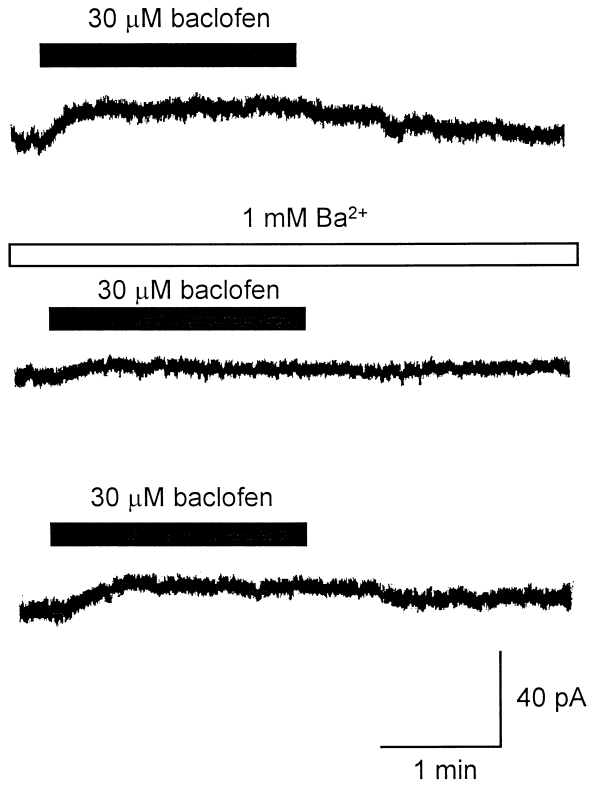

Fig. 5. The small outward current activated by 30 μM baclofen (upper trace) in this neurone was reduced by preincubation of 1 mM of Ba^{2+} in the extracellular medium (middle trace). Ba^{2+} itself caused an apparent inward current most likely due to blockade of some resting K^+-permeability. The response to baclofen partially recovered when Ba^{2+} was taken away from the artificial cerebrospinal fluid (lower trace). The neurone was clamped at -55 mV.

that the weak expression of baclofen-activated current in pars reticulata GABA neurones was not due to dilution of other critical cytoplasmic components by the internal solution, the postsynaptic action of baclofen on dopamine neurones in pars reticulata was also studied under identical experimental conditions. It was found that dopamine neurones expressed a more robust response to baclofen. Fig. 1B shows the concentration dependency of the effect of baclofen. A total of 61 GABA and 34 dopamine neurones were studied. The results reveal that the magnitude of the outward current activated by baclofen was consistently smaller in GABA neurones. For example, at 30 μM of baclofen, the average outward current activated in GABA neurones was 43.0 ± 4.5 pA ($n = 27$), significantly smaller than that of dopamine neurones (184.9 ± 19.3 pA, $n = 14$, $P < 0.001$). From the dose–response curve, the EC_{50} was estimated to be 4.5 μM for GABA neurones and 9.2 μM for dopamine neurones.

In current-clamp recordings, application of baclofen (3–30 μM) produced a dose-dependent and reversible membrane hyperpolarization in pars reticulata neurones at their resting membrane potentials (13 GABA neurones and 10 dopamine neurones). Consistent with the voltage-clamp data, the response of dopamine neurones to baclofen was bigger than that of GABA neurones. Examples of the responses of the two types of cells to 10 μM of baclofen are shown in Fig. 2. Although baclofen at this concentration could typically inhibit the firing of GABA neurones, the accompanying membrane hyperpolarization and decrease in input resistance were always small. In six GABA cells, baclofen at 10 μM caused a 14.4 ± 1.0% decrease in input resistance (control: 445 ± 67 MΩ; after baclofen: 381 ± 59 MΩ). On the other hand, the same concentration of baclofen caused a 44.5 ± 7.5% (control: 328 ± 38 MΩ; after baclofen: 182 ± 28 MΩ) decrease in the input resistance in five dopamine neurones.

