Effect of Bicuculline on Thalamic Activity: A Direct Blockade of $I_{\text{AHP}}$

in Reticularis Neurons

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Debarbieux, Franck, Jennifer Brunton, and Serge Charpak. Effect of bicuculline on thalamic activity: a direct blockade of $I_{\text{AHP}}$ in reticular neurons. J. Neurophysiol. 79: 2911–2918, 1998. The thalamic reticular nucleus (RTN) is the major source of inhibitory contacts in the thalamus and thus plays an important role in regulating the excitability of the thalamocortical network. Inhibition occurs through GABAergic synapses on relay cells as well as through GABAergic synapses between reticular neurons themselves. Here we report that the role and mechanisms of this inhibition, which are frequently used in studies of GABAergic neurotransmission, should be revisited. Using the whole cell patch-clamp technique in thalamic slices from young rats, we observed an enhancement by bicuculline methiodide, methobromide, and methochloride (collectively referred to as bicuculline-M; 5–60 μM) of the low-threshold calcium spike burst in RTN neurons that persisted in the presence of tetrodotoxin (1 μM) and was not reproduced in picrotoxin (100–300 μM). The effect did not involve activation of any GABA receptor subtype. Voltage-clamp recordings showed that bicuculline-M blocked the current underlying the low-threshold spike burst afterhyperpolarization (AHP), an effect that was mimicked by apamin (100 nM). Recordings from nucleated patches extracted from reticularis neurons demonstrated that this effect was not mediated by modulation of the release of an unidentified neurotransmitter but that bicuculline-M directly blocks small conductance (SK) channels. The AHP-blocking effect also was observed in other brain regions, demonstrating that although bicuculline-M is a potent GABA<sub>A</sub> receptor antagonist, it is of limited value in assessing GABAergic network interactions, which should be studied using picrotoxin or bicuculline-free base. However, bicuculline-M may provide a useful tool for developing nonpeptide antagonists of SK channels.

INTRODUCTION

The thalamic reticular nucleus forms a shell surrounding the anterior and lateral aspects of the thalamus. This population of GABAergic neurons projecting to the relay, midline, and intralaminar nuclei of the dorsal thalamus provides the main inhibitory input in this structure (Houser et al. 1980; Jones 1975). Synchronized activity in the thalamus, which has been extensively studied during sleep, appears to rely strongly on low-threshold calcium spike (LTS) bursting activity intrinsic to these neurons at hyperpolarized potentials (Jahn and Llinás 1984; Mulle et al. 1986; Steriade et al. 1985, 1993). More recent studies in thalamic slices have shown that not only the intrinsic properties of reticular nucleus (RTN) and thalamocortical neurons, characterized by the LTS and the hyperpolarization-activated cation current ($I_h$), but also the integrity of excitatory/inhibitory contacts in the dorsal thalamus-reticular nucleus relay are important in generating spindle activity similar to that seen in vivo during slow wave sleep (Bal et al. 1995; McCormick and Pape 1990; Steriade et al. 1993; von Krosigk et al. 1993). Modulation of this synchronous activity appears to be effected by a number of neurotransmitters (serotonin, histamine, noradrenaline, acetylcholine), which modify the membrane properties of both thalamic relay and reticularis neurons (McCormick 1992).

The role of GABAergic inhibition between relay and reticular neurons, and among reticular neurons, has been studied to elucidate the mechanisms of thalamocortical synchronization (Bal 1995; Kim et al. 1997; Sánchez-Vives and McCormick 1996; Thomson 1988a,b; Ulrich and Huguenard 1996; von Krosigk 1993). To this end, the γ-aminobutyric acid-A (GABA<sub>A</sub>) receptor antagonists, bicuculline methiodide, methobromide, and methochloride (collectively referred to as bicuculline-M), have been used extensively. An enhancement by bicuculline of the low-threshold calcium spike and overlying sodium spike burst was observed recently in the perigeniculate nucleus of the ferret and was attributed to disinhibition at intrareticular GABAergic synapses (Bal et al. 1995). However, discrepancies between the effects of bicuculline-M and another GABA<sub>A</sub> antagonist, picrotoxin, and the persistence of bicuculline-M effects in tetrodotoxin (TTX) have raised the possibility of another mechanism (Debarbieux et al. 1997; Sánchez-Vives and McCormick 1996). To address this question, we studied the effects of bicuculline on RTN neurons in thalamic slices from young rats using the whole cell patch-clamp technique, first on intact cells and then on nucleated patches. Our results show that the enhancement of the low-threshold spike burst does not involve the action of any neurotransmitter but is mediated by a direct block of apamin-sensitive small conductance (SK) potassium channels. This result not only has implications for studies of inhibition in thalamic network activity but suggests a new tool for developing nonpeptide SK channel blockers.

