Full-length article

Tonic activation of presynaptic $GABA_B$ receptors on rat pallidosubthalamic terminals[1]

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Key words

GABA-B receptor; baclofen; CGP55845; presynaptic inhibition; subthalamic nucleus; globus pallidus

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Abstract

Aim: The subthalamic nucleus plays a critical role in the regulation of movement, and abnormal activity of its neurons is associated with some basal ganglia motor symptoms. We examined the presence of functional presynaptic $GABA_B$ receptors on pallidosubthalamic terminals and tested whether they were tonically active in the *in vitro* subthalamic slices. **Methods:** Whole-cell patch-clamp recordings were applied to acutely prepared rat subthalamic nucleus slices. The effects of specific $GABA_B$ agonist and antagonist on action potential-independent inhibitory postsynaptic currents (IPSCs), as well as holding current, were examined. **Results:** Superfusion of baclofen, a $GABA_B$ receptor agonist, significantly reduced the frequency of $GABA_A$ receptor-mediated miniature IPSCs (mIPSCs), in a Cd^{2+}-sensitive manner, with no effect on the amplitude, indicating presynaptic inhibition on GABA release. In addition, baclofen induced a weak outward current only in a minority of subthalamic neurons. Both the pre- and post-synaptic effects of baclofen were prevented by the specific $GABA_B$ receptor antagonist, CGP55845. Furthermore, CGP55845 alone increased the frequency of mIPSCs, but had no effect on the holding current. **Conclusion:** These findings suggest the functional dominance of presynaptic $GABA_B$ receptors on the pallidosubthalamic terminals over the postsynaptic $GABA_B$ receptors on subthalamic neurons. Furthermore, the presynaptic, but not the postsynaptic, $GABA_B$ receptors are tonically active, suggesting that the presynaptic $GABA_B$ receptors in the subthalamic nucleus are potential therapeutic target for the treatment of Parkinson disease.

Introduction

Being the only nucleus in the basal ganglia containing glutamatergic neurons, the subthalamic nucleus occupies a critical position in the 'indirect' pathway by providing an excitatory drive to the output nuclei of this motor circuit. Anatomical studies have shown that the subthalamic nucleus receives GABAergic innervation from the globus pallidus and glutamatergic innervation from the cortex as well as the thalamus. The subthalamic nucleus then sends glutamatergic projection back to the globus pallidus, and to the substantia nigra pars reticulata and entopeduncular nucleus[1-7].

By influencing the output of the basal ganglia, the subthalamic nucleus plays a significant role in mediating movement in health and in diseased state. It has been demonstrated that the modification of the activity of subthalamic nucleus neurons constitutes the central origin of parkinsonian symptoms. For example, in Parkinson disease and its animal models, it is widely believed that depletion of dopamine in the basal ganglia leads to overactivity of the subthalamic nucleus. The resulting increased glutamatergic output of the subthalamic nucleus contributes to excessive inhibition of basal ganglia targets leading to akinesia and hypokinetic symptoms[8]. Recent studies on the firing properties of neurons from organotypic culture of the globus pallidus-subthalamic nucleus network[9], and from the *in vivo* brain[10,11] suggest that the reciprocally connected glutamatergic subthalamic and GABAergic pallidal neurons

are involved in the generation of low-frequency oscillatory activity in Parkinson disease, which is associated with tremor in parkinsonian subjects[12]. Indeed, disruption of the activity of the subthalamic nucleus could alleviate both the pathological neuronal activity and motor symptoms observed in Parkinson disease[13-15]. Thus, deep brain stimulation of the subthalamic nucleus has been introduced as a surgical procedure for the treatment of Parkinson disease[16].

