GABA\(_B\)-receptor-mediated suppression of sympathetic outflow from the spinal cord of neonatal rats

Yi-Wen Cheng,1 Min-Chi Ku,1 Chiu-Ming Ho,2 Chok-Yung Chai,1 and Chun-Kuei Su1

1Institute of Biomedical Sciences, Academia Sinica, and 2Department of Anesthesiology, Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan

Submitted 23 March 2005; accepted in final form 14 July 2005

In animal experiments, along the hierarchy of the neural axis, it has been shown that GABA\(_B\) receptors exert multifarious effects on sympathetic regulation. Administrations of Bac may reduce catecholamine or acetylcholine release by acting on the peripheral GABA\(_B\) receptors that are located presynaptically on the post- or preganglionic sympathetic nerve terminals (3, 34, 48, 51). Intrathecal administration of Bac to the thoracic spinal cord decreases blood pressure and heart rate (33). However, intraperitoneal or intracerebroventricular administration of Bac leads to a general excitation of sympathetic activity (49, 53) or elicits biphasic pressor and depressor responses (64). At the brain level, focal applications of Bac inhibit the presynaptic neurons in the rostral ventrolateral medulla (39). There, GABA\(_B\) receptors exert tonic inhibitory effect on sympahtoexcitation (4). Similarly, at the spinal level, GABA\(_B\) receptors are tonically active to inhibit the cardiovascular sympathetic preganglionic neuron (SPN) (28, 30). Although these in vivo studies had indicated a general GABA\(_B\)-receptor-mediated reduction of central sympathetic outflow, the underlying mechanisms were largely unknown.

GABA\(_B\) receptors are generally considered as one of the presynaptic elements that can reduce synaptic strength (2, 15, 16). The inhibitory effects of GABA\(_B\) receptors on neurotransmission could have been coupled with their modulations of voltage-gated channel activities. Activation of GABA\(_B\) Receptors attenuates high-voltage-activated Ca\(^{2+}\) currents in a variety of neurons (8, 13, 21, 27, 70). Bac also enhances voltage-dependent or the inwardly rectifying K\(^+\) currents (26, 38, 57, 63). With regard to the sympathetic control at the spinal levels, there is evidence indicating a presynaptic action of GABA\(_B\) receptors, which may attenuate excitatory synaptic inputs to SPNs (47, 75). However, there was no evidence indicating that, intrinsic to SPNs, GABA\(_B\) receptors would regulate their voltage-gated channel activities.

Activities of SPNs are not driven only by the commands from the brain. An isolated spinal cord can generate substantial amounts of sympathetic activities (54, 58, 65, 71). Using a splanchnic nerve-spinal cord preparation, we have demonstrated that three or less thoracic spinal segments in vitro contain sufficient neural components to generate a sympathetic nerve discharge (SND) (62). The display of SND patterns in either tonic or bursting form is regulated by a spontaneous GABAergic activity acting on ionotropic GABA\(_A\) receptors (60, 62). In the spinal cord of rats, metabotropic GABA\(_B\) receptors are present (10, 14, 66). Thus it would be interesting to know whether the intraspinal GABA\(_B\) receptors, being

Address for reprint requests and other correspondence: C.-K. Su, Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan (e-mail: csu@ibms.sinica.edu.tw).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
deprived from extraspinal synaptic inputs, were endogenously active to modulate sympathetic outflow.

In this study using an in vitro experimental model that had a better control of drug working concentrations, we aimed to determine GABA<sub>B</sub>-receptor-mediated effects on SND and the related ionic mechanisms underlying the regulation of sympathetic outflow from the thoracic spinal cord. We hypothesized that, similar to GABA<sub>A</sub> receptors, intraspinal GABA<sub>B</sub> receptors were endogenously active and would exert a tonic suppression of spinal SND. We also hypothesized that Bac-induced suppression of SND could be attributed to an attenuation of excitatory synaptic events to SPNs or a direct inhibition of SPN excitability due to an enhancement of K<sup>+</sup> or a reduction of Ca<sup>2+</sup> currents. Our data not only confirmed the presence of somatodendritic GABA<sub>B</sub> receptors on SPNs but also indicated complex ion events underlying Bac-induced SND suppression.

