GABA<sub>B</sub>-receptor-activated K<sup>+</sup> current in voltage-clamped CA<sub>3</sub> pyramidal cells in hippocampal cultures

(baclofen/inhibition/calium/barium)

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Communicated by Ewald R. Weibel, October 9, 1984

ABSTRACT GABA<sub>B</sub> receptors are a subclass of receptors for γ-amino-n-butyric acid (GABA) that are also activated by the antispastic drug β-p-chlorophenyl-GABA (baclofen). One effect of baclofen is to inhibit excitatory transmission from CA<sub>3</sub> to CA<sub>1</sub> hippocampal pyramidal cells. To identify the ionic mechanism of GABA<sub>B</sub>-receptor-mediated depression, we have studied the effect of baclofen and GABA on ionic currents in voltage-clamped CA<sub>3</sub> pyramidal cell somata in rat hippocampal slice cultures. Baclofen (10 μM) induced an inward rectifying outward current that reversed at −74 ± 4.3 mV (mean ± SD). This appeared to be a K<sup>+</sup> current since (i) its reversal potential showed the expected shift when extracellular K<sup>+</sup> concentration was changed and (ii) it was blocked by external Ba<sup>2+</sup> or internal Cs<sup>+</sup>. The action of baclofen was closely mimicked by GABA after the GABA<sub>B</sub>-mediated Cl<sup>−</sup> current had been abolished with picrotoxin (10 μM); under these conditions, GABA (100 μM) also produced an inward rectifying, Ba<sup>2+</sup>-sensitive current with a reversal potential identical to that of the baclofen-induced current. When outward currents were blocked with internal Cs<sup>+</sup>, the residual inward voltage-dependent Ca<sup>2+</sup> current was not changed by baclofen. It is concluded that the primary effect of GABA<sub>B</sub>-receptor activation in these neurones is to increase K<sup>+</sup> permeability rather than to reduce Ca<sup>2+</sup> permeability.

Two subclasses of receptors for the central inhibitory neurotransmitter 4-amino-n-butyric acid (GABA) have been defined and designated GABA<sub>A</sub> and GABA<sub>B</sub> receptors (1, 2). GABA<sub>B</sub> receptors are selectively activated by the GABA analogue muscimol and mediate an increased Cl<sup>−</sup> conductance (3, 4). Effects of GABA<sub>B</sub> activation are blocked by convulsants such as bicuculline and picrotoxin. A selective agonist for the bicuculline-insensitive GABA<sub>B</sub> receptor is the antispastic agent β-p-chlorophenyl-GABA (baclofen) (1). The principal effect of baclofen is to depress transmitter release from certain excitatory pathways, including intraspinal primary afferents (5-14) and Schaffer commissural fibers in the hippocampus (15-18).

The ionic mechanism responsible for this effect of baclofen action is not clear. In cell bodies of primary afferent fibers baclofen shortens the action potential (19, 20), from which it has been suggested that it reduces voltage-dependent Ca<sup>2+</sup> conductance (19). In hippocampal cell somata, on the other hand, baclofen induces a membrane hyperpolarization and an increased input conductance, suggesting an increased K<sup>+</sup> permeability (21-23); if such an effect occurred at nerve terminals it could also affect action potential duration and reduce Ca<sup>2+</sup> influx in the absence of a direct effect on Ca<sup>2+</sup> currents.

To obtain better resolution of the ionic permeability changes induced by baclofen, we have studied its effect on voltage-clamped hippocampal neurones under conditions in which actions on K<sup>+</sup> and Ca<sup>2+</sup> conductance can be discriminated. We find that baclofen induces a K<sup>+</sup> current without inhibiting directly the slow inward Ca<sup>2+</sup> current. We also show that the effect of baclofen can be replicated by GABA when GABA<sub>B</sub> receptors are blocked, so supporting the view (2) that GABA may be the endogenous ligand for baclofen receptors.