GABAergic innervation from the globus pallidus is the major inhibitory factor affecting the activity of the subthalamic nucleus. GABAergic input from the globus pallidus affects the oscillation frequency of burst firing cells in the subthalamic nucleus[17]. There are two types of GABA receptors in the central nervous system: the ionotropic, bicuculline-sensitive $GABA_A$ receptors and the metabotropic, G-protein coupled $GABA_B$ receptors which are activated by baclofen. By inhibiting calcium influx and facilitating potassium conductance, activation of $GABA_B$ receptors produces pre- and postsynaptic inhibitory effects, respectively. A previous report [18] showed the presence of presynaptic $GABA_B$ receptors in the subthalamic nucleus based on the changes of the paired-pulse ratio of evoked IPSCs. In the current study, the presence of presynaptic $GABA_B$ receptors is studied by examining the direct effect of a $GABA_B$ agonist on the constitutive release of GABA from the presynaptic terminals, with the aid of a more specific $GABA_B$ antagonist. Because the differential activation of pre- and postsynaptic $GABA_B$ receptors is a potential therapeutic strategy in the treatment of basal ganglia motor disorders, we also compared the degree of activation of the pre- and postsynaptic $GABA_B$ receptors and tested whether they were tonically active.

Materials and methods

Brain slice preparation Sprague-Dawley rats aged 13–15 d were used for the preparation of acutely prepared brain slices. The animals were killed by decapitation. The brains were then immediately removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mmol/L): 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 hemicoronal slices (250 μm) containing the subthalamic nucleus were sectioned using a vibrating microtome (Camden Instrument). After equilibration in a holding chamber for at least 30 min, the slices were transferred to a small volume chamber mounted on an upright microscope (Zeiss Axioskop), and superfused with ACSF at a rate of 1.5–2.0 mL/min maintained at a temperature of 34±1 ºC. Neuronal soma and proximal dendrities of neurons were directly visualized by a combination of differential interference contrast (DIC) optics and contrast-enhanced infrared (IR) video microscopy.

Whole-cell patch-clamp recordings Whole-cell patch-clamp recordings from the subthalamic nucleus neurons were obtained using a patch-clamp amplifier (LM/PCA, List Medical). Whole-cell pipettes had a resistance of 3-4 MΩ, when filled with an internal solution of the following composition (in mmol/L): KCl 140, HEPES 10, EGTA 1, $MgCl_2$ 2, Na_2ATP 2, and Tris GTP 0.4. The inclusion of 140 mmol/L of KCl in the recording pipettes reversed the polarity of the inhibitory postsynaptic currents (IPSCs) from outward to inward and enhanced their detection. Capturing of data and subsequent analysis followed the procedure of our previous report[19]. Monitoring through a television connected to the camera, a pipette was placed on the soma of a subthalamic nucleus neuron 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. The voltage and current signals were filtered at 3 kHz and were taped using a DAT recorder (Sony) modified for recording AC and DC signals at a sampling rate of 32 kHz. On- or off-line digitization (10 kHz) was made via the Digidata-pClamp system (Axon Instruments). Synaptic currents were analyzed by a program developed in our laboratory[19]. Once a synaptic current is detected, information on the time of occurrence, peak amplitude and kinetics are generated automatically. The program also performed statistical comparison of two cumulative probabilities using the Kolmogorov-Smirnov test.

Drugs and statistics (±)-Baclofen used in the present study will be referred to as baclofen and was obtained from RBI. CGP55845 was purchased from Tocris. (±)-2-Amino-5-phosphonopentanoic acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), bicuculline and tetrodotoxin (TTX) were also obtained from RBI.

The data were expressed as mean±SEM. Paired Student's *t*-test was used. The level of significance was presented by using a *P* value of 0.05.