MATERIALS AND METHODS

General procedures. Neonatal Sprague-Dawley rats (postnatal (P) days 1–7) were used in this study, and all of the experimental protocols were approved by the Institutional Animal Care and Utilization Committee (protocol no. RRAI.BMSC2003014). Methods in preparing a splanchnic nerve-thoracic spinal cord were modified from the procedures, as previously described (59, 62). Briefly, the nerve-cord preparation was fixed onto a recording chamber containing 8 ml 95% O<sub>2</sub>-5% CO<sub>2</sub> equilibrated artificial cerebrospinal fluid (aCSF; in mM: 128 NaCl, 3 KCl, 1.5 CaCl<sub>2</sub>, 1.0 MgSO<sub>4</sub>, 24 NaHCO<sub>3</sub>, 0.5 NaH<sub>2</sub>PO<sub>4</sub>, 30 g-glucose, and 3 ascorbate) and superfused at a rate of ~16 ml/min. A suction electrode placed on the proximal end of the spinal cord was used to record compound action potentials, which were then amplified and filtered (WPI, DAM50; band pass: 0.1–1 kHz). All signals were stored on a pulse-code modulation tape recorder (Neuro-Corder, DR-890) for offline analysis. The amount of nerve discharge was measured by a time-based integrator (Gould, 13–4615-70; resetting every 5 s) to obtain total SND (J<sub>0–7</sub> SND) or a leaky integrator (discharging time constant: 15 ms) to display the envelope of SND (J SND). The background level of neural recording was determined after a blockade of Ca<sup>2+</sup>-dependent neural activities by adding 24 mM Mg<sup>2+</sup> to the bath solution. During experiments, the bath temperature was maintained at 24.5 ± 1°C.

Whole cell, patch-clamp recording. The Bac-induced changes in electrophysiological properties of splanchnic SPNs were examined by using blind, whole cell, patch-clamp techniques. Patch pipettes were pulled from a borosilicate glass (AM-system, 6170) using a horizontal puller (P-97, Sutter Instrument, Navato, CA). The normal pipette solution contained (in mM) 145 potassium-glucurate, 5 NaCl, 10 EGTA, 1 CaCl<sub>2</sub>, 1 MgSO<sub>4</sub>, 4 ATP, and 10 HEPES (adjusted to pH 7.3 by 1 N KOH). When filled with normal pipette solution, the patch pipette had a resistance of 5–8 MΩ, with a liquid junction potential of ~8 mV. To examine Ca<sup>2+</sup> currents in some experiments, 145 mM potassium-glucurate in the normal pipette solution was replaced with 135 mM cesium-glucurate and 10 mM TEA acetate to reduce K<sup>+</sup> currents. Membrane currents or potentials were obtained with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) equipped with CV-203BU headstage, which was mounted on a three-dimensional micromanipulator. The patch pipette was advanced by steps (2 μm) into the tissue and positioned with a motion controller (PMC100, Newport), which also read the depth of pipette tip from the dorsal surface of the spinal cord. Signals were low-pass filtered at 5 kHz and processed by using a data-acquisition system (pClamp 6.0, Axon Instrument). The whole cell membrane capacitance (C<sub>m</sub>) or recording access resistance was compensated or determined by the patch-clamp amplifier. In voltage-clamp mode, the waveform of current response to a 5-mV pulse was displayed on an oscilloscope to monitor a possible change in the access resistance. Only the results of experiments without an apparent alteration of access resistance were included in the analysis.

Antidromic stimulation of splanchnic SPN and measurements of electrical membrane properties. To determine whether a spinal neuron was the SPN that had its axonal projection to the splanchnic nerves, the nerves were electrically stimulated by delivering square pulses (≤100 μA, 0.2 ms in duration, 0.5 Hz) to activate antidromic action potentials. A splanchnic SPN was then verified by the following criteria: 1) the spinal neuron had an evoked spike with a fixed onset latency following the stimulation; and 2) the evoked spike was collided or disappeared when a spontaneous spike appeared within the propagation time of an antidromic action potential (Fig. 1). In practice, following a spontaneous spike, the antidromic onset latency was taken as a conservative estimate of the critical delay for electrical stimuli to activate antidromic spikes (40). In voltage-clamp mode with a holding potential of ~60 mV (corrected for junction potential), we measured the electrical membrane properties, including whole cell membrane current (I<sub>mem</sub>) and input resistance (R<sub>i</sub>). The total resistance of whole cell, patch-clamp recording was determined by calculating the current responses elicited by a rectangular step pulse (~10 mV, 70 ms), whereas R<sub>i</sub> was acquired by subtracting the recording access resistance from the total resistance.