The outward current activated by baclofen was not sensitive to preincubation with tetrodotoxin (control: 54.1

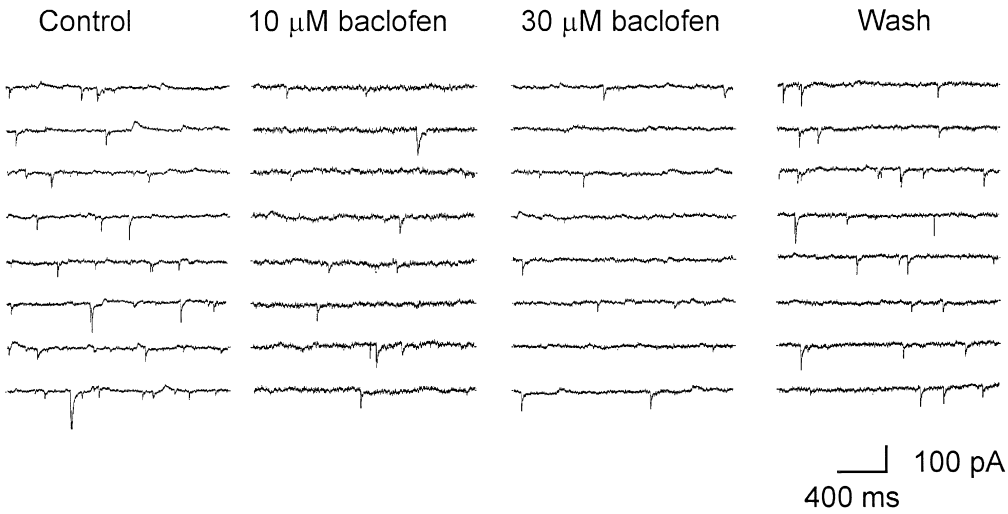

Fig. 6. Miniature inhibitory postsynaptic currents were recorded in the presence of tetrodotoxin (control), 10 μM baclofen, 30 μM baclofen and washout. Baclofen dose-dependently reduced the frequency of the currents. The effect was reversible when baclofen was washed out. In this GABA neurone, the membrane potential was clamped at -55 mV using a high chloride (130 mM) electrode. AP5 (20 μM) and CNQX (20 μM) were included in the artificial cerebrospinal fluid throughout the experiment.

$\pm$ 8.4 pA; tetrodotoxin: 47.0 $\pm$ 9.2 pA, $P > 0.05$, $n = 7$) or with Ca^{2+}-free artificial cerebrospinal fluid supplemented with 10 mM of Mg^{2+} (control: 46.0 $\pm$ 11.0 pA; Ca^{2+}-free: 42.8 $\pm$ 12.6 pA, $P > 0.05$, $n = 5$). These observations indicate that baclofen activated the outward current by a direct postsynaptic action. That the response to baclofen was mediated by $GABA_B$ receptor was supported by the antagonistic action of 2-OH-saclofen, a commonly used $GABA_B$ receptor antagonist. When preincubated for at least 3 min, 2-OH-saclofen reduced the current activated by 30 μM of baclofen (Fig. 3). The mean values in the absence (34.7 $\pm$ 3.3 pA) and presence (13.5 $\pm$ 1.9 pA) of 600 μM of 2-OH-saclofen were significantly different ($P < 0.001$, $n = 6$). However, even at this high concentration, 2-OH-saclofen could not completely eliminate the response to baclofen. In addition, 2-OH-saclofen when applied alone sometimes activated a small but observable outward current.

The involvement of K^+-channels in mediating the effect of baclofen was studied by determining the reversal potential of the current and the effect of extracellular Ba^{2+}. Fig. 4 shows the result of a typical experiment in which a 100-ms ramp pulse from -140 mV to -40 mV was applied to a GABA neurone during control and application of 30 μM of baclofen. The two current–voltage relationships are plotted in Fig. 4A and the difference between the two curves is plotted in Fig. 4B. Baclofen-activated current exhibited a clear inward rectification. In this cell, the reversal potential of the baclofen-activated current was found to be -98 mV. The average value obtained from five cells was -90.4 ± 8.4 mV, which was close to the calculated equilibrium potential of K^+. Fig. 5 shows the result of an experiment in which the current activated by 30 μM of baclofen was significantly reduced by adding 1 mM Ba^{2+} in the extracellular medium. In five cells tested, the control response to baclofen was 32.7 $\pm$ 3.7 pA. In the presence of Ba^{2+}, the current was reduced to 7.3 $\pm$ 1.6 pA ($P < 0.001$).