METHODS

Preparation of thalamic slices

Horizontal thalamic slices were prepared from young male Wistar rats (11–20 days). Rats were anesthetized with a ketamine and xylazine solution (100 and 16 mg/kg ip, respectively) before
FIG. 1. Bicuculline methiodide, methobromide, and methochloride (collectively referred to as bicuculline-M) enhance the low-threshold calcium spike and overlying sodium action potential bursts, increasing reticularis cell excitability. A: a series of hyperpolarizing current step injections (Δ0.1 nA) activate rebound calcium spike bursts. Bicuculline methiodide (40 μM), a γ-aminobutyric acid-A (GABA_A) receptor antagonist, enlarges rebound calcium spikes and reduces the slow afterhyperpolarization that follows. B: effect of bicuculline-M on rebound calcium spikes (activated on relief of a ~0.2-nA current step) is concentration dependent. Note that bicuculline-M alters intrinsic oscillatory activity. C: tetrodotoxin (TTX; 1 μM) abolishes action potential bursts but does not affect the underlying calcium spike enhancement.

decapitation. The skull was opened, and the brain was excised and placed in cold (4°C) oxygenated physiological solution containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 26.0 NaHCO₃, 10 glucose, and 5 pyruvic acid. A block of thalamic tissue was prepared by making the following cuts: two transversal (1 rostral to the optic chiasm and 1 caudal to the mammillary body) and one horizontal (through the 3rd ventricle). Horizontal slices (400 μM) were cut using a vibratome (Campden Instruments, Stiley, UK) and stored submerged in oxygenated solution in a small vial for 1 h. For recording, slices were transferred to a chamber perfused (~2 ml/min) with heated physiological solution (30–35°C).

Electrophysiological recordings

Whole cell current- and voltage-clamp recordings of membrane properties were obtained using borsilicate pipettes (2–6 MΩ resistance) filled with (in mM) 130 K-gluconate, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 10 KCl, 2 MgCl₂, 2 ATP-Mg, and 0.2 GTP-tris (hydroxymethyl)aminomethane. For some recordings, Cs-gluconate or KCl was substituted for K-gluconate. The omission of ATP and GTP from the intracellular solution had no effect on the result. Thalamic cells were patched under visual control, using an infrared-sensitive video camera (Hamamatsu, Hamamatsu-City, Japan), and recordings were carried out using an Axoclamp 200A amplifier (Axon Instruments, Foster City, CA) and a microcomputer equipped with pClamp software (Axon Instruments). Potential values were corrected for the junction potential (11 mV) during analysis. All drugs were applied by bath perfusion.

Chemicals

Drugs were obtained from either Sigma (St. Louis, MO), Tocris Cookson (Bristol, UK), or Latoxan (Rosnans, France) as follows: bicuculline free base, bicuculline methiodide, bicuculline methochloride, bicuculline methobromide, and picrotinox (Sigma or Tocris); GABA (Sigma); apamin (Sigma and Latoxan); TTX (Latoxan). The GABA_A receptor antagonists CGP 35348 and CGP 55845 were kindly provided by Novartis (Basel). The N-methyl derivatives of bicuculline (bicuculline methiodide, bicuculline methochloride, and bicuculline methobromide) were dissolved in water and aliquots were prepared from this stock solution. Bicuculline (free base) was dissolved in dimethyl sulfoxide, and aliquots of this stock were added to the perfusing medium (a dilution of 1:2,000).

RESULTS

Our study is based on electrophysiological recordings from 90 RTN neurons each with a stable resting potential negative to ~60 mV. Cells were not spontaneously active in our experimental conditions (KOUT = 2.5 mM), and their input resistance generally ranged from 100 to 200 MΩ. Reticularis neurons were distinguished based on their location.
BICUCULLINE BLOCKS $I_{\text{AHP}}$ IN THALAMIC CELLS

FIG. 2. Effect of bicuculline-M is not reproduced by picrotoxin although both drugs selectively block GABA$_A$ receptors. 