Chemicals. Bac (GABA<sub>B</sub> receptor agonist), saclofen hydrochloride (Sac; GABA<sub>B</sub> receptor antagonist), and CGP-46381 (CGP; another GABA<sub>B</sub> receptor antagonist) were purchased from Tocris, whereas glycine (Gly; glycine receptor agonist) and TTX (fast Na<sup>+</sup> channel blocker) were from Sigma. Concentrated solutions (in mM: 1 Bac, 10 CGP, 200 Gly, 10 Sac, or 0.25 TTX) were prepared by dissolving drugs in water and stored at ~20°C. The final concentration of drugs in the bath solution was obtained by adding an aliquot of concentrated solutions directly to the bath chamber. A cumulative concentration-response test was conducted by sequentially raising drug concentrations in the bath solution to determine drug sensitivity.
Data analysis. True neural signals were obtained by subtracting the background noise from the recorded signals. The height of $f_{0.5}$ SND under control conditions was taken as 100% activity. Drug effects on neural signals were then calculated as percent changes from control activity. For simple evaluation of the drug effects, a two-tailed Student’s $t$-test was used. The data from testing the concentration-dependent effects of Bac were analyzed with the aid of Origin (version 4.1) and fit with a sigmoidal Boltzman function, $y = A_2 + (A_1 - A_2)/[1 + \exp((x - x_0)/dx)]$, where $A_1$ and $A_2$ are the upper and lower asymptotes, respectively, $x_0$ is the half-maximal response, and $dx$ is the width. In practice, the SND before Bac application (100% activity) was set as a fixed asymptote for $A_1$. The effectiveness of Sac or CGP to antagonize Bac effects was evaluated first with ANOVA followed by post hoc multiple comparison, using adjusted $t$-test, with $P$ values corrected by Bonferroni procedure. All values are presented as means ± SE. A statistical probability ($P$) value $<0.05$ was considered significant.

RESULTS

Age-dependent Bac effects on the reduction of spinal SND. Effects of GABAB-receptor activation on spinal SND were investigated by bath applications of Bac (final concentration: 0.1–9.6 $\mu$M). The spinal SND was reduced by Bac in a concentration-dependent manner (Fig. 2). Although SND was always reduced, Bac concentration required to achieve a similar extent of inhibition varied substantially between experiments. Our preliminary observations indicated that Bac sensitivity was higher in younger neonates. In seven of the nine experiments (78%) using neonatal rats of younger ages (1–3 days old), a reduction of SND to <70% of the control activity only required Bac at a concentration of 0.4 $\mu$M. In all experiments using older neonates (5–7 days old; $n = 4$), a comparable SND inhibition could only be achieved by 1.6 $\mu$M Bac. Another series of experiments using neonates of different ages was, therefore, conducted to clarify a change in Bac sensitivity during early postnatal stages. Figure 3 shows an age-dependent shift of the concentration-response curves to the right. In older neonates, both effectiveness and potency of Bac on SND inhibition were attenuated. To minimize variations between

![Fig. 2. Baclofen (Bac)-induced reduction of panels in A and B sympathetic nerve discharge (SND). Bottom panels in A and B: SND at faster sweep speed. A: saclofen hydrochloride (Sac) pretreatment reversibly attenuates the Bac-induced reduction of SND. $f_{0.5}$ SND, integrated SND resetting every 5 s. Dashed line in middle panel indicates the noise level of recording. A rat of an age of postnatal day 4 (P4) was used in this experiment. Bac (final concentration: 0.8–6.4 $\mu$M) reduced SND in a concentration-dependent manner. In the presence of 100 $\mu$M Sac (open bar on top), the effect of 3.2 $\mu$M Bac on SND inhibition was markedly reduced. After washout, 3.2 $\mu$M Bac still reduced SND. The noise level of the neural recording was determined by adding 24 mM Mg$^{2+}$. B: a time control to demonstrate that the thoracic cord in vitro generates a stable SND.](http://jap.physiology.org/)

![Fig. 3. Age-dependent attenuation of the potency and effectiveness of Bac on SND inhibition. Bac was cumulatively applied to the bath solution to achieve final concentrations of 0.1–1.6 $\mu$M for P1, 0.1–3.2 $\mu$M for P3, and 0.4–9.6 $\mu$M for P5 rats ($n = 5$ for each age group). In each group, the reduction of SND in response to Bac was well described by a sigmoidal curve ($r = 0.9843$, 0.9672, and 0.9704 for P1, P3, and P5 group, respectively). The concentrations of Bac required to achieve half-maximal responses of SND inhibition for the age group of P1, P3, and P5 was estimated as 0.16, 0.34, and 2.67 $\mu$M, respectively. The effectiveness of Bac as revealed by the maximal response of SND inhibition was attenuated in an age-dependent manner. Values are means ± SE.](http://jap.physiology.org/)
results, the neonatal rats of similar ages were carefully selected for the same tests in the following experiments.