3.3. Presynaptic $GABA_B$ receptors in pars reticulata

When using Cl^- (130 mM) as the major anion in the internal solution, most of the GABA neurones displayed spontaneous inward going synaptic currents in the presence of the glutamate receptor antagonists AP5 (20 μM) and CNQX (20 μM). As reported before (Ye et al., 1997), these spontaneous postsynaptic currents were sensitive to a low concentration of the $GABA_A$ receptor antagonist bicuculline (1 μM) and reversed polarity at a potential close to the equilibrium potential of Cl ion. Thus, they were reversed inhibitory postsynaptic currents. The frequencies and average amplitudes of these spontaneous currents varied widely from one cell to another. Many of them were action potential dependent, as indicated by the effect of adding 0.5 to 1 μM of tetrodotoxin which eliminated an average of 61% of the events (control: 3.82 $\pm$ 0.84 Hz; tetrodotoxin: 1.50 $\pm$ 0.24 Hz, $n = 25$). The remaining synaptic currents are referred to as miniature inhibitory postsynaptic currents and presumably represent basal, quantal release of GABA from nerve terminals which synapse on the GABA neurones.

When baclofen (1–30 μM) was applied in the superfusion medium, the frequency of occurrence of the miniature inhibitory postsynaptic currents was quickly reduced. An example is shown in Fig. 6 in which 10 and 30 μM of baclofen strongly reduced the frequency of the inhibitory postsynaptic currents of a pars reticulata neurone. This effect was readily reversible when baclofen was taken out (Fig. 6) or when 2-OH-saclofen (400 to 600 μM) was applied in the presence of baclofen.

To analyse the effect of baclofen on the distribution of the frequency and amplitudes of the miniature inhibitory postsynaptic currents, histograms of the interevent intervals and peak amplitudes were constructed. As illustrated

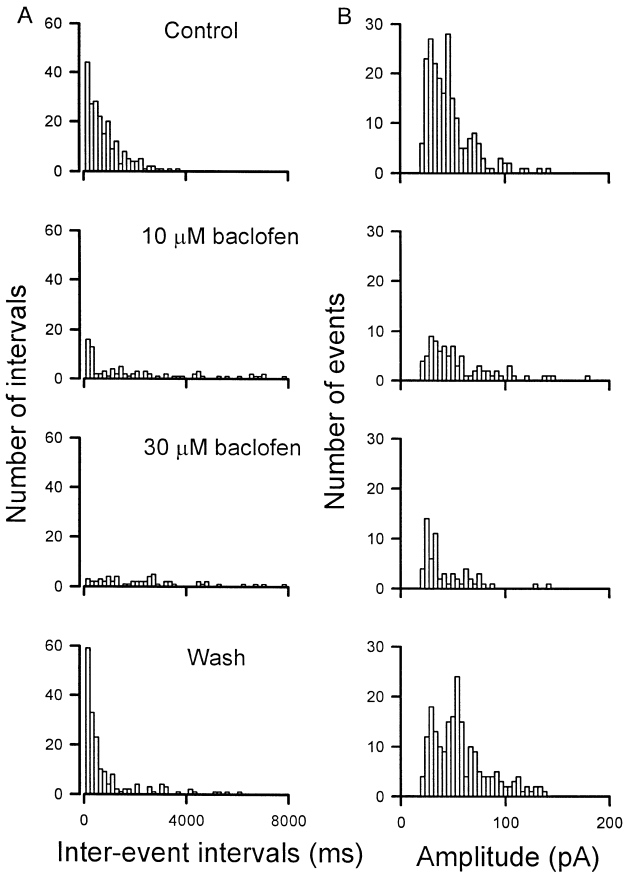

Fig. 7. (A) Histogram distributions of the interevent-intervals of the miniature inhibitory postsynaptic currents in control, 10 μM baclofen, 30 μM baclofen and washout. Baclofen increased the frequency of occurrence of longer intervals thus distorting the shape of the distribution. The number of events analysed were 215 for control, 84 for 10 μM baclofen, 62 for 30 μM baclofen and 200 for wash. The binwidth used was 160 ms. Intervals longer than 800 ms were not plotted. (B) On the other hand, despite a decrease in the total number of events, the shape of distribution of the amplitudes was not affected by baclofen. The bin width used was 4 pA. These effects of baclofen on the distributions were clearly reversible.