A: picrotoxin (300 $\mu$M), another antagonist for GABA$_A$ receptors, does not modify rebound calcium spike bursts. B: both picrotoxin (left, top 2 traces) and bicuculline-M (left, bottom 2 traces) block GABA depolarizing responses (resting membrane potential $-70$ mV). The I-V plot (right) shows that application of GABA increases a conductance that reverses at about $E_{Cl}$.

(a narrow band between the internal capsule and dorsal thalamus) and characteristic firing properties.

Bicuculline-M enhances the low-threshold calcium spike and overlying burst of sodium action potentials

Reticularis neurons were characterized by their accelerating-decelerating low-threshold spike burst and subsequent after-hyperpolarization (Mulle et al. 1986; Spreafico et al. 1988). Application of bicuculline-M (30–40 $\mu$M) in the bath evoked an enhancement of the low-threshold calcium spike and its overlying burst of action potentials activated on relief of a hyperpolarizing pulse ($n = 40$). Firing properties of a typical RTN neuron ($V_m = -70$ mV) are shown in Fig. 1A (top). Application of 40 $\mu$M bicuculline methiodide enhanced the calcium spike burst, an effect which was reversible (Fig. 1A, middle and bottom). Application of various concentrations of bicuculline methiodide from 5 to 40 $\mu$M, ($n = 3$) showed that the effect was concentration dependent (Fig. 1B). Furthermore, bicuculline-M markedly altered RTN cells’ intrinsic oscillatory activity (see Figs. 1B and 4A). To verify that these effects were not artifacts due to an impurity in the bicuculline methiodide, several different forms (bicuculline methochloride, methobromide, and methiodide, collectively referred to as bicuculline-M) from two different manufacturers (Sigma and Tocris) were tested. In all cases, the effect was similar to that initially observed, and the results were pooled. Bicuculline-M did not increase the width of sodium action potentials (control, 0.52 ± 0.12 (SE) ms; bicuculline-M, 0.51 ± 0.12 ms; $n = 8$; not shown).

To determine the site of bicuculline-M action, the same experiments were repeated in TTX (1 $\mu$M). Surprisingly, bicuculline-M still enhanced rebound calcium spikes in the presence of TTX (Fig. 1C; $n = 25$) and in the presence of glutamate receptor antagonists (6-cyano-7-nitroquinoxaline-2,3-dione, 40 $\mu$M; d-2-amino-5-phosphonovaleric acid, 20 $\mu$M; $n = 3$; not shown), suggesting that the effect did not involve the thalamocortical or reticularis-thalamic relay cell loop but was restricted to the RTN. The effect therefore was due to either a direct effect on the membrane currents underlying the calcium spike and AHP or to modulation of the TTX-independent release of an unidentified transmitter that would modulate the low-threshold spike and overlying action potential burst.

Bicuculline-M enhancement of the low-threshold spike burst is not reproduced by picrotoxin

The possible involvement of GABA$_A$ receptors in the effect was investigated by comparing the effects of picrotoxin and bicuculline-M on RTN cells. We first verified that both compounds antagonized GABA$_A$ responses in our preparation. Applications of 200 $\mu$M to 2 mM GABA led to membrane depolarization in our cells. Figure 2B (left) shows an experiment in which 500 $\mu$M GABA evoked a depolarizing response (+14 mV). This depolarization was due to the opening of a chloride conductance as revealed by the intersection of current-voltage curves in control and in GABA at about $E_{Cl}$ ([Cl]$_{IN} = 14$ mM, [Cl]$_{OUT} = 134.5$ mM) (Fig. 2B, right). The effect was blocked both by picrotoxin (200
Fig. 3. Excitatory effect of bicuculline-M does not involve GABA. A and B: picrotoxin and CGP 35348, a specific antagonist for GABA_B receptors, do not modify the enhancement by bicuculline-M of the low-threshold calcium spike evoked on relief of a hyperpolarizing pulse (A) or depolarization from a hyperpolarized potential (B). C: GABA (in the presence of picrotoxin and CGP 55845) did not modify the low-threshold calcium spike and overlying action potential burst or displace its enhancement by bicuculline-M.