Antagonizing Bac-induced SND suppression by Sac or CGP.
To evaluate the specificity of Bac in activating GABA\(_B\) receptors, Bac effects on SND suppression were challenged by adding Sac or CGP. Due to the age-dependent sensitivity to Bac, nerve cord preparations from neonates of various ages were treated with Bac concentrations to produce a SND suppression to 27 ± 4% of the control activity (Fig. 4A–C). The specificity of CGP in antagonizing Bac effects was further verified by examining whether CGP could reduce Gly-induced SND suppression (Fig. 4D). Bac-induced SND suppression was not affected by the pretreatment of 100 μM CGP (Fig. 4D).

Bac effects on the spontaneous firing and membrane potential of splanchnic SPNs. SPNs were recorded by using blind whole cell, patch-clamp techniques to elucidate the causes of Bac-induced SND suppression. Electrical stimulations of the splanchnic nerves successfully elicited antidromical action potentials with fixed onset latencies of ∼22–56 ms in 44 spinal neurons (Fig. 1). Under extracellular recording conditions, most splanchnic SPNs (66%) were spontaneously active, with the average firing rate of 0.1–2 Hz. Under voltage-clamp conditions, we determined passive membrane properties, including \(C_m\) and \(R_i\), which varied substantially in different SPNs (\(C_m\): 0.5–4.2 pF; \(R_i\): 180–650 MΩ). Under current-clamp conditions, applications of Bac always reduced SND and SPN firing (Fig. 5). However, the reduction of SPN firing might not always be accompanied with a membrane hyperpolarization. Prominent membrane hyperpolarizations (∼6.5 ± 2.1 mV) were observed in three SPNs, an example shown in Fig. 5. Figure 5 also shows that application of Bac reduces the occurrence of excitatory postsynaptic potentials.

Bac-induced changes in baseline \(I_{m}\) and whole cell \(R_i\). Under voltage-clamp conditions, SPNs were held at −60 mV. A time series plot was constructed by sampling \(I_{m}\) and \(R_i\) with a frequency of every 10 s to examine Bac-induced changes along the time course (Fig. 6). Bac was applied to the nerve cord preparation when its synaptic transmission was intact or when it was interrupted by adding 12 mM Mg\(^{2+}\) or 0.5 μM TTX to aCSF. Figure 7 summarizes the changes of \(I_{m}\) and \(R_i\) elicited by Bac.
Bac-induced changes of whole cell, current-voltage relationships. Previous studies have indicated that all of the SPNs innervating the adrenal medulla express a Ba\(^{2+}\)/Cs\(^{+}\)-sensitive and hyperpolarization-activated inwardly rectifying conductance (74). To decipher whether splanchic SPNs had similar currents that might be enhanced by Bac, the whole cell, current-voltage (I-V) relationship was determined by measuring the current responses elicited by voltage pulses from \(-100\) to \(-40\) mV, with a step increment of \(10\) mV. Experiments were conducted in the presence of \(0.5\) \(\mu\)M TTX, using normal pipette solution, and without the leak subtraction of passive currents. Figure 8 shows that the whole cell I-V curve is linear, indicating that this SPN does not have inwardly rectifying conductances being activated by hyperpolarization. In this series of experiments \((n = 4)\), an application of \(5\) \(\mu\)M Bac always induced outward baseline \(I_m\) \((26 \pm 7\) pA), but it might either decrease \((-19 \pm 4\%\), \(n = 3\)) or increase \((+22\%, \ n = 1)\) the slope of I-V curves. The I-V curves for control and Bac converged at \(-29 \pm 6\) mV \((n = 4)\), a potential that was very different from K\(^+\) equilibrium potential \((E_K = -99.7\) mV). In Cs\(^{+}\)-loaded SPNs with external \(0.5\) \(\mu\)M TTX, applications of \(5\) \(\mu\)M Bac still caused an outward baseline \(I_m\) \((19 \pm 4\) pA, \(n = 5)\), which was reversed by application of \(100\) \(\mu\)M CGP.