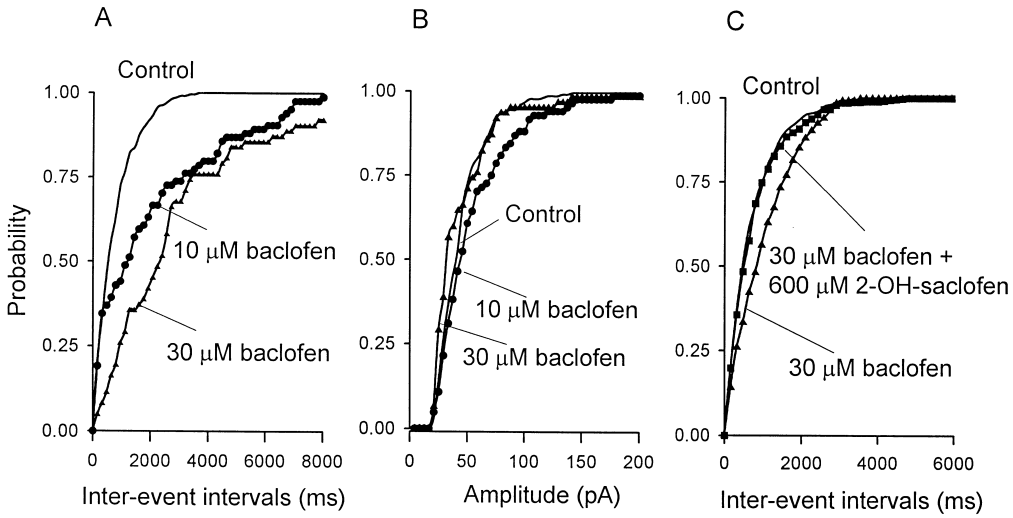

Fig. 8. (A) Cumulative probability of interevent intervals using the same set of data shown in Fig. 7A. Baclofen at 10 and 30 μM shifted the trace to the right indicating an increase in the mean interevent intervals. These distributions were significantly different from the control ($P < 0.01$, Kolmogorov–Smirnov test). (B) The amplitudes in the presence of 10 and 30 μM baclofen did not show a large and consistent deviation from the control ($P > 0.05$, Kolmogorov–Smirnov test). (C) In another cell, 2-OH-saclofen (600 μM) antagonized the effect of baclofen.

in Fig. 7A, 10 and 30 μM of baclofen reduced the total number of miniature inhibitory postsynaptic currents occurred in a period of time and therefore shifted the interevent intervals to longer values. However, despite a reduction in frequency, the distributions of the peak amplitudes were unaffected (Fig. 7B). Fig. 8A and B show the cumulative probability distributions generated from the same set of data shown in Fig. 7. The Kolmogorov–Smirnov test showed that there was no statistical difference ($P > 0.05$) among the amplitude distributions but a significant difference ($P < 0.001$) was found among the interevent interval distributions. Out of 25 cells tested, statistical differences between the frequency distributions ($P < 0.01$) were found in 22 cells treated with 1–30 μM of baclofen. In contrast, statistical differences in the amplitude distributions were found in seven cells only. The amplitudes in four cells were increased but decreased in the remaining three cells. The inhibitory effect of baclofen on the synaptic current frequency was also antagonized by 2-OH-saclofen. A typical example is illustrated in Fig. 8C. The shift in the interevent intervals towards longer value in the presence of baclofen was reversed by 600 μM of 2-OH-saclofen. In a total of five cells tested, the mean frequency of the miniature inhibitory postsynaptic currents before application of baclofen was 1.13 ± 0.20 Hz, which was reduced by 30 μM of baclofen to 0.47 ± 0.16 Hz ($P < 0.01$). 2-OH-saclofen at 600 μM restored the frequency to 0.78 ± 0.22 Hz ($P < 0.05$, compared to baclofen alone; $P > 0.1$, compared to control).