GABA is not involved

We then tested the possible involvement of a modulation of GABA release by bicuculline-M because synaptic terminal release of GABA in the presence of TTX and dendrodendritic synapses between GABAergic neurons in the RTN have been reported (Pinault et al. 1997; Ulrich and Huguenard 1996). To rule out effects mediated by GABA_A and GABA_B receptors, bicuculline-M was coapplied with picrotoxin (100 μM) and CGP 35348 or 55845 (1 mM, 13 μM), selective antagonists of GABA_B receptors (Fig. 3, A and B, respectively; n = 3, n = 3). The effect persisted in both cases. However, the participation of GABA could not yet be excluded, owing to the diversity of identified GABA receptor subtypes (Johnston 1996; Whiting et al. 1995) and the possibility of the activation of a new GABA receptor subtype. We studied this possibility in an experiment testing the ability of high concentrations of GABA (0.5–2 mM; in the presence of GABA_A and GABA_B receptor antagonists) to modify the low-threshold spike or displace the bicuculline-M effect (n = 4). Figure 3C shows such an experiment in which long applications of GABA (500 μM) fail to alter the low-threshold spike burst and cell sensitivity to bicuculline-M. Therefore, GABA was not involved in the effect of bicuculline-M.

At this stage, it was still not possible to conclude whether bicuculline-M acted presynaptically to modulate the spontaneous release of an unidentified neurotransmitter or whether bicuculline-M acted directly on the postsynaptic membrane of the cell. To distinguish between these two possibilities, we decided to use a different preparation (a nucleted patch held away from the slice; see further), but we first had to characterize the current(s) modulated by bicuculline-M.

Bicuculline-M blocks I_AHP in a voltage-dependent manner

It is established that small conductance calcium-activated potassium channels play a major role in the slow repolarization that follows calcium spike activation (Alger and Nicoll 1980; Schwartzkroin and Stafstrom 1980). SK channel openings underlie the current I_AHP and can be selectively blocked with apamin (Blatz and Magleby 1986; Köhler et al. 1996). In current-clamp recordings, bath application of 100 nM apamin mimicked the bicuculline-M effect (Fig. 4A; n = 7), suggesting that bicuculline-M acts by directly blocking I_AHP. Voltage-clamp recordings were conducted to study the effect on I_AHP further.
We activated $I_{\text{AHP}}$ with protocols designed to activate both low-threshold and high-threshold calcium currents. A voltage step from $-61$ to $-11$ mV activated a high-threshold calcium current and consequently a slow, outward potassium current, $I_{\text{AHP}}$, whereas a smaller voltage step from $-61$ to $-56$ mV had no effect (Fig. 4B, left). When the same voltage step was preceded by a hyperpolarizing step to $-91$ mV, to deactivate low-voltage-activated calcium channels, activation of a low-threshold calcium current and subsequent $I_{\text{AHP}}$ was seen (Fig. 4B, right). The latter protocol was used to test the effect of bicuculline-M on $I_{\text{AHP}}$ activated by both high- and low-threshold calcium currents. Bicuculline-M was found to reversibly block $I_{\text{AHP}}$ independently of the calcium current used to activate it (Fig. 4C; $n = 15$). Experiments carried out with external TTX (1 μM), tetraethylammonium chloride (10 mM), and cesium gluconate intracellular solution showed that the inhibition of $I_{\text{AHP}}$ did not appear to decrease calcium entry because bicuculline-M had no effect on calcium currents evoked by the protocols in Fig. 4B ($n = 10$, not shown).

The voltage dependency of the blockade was studied using two protocols. First, a step was given from $-61$ to $-1$ mV to activate a high-threshold calcium current and subsequent $I_{\text{AHP}}$, which then was measured at various membrane potentials. Then a similar protocol was performed in which calcium entry was not activated (the first step was omitted). Subtraction of the second protocol from the first yielded $I_{\text{AHP}}$ (Fig. 5A, left). We found that bicuculline-M blocked $I_{\text{AHP}}$ in a voltage-dependent manner because the blockade was not symmetrical (Fig. 5A). When the AHP current blocked calcium current and consequently a slow, outward potassium current, $I_{\text{AHP}}$, whereas a smaller voltage step from $-61$ to $-56$ mV had no effect (left). The same step, when preceded by a voltage step to $-91$ mV to deactivate low-voltage-activated calcium channels, is followed by an $I_{\text{AHP}}$ (right).

C: bicuculline-M decreases the $I_{\text{AHP}}$, regardless of whether it is evoked by the activation of low- or high-voltage-activated calcium channels (+, control; *, bicuculline-M 30 μM).