Bac reversibly reduces Ca\(^{2+}\)-sensitive and high-voltage-activated inward currents in Cs\(^{+}\)-loaded SPNs. Bac effects on high-voltage-activated Ca\(^{2+}\) current were examined in Cs\(^{+}\)-loaded SPNs. To acquire high-voltage-activated Ca\(^{2+}\) currents, the passive current responses elicited by the voltage pulses were subtracted by using a P/4 protocol, i.e., 4 prepulses with \(\frac{1}{4}\) amplitude and reversed polarity were given before the full-size test pulse. The voltage pulses depolarizing Cs\(^{+}\)-loaded SPNs less than or equal to \(-20\) mV always induced inward currents. Without any treatment, the run-down of high-voltage-activated inward currents was apparent along the time course (decaying rate: \(-4.4 \pm 1.1\%\) \(\text{min}, n = 5\)). Therefore, we examined the changes induced by drug applications in a time series plot, which was acquired by determining the voltage-gated currents in response to successive rectangular depolarizing voltage pulses \((-10\) mV, \(76\) ms, \(0.1\) Hz). As shown in Fig. 9, application of \(5\) \(\mu\)M Bac promptly reduced high-voltage-activated inward currents, which were reversed by adding \(100\) \(\mu\)M CGP. This high-voltage-activated inward cur-

---

**Fig. 6.** Time series plot of Bac-induced changes of whole cell membrane current \((I_m)\) and input resistance \((R_i)\) in two SPNs. Experiments were conducted in the presence of \(0.5\) \(\mu\)M TTX. \(V_m\). Holding potential. A: bath application of Bac induced an outward baseline current \((-23\) pA), but it did not cause a concurrent change in \(R_i\), \(a1\) and \(a2\): Original traces showing changes of \(I_m\) in response to \(-10\)-mV step pulses. Each trace was acquired by averaging 12 current responses as labels \((a1, a2)\) indicated. Except for a vertical shift of the baseline current after Bac application, the step current responses of \(a1\) and \(a2\) were not different. B: Bac-induced transient and steady-state responses in \(I_m\) and \(R_i\). Steady-state membrane responses in A and B were similar. However, the time series plot shows a transient inward \(I_m\) concomitant with a transient rise of \(R_i\). Superimposed traces \((b1, b2)\) show a reduction of step-current response, indicating a transient rise of \(R_i\) (b2, single trace without averaging, shaded arrows). Traces of \(b1\) and \(b2\) (average of 6 current responses) were not different.

---

**Fig. 7.** Overall effects of Bac-induced changes in \(I_m\) and \(R_i\) among SPNs with intact or interrupted synaptic transmission. Different to the status of synaptic transmissions, application of \(5\) \(\mu\)M Bac consistently elicited outward baseline currents. In the presence of \(12\) mM Mg\(^{2+}\) to reduce Ca\(^{2+}\)-dependent synaptic transmission, application of Bac significantly increased \(R_i\) \((18 \pm 6\%\). Values are means \(\pm\) SE. Significant changes induced by Bac: \(*P < 0.05\), \(**P < 0.01\).
Application, the equation, showing correlation coefficient ($r$ B).

GABAB receptors and inhibits the spinally generated SND. The appearance of this hyperpolarizing current of outward baseline currents that directly hyperpolarized excitation of excitatory synaptic potentials to SPNs and the elicitation of inward baseline current of 19 pA (a shift of 1 pA). After Bac, the magnitudes of pulse-evoked $I_m$ responses were reduced. B: I-V curves before and after Bac. Insets indicate curve fitting with a linear equation, showing correlation coefficient ($r$) that approximates 1. After Bac application, the I-V curve declined by 27% in slope and was shifted to the left, leading to a converging point of the two straight lines at $-35.6$ mV.

**DISCUSSION**

We have demonstrated that Bac activates intraspinal GABA$_B$ receptors and inhibits the spinally generated SND. Surprisingly, Bac sensitivity in SND suppression is age dependent, suggesting a functional change of intraspinal GABA$_B$ receptors in sympathetic regulation at early postnatal stages. The inhibition of SND could be attributed to the diminution of excitatory synaptic potentials to SPNs and the elicitation of outward baseline currents that directly hyperpolarized SPNs themselves. The appearance of this hyperpolarizing current in SPNs seems to depend on a mixture of ion events that may not lead to a concomitant change in passive membrane properties. Another new finding is that activation of somatodendritic GABA$_B$ receptors on SPNs reduces high-voltage-activated Ca$^{2+}$ currents. Our findings indicate that intraspinal GABA$_B$-receptor activities suppress central sympathetic outflow through mechanisms both antecedent and intrinsic to SPNs.