In contrast to the postsynaptic effect of baclofen, the ability of baclofen in reducing the frequency of miniature inhibitory postsynaptic currents was clearly seen at concentrations as low as 1 μM, achieving an inhibition of $50.0 \pm 5.4\%$ ($n = 4$). Inhibition of the frequency of synap-

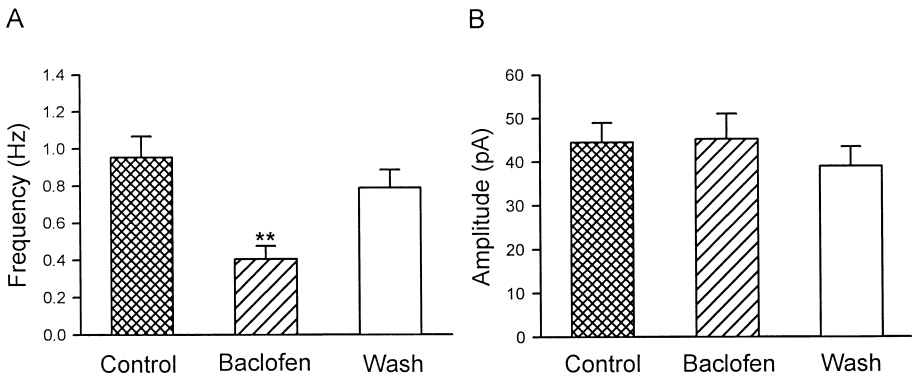

Fig. 9. Mean values obtained from 10 neurones with wash revealed that baclofen significantly reduced the frequency (A) but not the amplitude (B) of the miniature inhibitory postsynaptic currents (** $P < 0.01$, Student's paired t-test). The responses to 10–30 μM of baclofen were pooled.

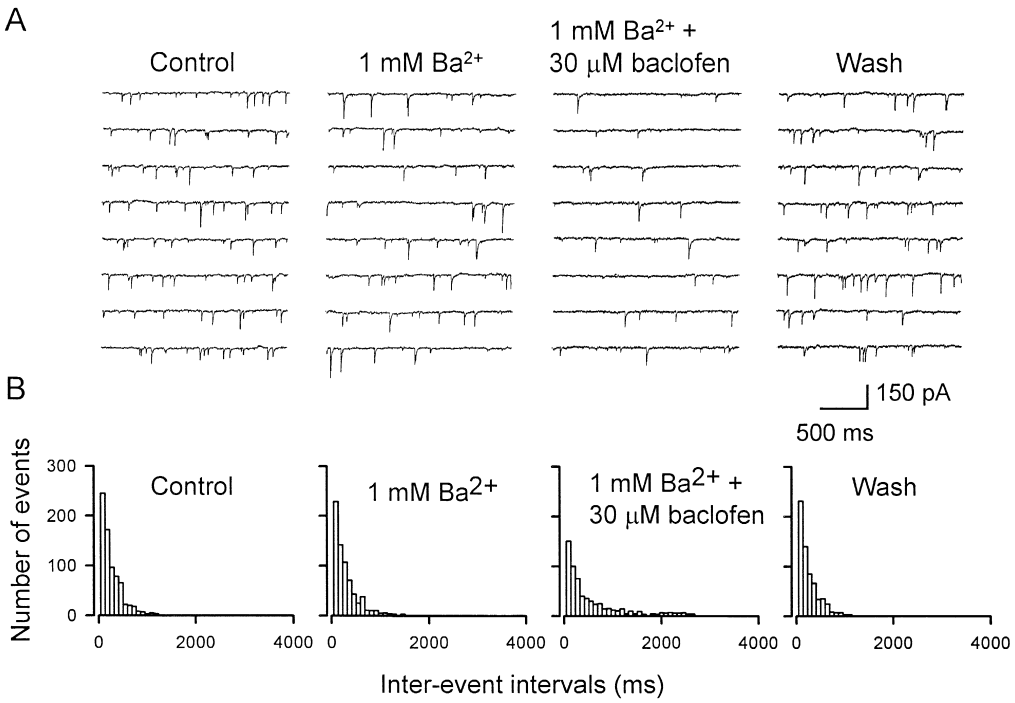

Fig. 10. (A) Typical recordings showing that 1 mM of Ba^{2+} did not affect the inhibitory action of baclofen on the miniature inhibitory postsynaptic currents recorded in a pars reticulata neurone. In the presence of 1 mM of Ba^{2+}, baclofen (30 μM) still caused a significant and reversible decrease in the frequency. (B) Histogram distributions of the interevent intervals. The sampling periods were 170 s for control, 184 s for Ba^{2+}, 222 s for Ba^{2+} plus baclofen and 144 s for wash. Note that in the presence of baclofen, a longer period was analysed to capture more events.