Results

Pre- and postsynaptic $GABA_B$ receptors activated by baclofen The $GABA_A$ receptor-mediated miniature IPSCs (mIPSCs) were isolated by the addition of AP5 50 μmol/L and CNQX 20 μmol/L to eliminate glutamate receptor-

mediated synaptic currents and TTX 0.5 μmol/L to block action potential-dependent transmitter release. These currents were sensitive to 10 μmol/L $GABA_A$ receptor antagonist bicuculline, confirming their GABAergic nature. Spontaneous synaptic currents were found in most subthalamic nucleus neurons. Superfusion of baclofen, a specific $GABA_B$ receptor agonist, at 30 μmol/L significantly reduced the frequency of mIPSCs. This effect was reversible when baclofen was removed (Figure 1A, 1B). The inhibitory effect of baclofen was selective to the frequency (control: 1.69±0.27 Hz; baclofen: 0.64±0.09 Hz; wash: 1.08±0.16 Hz, n=12, P< 0.01) but not the amplitude of the mIPSCs (control: 31.4± 1.9 pA; baclofen: 32.5±2.3 pA, n=12, P>0.05), indicating that the effect was presynaptic (Figure 1C). Furthermore, in five cells, application of 200 μmol/L of $CdCl_2$ reduced the mIPSC frequency (control: 1.58±0.22 Hz; Cd^{2+}: 0.86±0.38 Hz; P<0.01). In this case, the effect of balcofen was largely abolished (0.82±0.38 Hz; P>0.05 *vs* Cd^{2+} alone).

To study whether baclofen directly inhibits the subthalamic nucleus neurons through activation of postsynaptic $GABA_B$ receptors, we also quantified the effect of baclofen in inducing an outward current in subthalamic nucleus neurons in the brain slice. In contrast to its presynaptic effect, which was observed in all neurons tested, baclofen at 30 μmol/L induced a weak outward current in only six out of 23 neurons (21.7 %), with a mean of 20.1±3.1 pA.

Receptor specificity and tonic activity revealed by CGP55845 To confirm that baclofen acts on $GABA_B$ receptors and to test whether the receptors are tonically active in the subthalamic nucleus, the effects of a recently introduced potent and specific $GABA_B$ receptor antagonist, CGP55845, were studied. When CGP55845 2 μmol/L was applied into the superfusion solution, there was no changes in the holding current in all six cells tested. In contrast, CGP55845 caused a clear increase in the frequency of the mIPSC, from 1.22±0.29 Hz to 2.14±0.56 Hz (n=6, P<0.05). These data suggest that the pre- but not the postsynaptic $GABA_B$ receptors are tonically active (The results from a typical cell were shown in Figure 2A, 2B). Consistent with a presynaptic site of action, the increase in the mIPSC frequency induced by CGP55845 was not accompanied by a change in the amplitudes (control: 38.3±3.7 pA; CGP55845: 39.2±3.7 pA; CGP55845+baclofen: 36.7±3.5 pA, n=6, P> 0.05). Furthermore, in the presence of CGP55845, baclofen did not decrease the frequency of mIPSCs in these neurons (CGP55845+baclofen: 2.26±0.48 Hz, P>0.05 *vs* CGP55845 alone, Figure 2). These data indicate that the presynaptic inhibitory effect observed when baclofen was applied alone was mediated by $GABA_B$ receptors (Figure 2).

In the presence of CGP55845, baclofen did not activate any outward current (n=6), suggesting that CGP55845 prevented the activation of postsynaptic $GABA_B$ receptors. The receptor specificity of the postsynaptic effect of baclofen was also tested in those neurons that responded to baclofen. Addition of CGP55845 in the presence of baclofen completely reversed the effect of baclofen (n=3). A typical result was shown in Figure 3.

Discussion

In the present study, we provide evidence for the existence of functional presynaptic $GABA_B$ receptors in the pallidosubthalamic pathway. This conclusion is based on the effect of balcofen on mIPSCs recorded from subthalamic neurons. This effect of balcofen is sensitive to specific $GABA_B$ receptors antagonist CGP55845, and also the broad-spectrum calcium channel blocker Cd^{2+}, which presumably blocks the influx of calcium into nerve terminals necessary for the release of GABA. Thus, Ca^{2+}-influx is likely to be involved in the presynaptic effect of baclofen.