**Bicuculline-M acts by directly blocking potassium channels**

Finally, to rule out bicuculline-M modulation of TTX-independent neurotransmitter release (for instance of an unidentified neurotransmitter acting on metabotropic receptors, the activation of which would affect the low-threshold spike and overlying burst), we tested the action of bicuculline-M on nucleated patches withdrawn from RTN neurons. After achieving a conventional whole cell configuration, the pipette was slowly drawn away from the cell while maintaining suction. It was thus possible to extract the nucleus of the cell surrounded by a large piece of membrane. The patch then was withdrawn slowly from the slice. In these large membrane patches, we could record both calcium and calcium-activated potassium macroscopic currents. However, only one of the four extracted nucleated patches had both low- and high-threshold calcium channels. The high-thresh-
old calcium channels were easier to extract, an observation that is in accord with the hypothesis that low-threshold calcium channels are localized in the dendrites of RTN neurons (Destexhe et al. 1996).

Using the same stimulation protocol as in whole cell recordings, we observed that bicuculline-M still reversibly blocked the $I_{\text{AMP}}$ (Fig. 6A, $n = 4$) elicited by the activation of both low-threshold (6A, left) and high-threshold (Fig. 6A, right) calcium channels. These results clearly show that bicuculline-M has a direct effect on RTN cells, blocking a current mediated by apamin-sensitive SK potassium channels. The schematic diagram in Fig. 6B summarizes the results of this study. The enhancement of the low-threshold calcium spike and overlying action potential burst by bicuculline-M is not dependent on TTX-dependent neurotransmitter release in the thalamocortical network (1), TTX-independent GABA release at dendrodendritic synapses (2) or synaptic terminals (3), or TTX-independent release of glutamate or an unidentified neurotransmitter (4), but results from a direct block of SK potassium channels (5).

Should the use of bicuculline be discontinued?

We then tested whether the effect of bicuculline-M was specific to the RTN in the young rat or if it was a general phenomenon occurring in other brain regions and in adult animals. In brain slices from young rats, thalamic relay cells (in the centrolateral ($n = 3$) and dorsal lateral geniculate nuclei ($n = 3$)) and hippocampal interneurons ($n = 3$) were selected arbitrarily and recorded in whole cell current- or (Destexhe et al. 1996). Using the same stimulation protocol as in whole cell voltage-clamp mode. Cortical pyramidal cells ($n = 4$) were recorded similarly from brain slices taken from female adult rats. In all cases bicuculline-M, in the presence of picrotoxin (100 μM), depressed $I_{\text{AMP}}$, indicating that the use of bicuculline-M should be discontinued not only in studies of the thalamus but in studies concerning other brain regions. Results from three cell types are shown in Fig. 6C. Bicuculline-M depressed $I_{\text{AMP}}$ activated by a depolarizing voltage step in hippocampal interneurons (Fig. 6C, a), and centrolateral (Fig. 6C, b) and dorsal lateral geniculate (Fig. 6C, c) thalamic neurons.

To determine whether the effect of bicuculline-M was dependent on the presence of the methyl group, we investigated the action of bicuculline free base (bicuculline-FB) on RTN neurons. The ability of bicuculline-FB to block GABA$_A$ responses was confirmed first ($n = 3$; not shown). When bicuculline-FB (60 μM) was added to the perfusing medium in the presence of picrotoxin (100 μM), no effect on the low-threshold spike burst was observed ($n = 3$). However, when bicuculline methiodide was perfused at the same concentration, the low-threshold spike burst was enhanced ($n = 3$; Fig. 6D). Thus bicuculline-FB is still valuable as a specific GABA$_A$ receptor antagonist.
FIG. 6. Bicuculline-M blocks $I_{\text{AMP}}$ directly. A: a nucleated patch was withdrawn from a reticularis neuron and held away from the slice to avoid diffusion of unidentified transmitters. As in whole cell recordings, bicuculline-M reversibly depressed $I_{\text{AMP}}$. B: a schematic diagram demonstrating the possible actions of bicuculline-M on $I_{\text{AMP}}$. It does not block $I_{\text{AMP}}$ through modulation of TTX-dependent release in the thalamic network (local or distant) (1). GABA release at dendrodendritic synapses is not involved (2) nor TTX-independent GABA release from synaptic terminals (3). Action of glutamate or an unidentified transmitter (4) released in a TTX-independent manner is also ruled out. Bicuculline-M therefore acts directly on potassium channels (5). C: bicuculline-M (30–60 μM) blocks $I_{\text{AMP}}$ (*) in hippocampal interneurons (a), centrolateral thalamic neurons (b), and dorsal lateral geniculate thalamic neurons (c). $I_{\text{AMP}}$ was activated by depolarizing voltage steps of 50, 50, and 45 mV, respectively. D: bicuculline free base (60 μM), applied in the presence of picrotoxin (100 μM) fails to enhance the low-threshold spike burst (middle). However, bicuculline methiodide (60 μM), also applied in the presence of 100 μM picrotoxin, enhances the low-threshold calcium spike and overlying burst (bottom).