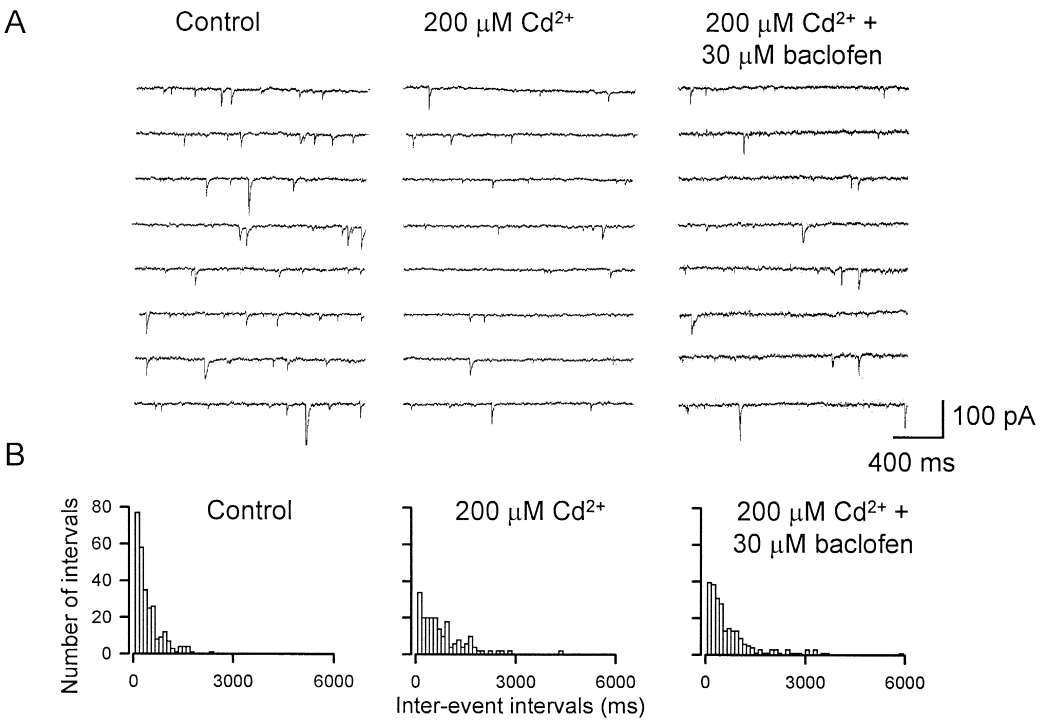

Fig. 11. (A) In this set of typical recordings from a pars reticulata GABA neurone, Cd^{2+} (200 μM) caused a significant reduction in the number of tetrodotoxin-resistant inhibitory postsynaptic currents. In the presence of Cd^{2+}, a saturating concentration of baclofen (30 μM) did not further reduce the miniature inhibitory postsynaptic current frequency indicating that its effect was largely occluded by Cd^{2+}. (B) Histogram distributions of the interevent intervals in the corresponding test periods shown in (A). The sampling periods were 104 s for control, 164 s for Cd^{2+} and 166 s for Cd^{2+} plus baclofen.

tic currents at other concentrations of baclofen were: 56.0 ± 8.0% (3 μM, $n = 4$); 62.6 ± 4.9% (10 μM, $n = 5$); 62.3 ± 5.5% (30 μM, $n = 7$). Fig. 9 summarized the presynaptic effect of baclofen on 10 pars reticulata GABA neurones that were stable enough to allow the effect of wash to be recorded. The effect of baclofen was clearly selective on the frequency but not the amplitudes. The data were pooled from results using 10 and 30 μM of baclofen, as 10 μM of baclofen is already saturating.