The pallidosubthalamic GABAergic pathway is the major inhibitory pathway controlling the activity of the subthalamic nucleus neurons. The present study revealed that activation of $GABA_B$ receptors in this pathway could, in principle, exert two opposite effects on subthalamic nucleus neurons. On the one hand, by activating presynaptic $GABA_B$ receptor, baclofen reduces the release of GABA from pallidosubthalamic GABAergic terminals and then disinhibits the subthalamic nucleus neurons. On the other hand, by activating postsynaptic $GABA_B$ receptors, baclofen directly inhibits the subthalamic neurons. These electrophysiological results corresponded with anatomical observations in primates, which revealed the existence of pre- and postsynaptic $GABA_B$ receptor type 1 subunits in the subthalamic nucleus[20]. Since only a minority of subthalamic neurons responded to baclofen by exhibiting an outward current, which were nevertheless small in the amplitude, this implies that either there is a smaller number, or a less efficient coupling and signaling mechanism, of the postsynaptic $GABA_B$ receptors compared with their presynaptic counterpart. However, subcellular immunolabelling under an electron microscope showed a dense postsynaptic $GABA_B$ receptor labeling in the monkey subthalamic nucleus[20]. Also, our recent pre-embedding immunolabelling in the adult rat globus pallidus showed a similar level of $GABA_{B1}$ and $GABA_{B2}$ subunits at pre- and postsynaptic sites of GABAergic synapses[21] although the pharmacological data in young rats revealed a stronger presynaptic effects in this nucleus[22,23].