Age-dependent sensitivity of Bac: a clinical implication. The age difference of Bac sensitivity in SND inhibition suggests that GABA$_B$ receptors undergo postnatal changes. Molecular biological cloning of GABA$_B$ receptors has demonstrated the presence of different clones coding for GABA$_B$-receptor subunits, namely, GABA$_B$R1a/b and GABA$_B$R2. The combination of these two clones to form a heterodimer (GABA$_B$R1 + GABA$_B$R2) is required to obtain full biological activity (11, 21, 72, 76). Developmentally, the expression of GABA$_B$R1a/b splice variants is undergoing a postnatal change, showing a shift from GABA$_B$R1a in neonatal to GABA$_B$R1b in adult stages (22). The functions of GABA$_B$ receptors also undergo postnatal development (7, 22, 41, 43, 67, 78). Intriguingly, the autonomic nervous system is not mature at birth (5, 25, 31). Thus, in terms of the sympathetic control at the spinal level, alterations of Bac sensitivity may occur, because the reconstitution of the receptor subunits can, in turn, affect the binding affinity of Bac (43) or even their coupling with G protein (1). Although we have not resolved the exact mechanisms, our results strongly suggest that a prominent change of spinal GABA$_B$ receptor plays a role in modulating the sympathetic outflow during postnatal maturation.

Our findings have some clinical implications. In our experiments, bath application of Bac to an in vitro spinal cord preparation simulates the regimen that uses intrathecal Bac to alleviate autonomic dysfunction (6). We observed that Bac inactivating GABA$_B$ receptor consistently reduces SND. This observation clearly suggests an overall inhibition of the sympathetic outflow at the spinal level during intrathecal administration of Bac. However, the ability of Bac to inhibit SND largely depends on the age of neonates. The age-dependent Bac sensitivity in SND inhibition could not be due to the thickness of tissue as animals age. In one of our laboratory’s previous studies, bath applications of N$^\circ$-cyclopentyladenosine (CPA; adenosine A$_1$-receptor agonist) also reduced SND (52). We did not find any age-dependent CPA effect as Bac induced. Besides, compared with CPA, Bac has a lower molecular weight (of Bac and CPA: 213.7 and 335.4, respectively), which presumably makes Bac a better permeate than CPA and allows Bac to sink into the tissue faster than CPA. Moreover, as shown in Fig. 2, the reduction of SND reached a plateau 3–5 min after Bac applications. Thus, given 15 min for each concentration test here, the age difference in Bac sensitivity is unlikely due to the lack of equilibrated time required for drug diffusion. Similar to our findings in age-dependent sympathetic suppression, Bac as an anticonvulsant has a distinct age-related difference in its effectiveness (69). The evidence suggests a necessity to carefully assess the dose that is required for a Bac regimen on younger individuals.

Activation of somatodendritic GABA$_B$ receptors elicited hyperpolarizing outward baseline currents without consistent changes in $R_i$. There were discrepant observations with regard to a direct postsynaptic GABA$_B$-receptor activity in SPNs. In CA3 hippocampal regions, it seems that only the presynaptic...
GABAB receptors function in early postnatal stages (24). Similar findings in the SPNs of immature rats (12–20 days old) also have reported that Bac only attenuates excitatory synaptic transmission to SPNs without affecting \( R_i \), suggesting a lack of somatodendritic GABAB-receptor-mediated effects on SPNs (75). However, a recent study by Whyment et al. (73) indicates that applications of 50 \( \mu \text{M} \) Bac induce a dramatic decrease of \( R_i \) (~34%), which persists in the presence of TTX and is

Fig. 9. Time series plot of Bac and CGP effects on the Cd\(^{2+}\)-sensitive and high-voltage-activated Ca\(^{2+}\) current (\( I_{Ca} \)) in a Ca\(^{2+}\)-loaded SPN. Experiments were conducted in the presence of 0.5 \( \mu \text{M} \) TTX. Bars on top indicate applications of Bac, CGP, or Cd\(^{2+}\). Data were collected 5 min after achieving whole cell, patch-clamp configurations to allow diffusion of pipette Cs\(^+\) into the neuron. Holding potential was ~60 mV. Consecutive rectangular test pulses (76 ms) were given every 10 s to depolarize membrane from holding potential to ~10 mV. Along the time course of tests, each circle represents the peak \( I_{Ca} \) elicited by the test pulse with leak current subtraction by a P/4 protocol. Raw \( I_{Ca} \) traces labeled by 1–7 in middle insets are the average responses of 6 consecutive test pulses and correspond to the numbered data points in the time series plot. The plot and raw \( I_{Ca} \) traces indicate that the high-voltage-activated \( I_{Ca} \) gradually run down along the time course (1–2), drop abruptly after Bac application (2–3), recover substantially after addition of CGP (4–5), and are virtually abolished by Cd\(^{2+}\) (6–7). The I-V curves in the bottom right inset were obtained by applying test pulses to depolarize membrane to potentials between ~35 and 25 mV (400 ms, 15-mV increments) at the timing, as solid symbols and arrows indicated in the time series plot.