To test the involvement of potassium and calcium channels in the presynaptic effect of baclofen, the effects of Ba^{2+} and Cd^{2+} were also examined. It was found that 1 mM of Ba^{2+}, which largely blocked the baclofen-activated postsynaptic outward current, could not reverse the inhibitory action of baclofen on the miniature inhibitory postsynaptic current frequency (control: 1.62 ± 0.19 Hz; baclofen: 0.72 ± 0.16 Hz; plus Ba^{2+}: 0.59 ± 0.13 Hz, $n = 5$). Similarly, preincubation with 1 mM of Ba^{2+} cannot prevent the inhibitory effect of baclofen (control: 2.18 ± 0.98 Hz; Ba^{2+}: 1.64 ± 0.62 Hz; plus baclofen: 0.78 ± 0.55 Hz, $n = 5$). Ba^{2+} itself did not have a consistent and significant effect on the miniature inhibitory postsynaptic current frequency ($P > 0.05$) while baclofen still caused a significant reduction ($P < 0.05$). An example is shown in Fig. 10. On the other hand, in five other cells tested, 200 μM of Cd^{2+} itself reduced the synaptic current frequency from 2.35 ± 0.75 Hz to 0.99 ± 0.20 Hz. In the presence of Cd^{2+}, baclofen at 30 μM reduced the frequency to 0.75 ± 0.20 Hz (19.9 ± 13.9%; $P > 0.05$). A set of typical data is shown in Fig. 11. In contrast, baclofen at the same concentration alone in seven other cells caused a 62.3 ± 5.5% inhibition in the frequency.

4. Discussion

4.1. Postsynaptic $GABA_B$ receptors are weakly expressed in pars reticulata

Baclofen even at a saturating concentration (≥ 30 μM) can only activate a small outward current in substantia nigra pars reticulata GABA neurones at their resting membrane potentials. Nevertheless, the EC_{50} obtained in the present study (4.5 μM and 9.2 μM for GABA and dopamine neurones respectively) is comparable to the value of 3 μM reported in a recent study on dissociated hippocampal CA3 neurones using the more active (−)-baclofen (Sodickson and Bean, 1996). The properties of the baclofen-activated postsynaptic current are also consistent with those reported in other brain areas (Gähwiler and Brown, 1985; Misgeld et al., 1995; Sodickson and Bean, 1996). On the other hand, dopamine neurones in pars reticulata expressed a more robust response to baclofen. It is possible that differences in the dialysis properties between the two types of cells may affect the relatively availability of GTP and therefore underlie the apparent differences in their responses to baclofen. Alternatively, a simple explanation of the much weaker current in GABA neurones is that fewer $GABA_B$ receptors are expressed. This conclusion would suggest that, in the GABA neurones of pars reticulata, fast postsynaptic $GABA_A$ receptor-mediated inhibition plays a more important role than that mediated by $GABA_B$ receptors. The result is consistent with the observation that spontaneous and evoked synaptic currents recorded in these cells are dominated by the fast, $GABA_A$ receptor-mediated inhibitory postsynaptic currents (Stanford and Lacey, 1996; Leung et al., 1996; Ye et al., 1997).

4.2. Presynaptic $GABA_B$ receptors dominate in pars reticulata

The presynaptic effect of baclofen is demonstrated by its ability to selectively reduce the frequency of miniature inhibitory postsynaptic currents without a major effect on the distribution of the amplitudes. In contrast to the postsynaptic action, this effect is prominent even at a concentration of baclofen as low as 1 μM. The results are therefore consistent with experiments on rat-brain slices (Floran et al., 1988) and synaptosomes (Giralt et al., 1990) showing that the release of GABA from nerve terminals in pars reticulata is sensitive to baclofen. Stanford and Lacey (1996) demonstrated that baclofen not only reduced the magnitude of evoked inhibitory postsynaptic currents in pars reticulata neurones but also reduced the amount of paired-pulse depression. The latter action has generally been accepted as an indication of a presynaptic origin, and is believed to be a consequence of reduced transmitter release (Stuart and Redman, 1991). However, the mechanism of this phenomenon has not been further elucidated. Our results therefore extend the above findings by directly demonstrating that the basal release of GABA in pars reticulata is also modulated by presynaptic $GABA_B$ autoreceptors.

The GABA inputs to the substantia nigra pars reticulata neurones are heterogeneous. It is known that inhibitory synaptic inputs originate not only from the striatum but also from the globus pallidus (Smith and Bolam, 1989), neighbouring pars reticulata GABA neurones (Rick and Lacey, 1994) and interneurones (Nitsch and Riesenberg, 1988). Since it has been estimated that the striatonigral terminals contributed about 80% of spontaneously released GABA (Lantin Le Boulch et al., 1991), it is reasonable to expect that a significant portion of the presynaptic $GABA_B$ receptors are on these terminals. Thus, these receptors could be important in controlling activity-dependent GABA release, if any, from the striatonigral terminals.