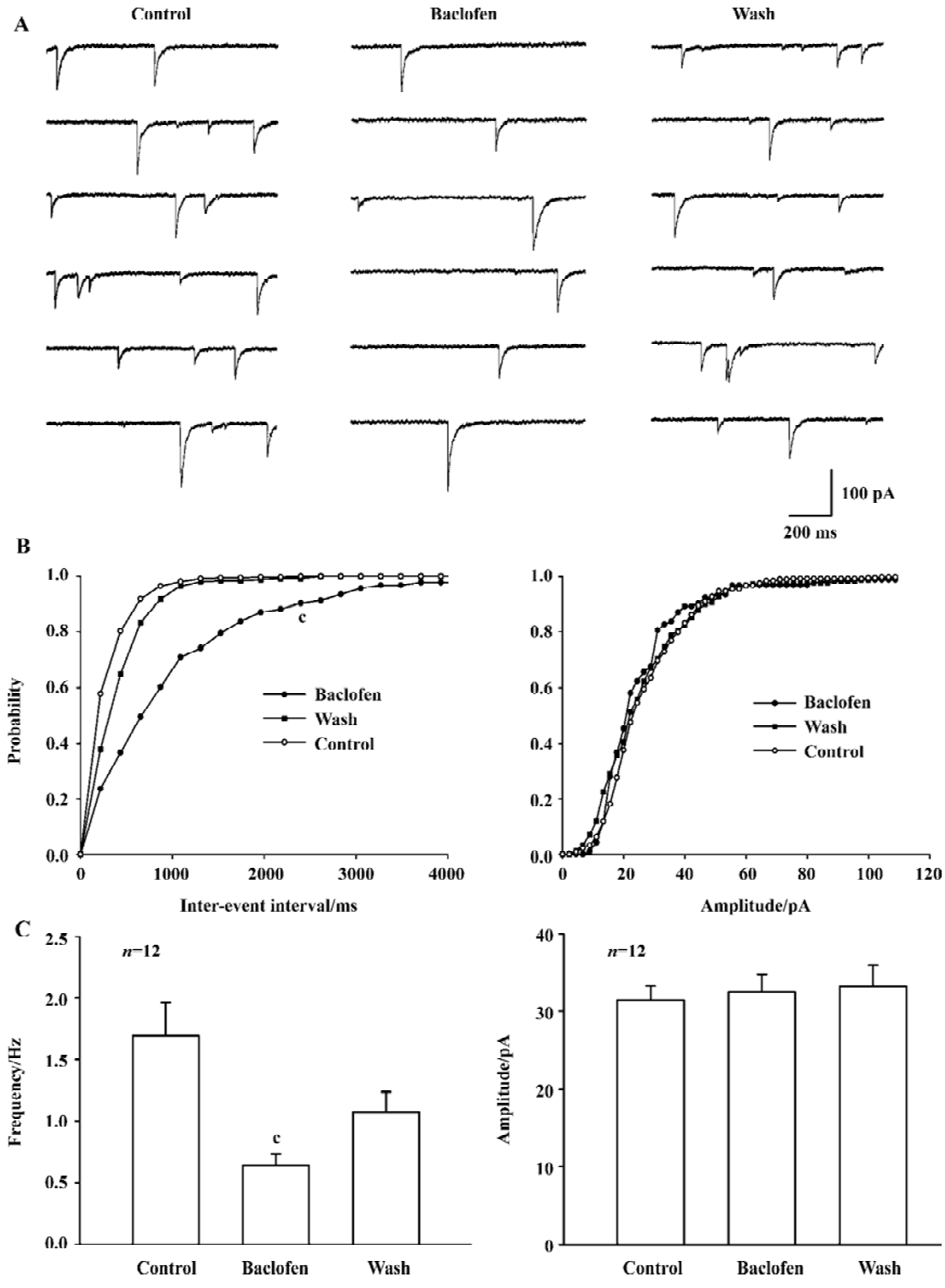

Figure 1. Presynaptic inhibition of GABA release by baclofen. (A) Typical traces showing that 30 μmol/L baclofen significantly and reversibly reduced the frequency of bicuculline-sensitive mIPSCs in the rat subthalamic nucleus neurons. (B) Cumulative probability distributions of the inter-event intervals and amplitudes of the mIPSCs from the experiment shown in panel A. Significant reduction was found in the distribution of the inter-event intervals. (C) Means values obtained from 12 neurons showing that the inhibitory effect of baclofen was selective to the frequency but not the amplitude. Mean±SEM. $^{c}P<0.01$ *vs* control.