Fig. 10. Time series plot of high-voltage-activated outward currents (\( I_{out} \)) in a SPN. Bars on top indicate applications of Bac and CGP. Experiments were conducted by using normal pipette solution and in the presence of external 0.5 \( \mu \text{M} \) TTX and 100 \( \mu \text{M} \) Cd\(^{2+}\). Holding potential was ~60 mV. Consecutive test pulses (76 ms) to depolarize membrane from holding potential to 30 mV were given every 10 s. Raw \( I_{out} \) traces in the bottom insets are responses with leak current subtraction by a P/4 protocol and correspond to numbered data points in the time series plot. Each circle in the time series plot represents the peak \( I_{out} \) elicited by the test pulse. Along the time course of tests, \( I_{out} \) was not changed by application of Bac or CGP.

J Appl Physiol • VOL 99 • NOVEMBER 2005 • www.jap.org
caused by an increase of K⁺ conductance (73). Under conditions with intact or interrupted synaptic transmissions, given a holding potential at −60 mV, we found that Bac consistently elicited hyperpolarizing outward baseline currents with inconsistent changes in Rᵢ among SPNs. This inconsistency in Bac-induced changes of Rᵢ has also been observed in the neurons located in the rostral ventrolateral medulla (39) or some of the SPNs in the thoracic spinal cord (See Fig. 5 in Ref. 73). Compared with observations from other studies, our results clearly indicate a heterogeneous response in different SPNs that may or may not reveal a discernible postsynaptic GABAB-receptor-mediated effect on Rᵢ. We believe that the discrepancy of observations may be due to a complex ion mechanism underlying the hyperpolarizing outward baseline current. This might explain why activation of the somatodendritic GABAB receptors on SPNs did not elicit a concurrent change in Rᵢ.

Compared with the inconsistent effects of Bac on Rᵢ when the tests were conducted in the presence of 0.5 μM TTX, Bac consistently increased Rᵢ when 12 mM Mg²⁺ was used to reduce Ca²⁺-dependent synaptic transmission. This observation implies that an activation of Ca²⁺-dependent channel activities by Bac, which can be blocked by the presence of high Mg²⁺, may contribute to a decrease of Rᵢ in some Bac tests. On the other hand, Bac-induced outward baseline currents persisted in the presence of external TTX and Cd²⁺ or when most K⁺ currents were removed by intracellular Cs⁺ loading. These findings exclude the possible involvement of TTX-sensitive Na⁺, high-voltage-activated Ca²⁺, or leak K⁺ conductance in causing the hyperpolarizing outward baseline current.

Reduction of Ca²⁺-sensitive and high-voltage-activated Ca²⁺ currents by GABAB-receptor activation plays the key role in SND inhibition. Activation of GABAB receptors in various neurons has been reported to activate inwardly rectifying K⁺ currents (23, 57), facilitate L-type but attenuate N-type Ca²⁺ currents (13, 36, 55), reduce low-voltage-activated Ca²⁺ currents (45), or cause an indirect suppression of Ca²⁺-dependent K⁺ currents (12, 46). All of these ion events may occur at either presynaptic nerve terminals or postsynaptic somatodendritic area to alter the neuronal excitability. In our studies, we did not find a discernible inwardly rectifying conductance when splanchnic SPNs were hyperpolarized between −80 and −100 mV (Fig. 8). In the SPNs that innervate the adrenal medulla, there is a hyperpolarization-activated inwardly rectifying current, which is sensitive to external Ba²⁺ or intracellular Cs⁺ and is reduced in K⁺-free aCSF (74). It was not clear, in our experimental conditions, why we did not find a likely current in splanchnic SPNs. Under physiological conditions, the functional operation of inwardly rectifying K⁺ conductances falls in a potential range between resting level and E_kv, lending a more hyperpolarized resting level than those neurons without such conductances (44). Compared with the resting potential of phrenic motoneurons at approximately −65 mV (61), the SPNs in our studies here, which were examined under the same experimental conditions as for phrenic motoneurons, had a fairly depolarized resting potential at approximately −50 mV. The resting potentials of those SPNs examined in thin slice preparations are −48.2 ± 1 mV (17). Thus the lack of hyperpolarization-activated inward rectification and the fairly depolarized resting potential did not indicate an existence of inwardly rectifying K⁺ conductance in splanchnic SPNs. In our studies, application of Bac still induced hyperpolarizing outward baseline current in Cs⁺-loaded splanchnic SPNs. Moreover, the I–V curves of control and after Bac were linear and did not converge toward E_kv. Taken together, our results did not support the notion that Bac reduced SND through an intrinsic mechanism of SPNs attributing to the inwardly rectifying K⁺ conductance. The possibility that Bac reduces SND by an enhancement of inwardly rectifying K⁺ conductances on the pre-SPN spinal neurons, however, cannot be excluded.