Although an inhibitory effect of baclofen on tetrodotoxin-resistant inhibitory postsynaptic currents has been demonstrated in a number of cell types including thalamic neurones (Ulrich and Huguenard, 1996; Le Feuvre et al., 1997), cultured midbrain neurones (Jarolimek

and Misgeld, 1992; Rohrbacher et al., 1997) and hippocampal CA1 neurones (Doze et al., 1995; Jarolimek and Misgeld, 1997), the mechanism of this process is still unsettled, and may vary from one synapse to another (Thompson and Gähwiler, 1992; Doze et al., 1995; Rohrbacher et al., 1997). In the present study, the lack of effect of Ba^{2+} on the action of baclofen indicates that opening of K^+-channels is not involved. On the other hand, in the presence of Cd^{2+}, the inhibitory effect of baclofen was largely occluded. Under this condition, baclofen could only reduce the frequency of the miniature inhibitory postsynaptic currents by 20%, much less than the 62% inhibition when baclofen was used alone. Thus, the data suggest that baclofen reduces the frequency of these synaptic currents by inhibition of Cd^{2+}-sensitive Ca^{2+}-channels. Inhibition on the release process downstream of Ca^{2+}-influx may play a minor role only. Since the basal release of GABA was reduced significantly by Cd^{2+} itself and not Ba^{2+}, we conclude that the miniature inhibitory postsynaptic currents recorded were to a large extent dependent on resting Ca^{2+}-influx and that baclofen reduced them by mainly inhibiting this process. This mechanism may also contribute to the presynaptic effect of baclofen on evoked and spontaneous, action potential-dependent inhibitory postsynaptic currents observed by Stanford and Lacey (1996). The pars reticulata neurones are different from the midbrain cultured neurones (Rohrbacher et al., 1997) and neurones in the thalamic sensory nuclei (Le Feuvre et al., 1997) in which the inhibitory effects of baclofen on tetrodotoxin-resistant currents are mainly due to inhibition of a mechanism downstream of Ca^{2+}-influx. This situation is also in contrast to the result shown by Doze et al. (1995) in hippocampal CA1 neurones that a Cd^{2+}-sensitive mechanism was observed only after facilitation of miniature inhibitory postsynaptic currents by raising the extracellular concentration of K^+. Nevertheless, the data are consistent with the notion that the effect of baclofen is stronger when Ca^{2+} influx contributes to the release of transmitter (Misgeld et al., 1995).

4.3. Relation to $GABA_B$ binding sites and epileptic seizures

Previous studies by autoradiographic techniques (Bowery et al., 1987; Chu et al., 1990) have shown that binding sites for baclofen in substantia nigra pars reticulata are always higher in density than that in the pars compacta. However, a recent study on the cloning of $GABA_B$ receptor (Kaupmann et al., 1997) showed that $GABA_B$ receptor mRNA is highly expressed in the pars compacta and much less so in pars reticulata. These data suggest that the $GABA_B$ binding sites in pars reticulata may originate from postsynaptic $GABA_B$ receptors located on the dendrites of the pars compacta dopamine cells or from presynaptic $GABA_B$ receptors on nerve terminals projecting from other brain areas. The present finding that the GABA neurones expressed only a weak postsynaptic $GABA_B$ response is consistent with these scenarios.

It is well known that inhibitory synaptic inputs are the major factor in controlling the activities of substantia nigra pars reticulata neurones. Since a saturating concentration of baclofen can only produce a weak postsynaptic current in these neurones while a low concentration ($< 3\ \mu M$) already reduces the frequency of the miniature inhibitory postsynaptic currents by half, the present study suggests that the presynaptic receptors may be the major subtype of $GABA_B$ receptors mediating the anti-convulsant effect of baclofen in young rats. However, it is obvious that in order to better define the role of $GABA_B$ receptors in epileptic seizures, the expression of both pre- and postsynaptic $GABA_B$ receptors in different ages need to be examined.

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