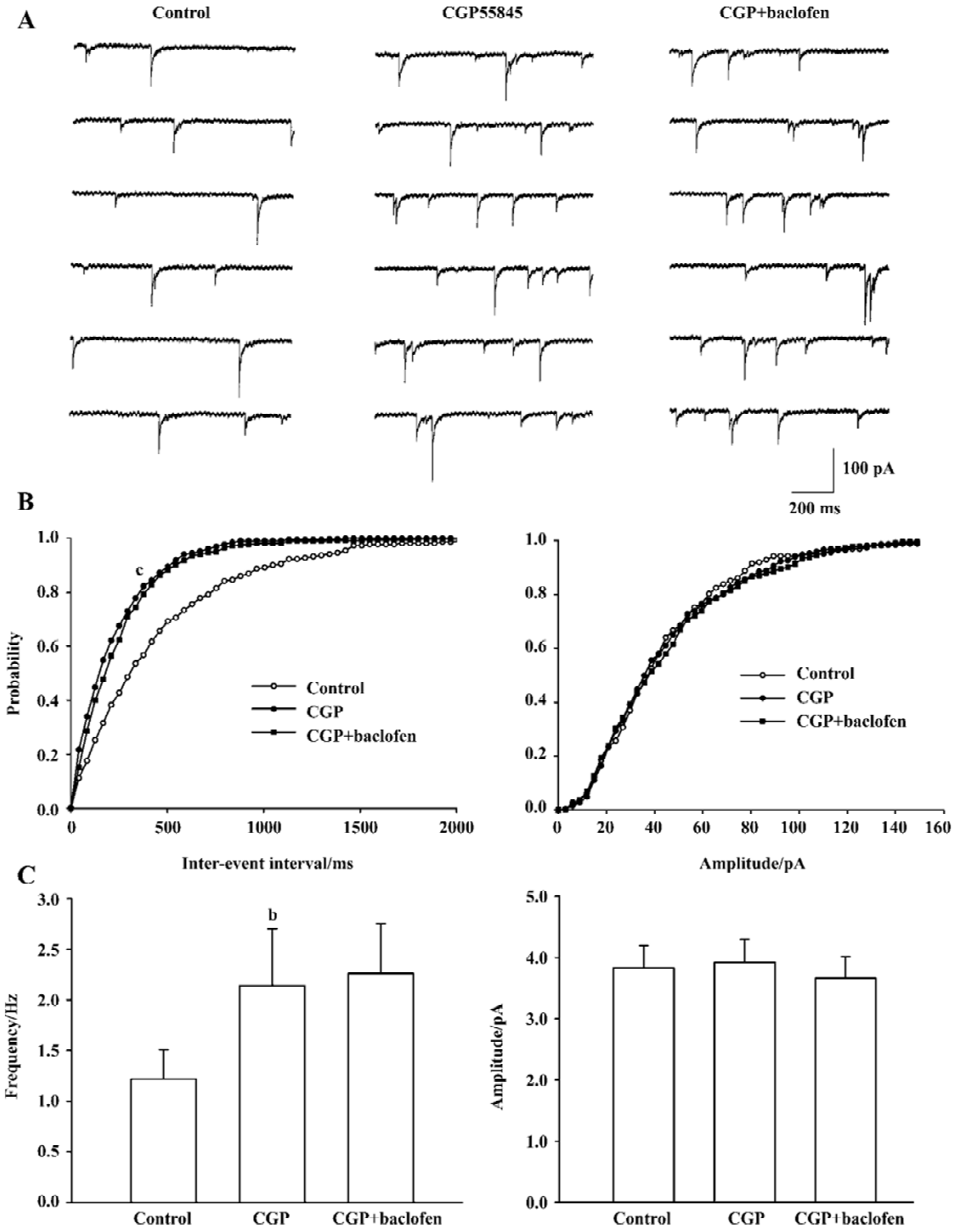

Figure 2. Presynaptic effect of baclofen was sensitive to CGP55845. (A) Typical traces showing that CGP55845 2 μmol/L increased the frequency and prevented the inhibitory effect of baclofen on the mIPSCs. (B) Cumulative probability distribution of the inter-event intervals and amplitudes of the mIPSCs from panel A. $^{c}P<0.01$ *vs* control. (C) Pooled data obtained from 6 cells showing that CGP55845 significantly increased the frequency of mIPSCs and blocked the presynaptic inhibition of baclofen. Mean±SEM. $^{b}P<0.05$ compared with control.