Applications of Bac to Cs⁺-loaded SPNs reduced the Cd²⁺-sensitive and high-voltage-activated inward currents. This result indicates a downregulation of high-voltage-activated Ca²⁺ channel activities by GABAB receptors. In contrast, with TTX and Cd²⁺ to reduce voltage-gated Na⁺ and Ca²⁺ currents, activation of GABAB receptors did not affect the high-voltage-activated outward current, thus excluding the involvement of delayed-rectified K⁺ current (Fig. 10). Bac effects on high-voltage-activated Ca²⁺ current may not be unique to SPNs. In three of three Cs⁺-loaded spinal neurons that did not respond to antidromic stimulation of splanchnic nerves, applications of 5 μM Bac reduced high-voltage-activated Ca²⁺ currents (data not shown). Thus it is likely that Bac exerts its effect on SND inhibition via a universal reduction of Ca²⁺ influx at multiple levels, including SPN, non-SPN, and even pre-SPN spinal neurons. Although the diminution of high-voltage-activated Ca²⁺ influx into somatodendritic area of some spinal neurons could reduce SND was unknown, an attenuation of Ca²⁺ influx into the presynaptic nerve terminals opposing SPNs would decrease excitatory postsynaptic potentials, as we observed in Fig. 5. Previous studies have shown that SPNs receive prominent intraspinal GABAergic, glycinergic, and glutamatergic inputs (9, 18, 35, 42, 60). The release of these amino acid neurotransmitters might, therefore, be regulated by presynaptic GABAB receptors.

The source of tonic GABAB-receptor-mediated activity under in vivo conditions is extrinsic to the spinal cord. Under the present in vitro conditions, we did not find significant effects of Sac or CGP. The lack of antagonistic effect suggests an absence of endogenous GABAB-receptor-mediated activity within the spinal cord. This observation is not consistent with those obtained from other in vivo studies that support the notion of a tonic GABAB-receptor-mediated activity at the spinal level (28, 30). The existence of spinal GABAergic activity is evident, because our laboratory has previously demonstrated that bicuculline or picrotoxin, by eliminating endogenous GABA_A-receptor-mediated activities, induces a sympathetic output in bursting forms (60, 62). Thus the discrepancy of observations between our in vitro and other’s in vivo studies cannot be attributed to the lack of endogenous GABAergic activity under the present in vitro conditions. It has been reported that the excitation of SPNs elicited from the dorsal root stimulation is suppressed by Bac (47). Therefore, the spontaneous GABA_B-receptor activity may arise from the local spinal reflex under in vivo conditions. In contrast to the actions of intraspinal GABA_A receptors that mainly affect the patterns, rather than the amounts of SND (60), activation of intraspinal GABA_B receptors causes a general reduction of sympathetic outflow. Thus intraspinal GABA_B receptors act as a simple target subserving the commands from the extraspinal GABAergic sources for sympathetic regulation.

J Appl Physiol • VOL 99 • NOVEMBER 2005 • www.jap.org
ACKNOWLEDGMENTS

We are grateful to Dr. C.-Y. Tang for insightful discussion, S.-L. Phoon for technical assistance, C. C.-J. Hsieh for editorial service, and M. Seah for critical reading of the manuscript.

GRANTS

This work was supported by the National Science Council of the Republic of China (NSC 90–2320-B-001–043 and NSC 92–2320-B-001–025).

REFERENCES


J Appl Physiol • VOL 99 • NOVEMBER 2005 • www.jap.org