**DISCUSSION**

**Mechanism of bicuculline-M enhancement of the low-threshold spike burst**

Our results both in whole cell and nucleated patch recordings unequivocally demonstrate that bicuculline-M directly blocks $I_{\text{AMP}}$ in RTN neurons, thus prolonging the low-threshold calcium spike and overlying burst of sodium action potentials. Bicuculline was shown to prolong calcium-dependent action potentials in spinal cord and dorsal horn neurons (Heyer et al. 1982) and to potentiate $N$-methyl-$d$-aspartate–dependent burst firing in dopaminergic neurons of the midbrain and burst firing in nucleus accumbens neurons (Johnson and Seutin 1997; Shi and Rayport 1994). These reports clearly suggested that the effect resulted from a blockade of a calcium-activated potassium current. However, none of these studies demonstrated the direct effect of bicuculline by ruling out the possibility that it alters a TTX-insensitive tonic release of a neurotransmitter that would modulate $I_{\text{AMP}}$. Recordings of isolated SK channels in outside-out patches or in nucleated patches held distant from the slice were necessary to definitely establish that bicuculline-M blocks these channels. The voltage dependency of the block suggests that bicuculline-M acts as a plug, an effect that requires the presence of a methyl group. Bicuculline-M blockade of $I_{\text{AMP}}$ is a general phenomenon. Indeed, when we recorded from cells in other regions of the brain (hippocampus, dorsal thalamus, cortex) in the presence of bicuculline methiodide, we saw similar $I_{\text{AMP}}$-blocking effects. Taking into account the effects in the reticularis and various other brain regions, we suggest that the use of bicuculline-M should be discontinued in the study of inhibition in the entire brain.

**Implications for the study of inhibition in the thalamocortical network**

Anatomic and physiological evidence suggest that lateral inhibition occurs in the RTN. Both GABA$_A$ and GABA$_B$ responses of ferret perigeniculate neurons stimulation in the perigeniculate nucleus, and the presence of axodendritic and dendrodendritic synapses in the cat and rat, have been demonstrated (Deschénes et al. 1985; Pinault et al. 1997; Sánchez-Vives and McCormick 1996). The strong enhancement by bicuculline methiodide of the low-threshold calcium spike and overlying burst of action potentials previously
observed in slices most probably reflects a combination of effects: a relief of lateral inhibition and a direct effect on $I_{\text{AMP}}$. The effect of a $\text{GABA}_A$ receptor blockade on thalamic activity (lateral inhibition, thalamic-generated epilepsy) therefore should be reinvestigated using either bicuculline-FB or picrotoxin. The persistence of a burst enhancement induced by picrotoxin then would be due to the block of a shunt caused by the continuous release of $\text{GABA}$ because $E_C$ is near the membrane potential of RTN cells (Ulrich and Huguenard 1997) and because low concentrations of $\text{GABA}$ reduce the low-threshold calcium spike and burst (not shown). However, we did not see any dramatic effect of picrotoxin on cell activity in our preparation, most probably due to the differences between the thalamic slice preparation in the rat and the ferret, in which intrathalamic connections are especially well preserved and the effect of tonic $\text{GABA}$ release is enhanced.

**Possible development of bicuculline-M as an SK channel blocker**

Although bicuculline-M is of limited value in assessing network inhibition, the elucidation of its blocking mechanism could lead to the development of new nonpeptide SK channel blockers. A diversity of SK channel subtypes has been demonstrated in the CNS, and these channels have different sensitivities to apamin (Köhler et al. 1996). Bicuculline-M would provide a good tool for the development of antagonists distinguishing between these different subtypes.

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