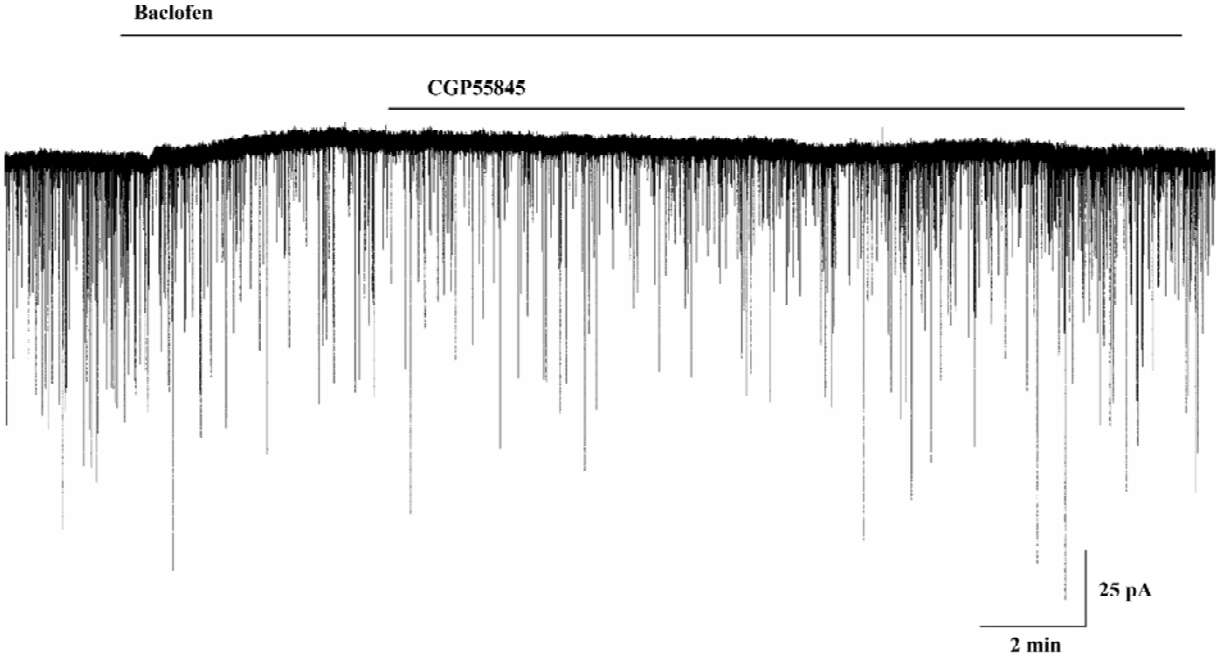

Figure 3. Postsynaptic effect of $GABA_B$ receptor activation. Typical trace showing the outward current induced by 30 μmol/L baclofen which was reversed by CGP55845 2 μmol/L.

Therefore, other factors like species variation and age might affect the outcome of the electrophysiological studies.

The presence of presynaptic $GABA_B$ receptors in the rat subthalamic nucleus has been reported by Shen and Johnson[18], based on a study of evoked IPSCs and their paired-pulse ratio. Our present study confirmed their work by showing that baclofen directly decreased the probability of action potential-independent release of GABA from presynaptic terminals. The conclusion was also enhanced by the use of a more specific and potent $GABA_B$ receptor antagonist, CGP55845. One surprising and intriguing finding of the present study is that CGP55845 increased the frequency of the mIPSCs but had no effect on the holding current, indicating that there is tonic activation of the pre- but not postsynaptic $GABA_B$ receptors. These data therefore suggest that the tonic release of GABA from pallidosubthalamic terminals, either from tonic firing of pallidal neurons, or from action potential-independent activities, may play a significant role in controlling the activity of subthalamic neurons. This finding has interesting implications in the therapeutic management of Parkinson disease. It is known that, in parkinsonian subjects, decreased activity of the GABAergic projection from the globus pallidus disinhibits the activity of subthalamic nucleus neurons, which results in enhanced inhibition on the basal ganglia targets. Since the tonic activity of presynaptic $GABA_B$ receptor on GABA release is expected to maintain the excitability of subthalamic nucleus, selective blockade of this tonic inhibition would help suppressing the subthalamic nucleus hyperactivity and therefore beneficial to parkinsonian subjects. This reasoning is supported by recent morphological evidence that parkinsonism is associated with an increased $GABA_B$ receptor immuno-reactivity, especially in the neurophil, of the subthalamic nucleus, reflecting an upregulation of presynaptic $GABA_B$ receptors[24].

In addition to the involvement in Parkinson's disease, the subthalamic nucleus has been reported to be involved in the genesis of epilepsy. Focal inhibition of the activity of the subthalamic nucleus by $GABA_A$ receptor agonist or high frequency deep brain stimulation exerted anticonvulsant effect in animal model and epilepsy patients[25-28]. Therefore, the results of the present study also provide a rationale for exploring the role of subthalamic $GABA_B$ receptor systems in the etiology and the treatment of epilepsy.

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