MATERIALS AND METHODS

Experiments were performed on hippocampal slices prepared from 7-day-old Wistar rats and cultured organotypically for 3-5 weeks in vitro as described previously (24, 25). The slice was superfused with Hanks’ balanced salt solution at 35°C at a rate of 1 ml/min. The composition of the solution was (mM): Na<sup>+</sup>, 141.7; K<sup>+</sup>, 5.8; Cl<sup>−</sup>, 147.8; Ca<sup>2+</sup>, 2.8; Mg<sup>2+</sup>, 0.9; HCO<sub>3</sub> -, 4.2; SO<sub>4</sub><sup>2-</sup>, 0.4; H<sub>2</sub>PO<sub>4</sub> -, 0.4; d-glucose, 5.6. Individual CA<sub>3</sub> pyramidal cells were impaled with a single microelectrode containing either 3 M KCl or 2 M CsCl (tip resistance 25-35 MΩ or 15-25 MΩ, respectively) and voltage-clamped with an amplifier (Axoclamp-2, Axon Instruments, Burlingame, CA) switching between current-passing and voltage-sampling modes at 2-6 kHz. A continuous voltage-output display was monitored to ensure that the voltage drop across the electrode induced by the current pulses had dissipated between voltage-sampling times, so that the measured voltage referred solely to the cell membrane potential. Fast Na<sup>+</sup> currents were eliminated by adding 1 μM tetrodotoxin to the perfusion fluid. During the slow currents recorded in these experiments, measured voltages were within 1-2 mV of the nominal command voltages. All potentials are corrected for electrode potentials registered after withdrawing the electrode from the cell. Baclofen was obtained from CIBA-Geigy in (+), (−), and (±) forms; in most experiments we used the (±) form.

RESULTS

Baclofen-Induced Outward Currents. In cells impaled with KCl-filled microelectrodes and voltage-clamped at or near their resting potential (−55 to −65 mV), (±)-baclofen (10 μM) always produced an outward (hyperpolarizing) membrane current (Fig. 1). During this current the amplitudes of the current deflections produced by superimposed short (1 s) voltage commands were greatly increased, showing that baclofen approximated doubled the cell’s input conductance at the holding potential. This effect was readily reversible on washing out the baclofen and could be induced several times on the same cell at intervals of 10 min or so without loss of sensitivity. The action of (±)-baclofen was inhibited by 10 μM (−)-baclofen but not by 10 μM (±)-baclofen.

Outward Current Reversal Potential. "Steady-state" cur-

Abbreviation: GABA, γ-aminobutyric acid.

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rent–voltage (I/V) curves were constructed by measuring the currents attained at the end of the 1-s commands and adding these to the holding current. The net current induced by baclofen \( I_{bac} \) was calculated by subtracting the I/V curves obtained before and after adding baclofen from the curve obtained during baclofen perfusion. In the cell illustrated in Fig. 1, \( I_{bac} \) reversed from outward to inward at –82 mV—i.e., 21 mV hyperpolarized to the resting potential of –61 mV (Fig. 2). This is also apparent from the record in Fig. 1, since the current level attained during the 20-mV hyperpolarizing commands was the same in the absence and presence of baclofen. The mean (±SD) reversal potential for \( I_{bac} \) was –74 ± 4.3 mV in 11 cells.

\( I_{bac} \) Is a K⁺ Current. When [K⁺]o was raised 3-fold, from 5.8 to 17.4 mM, the reversal potential shifted +26 mV, to –56 mV (Fig. 2); this is close to the +29-mV shift predicted from the Nernst equation for K⁺, suggesting that \( I_{bac} \) is substantially or exclusively a K⁺ current.

Rectification of \( I_{bac} \). The curves in Fig. 2 indicate that \( I_{bac} \) shows appreciable inward rectification—that is, the current becomes larger in the inward direction. A small time-dependent component of this inward current is visible in Fig. 1, but inward rectification was still apparent when currents were measured 20 ms after the imposed voltage step, immediately after the capacity transients had settled.

External Ba²⁺ Blocks \( I_{bac} \). Some inwardly rectifying K⁺ currents are blocked by external Ba²⁺ (30, 31). Fig. 3 shows that \( I_{bac} \) is also effectively blocked by Ba²⁺. Ba²⁺ ions also block the voltage-dependent K⁺ current \( I_M \) in hippocampal neurones (28, 32). However, \( I_{bac} \) differs from \( I_M \) in that \( I_{bac} \) became larger with hyperpolarization instead of smaller and was not inhibited by 10 μM acetylcholine, which suffices to inhibit \( I_M \) in these cultured hippocampal cells (unpublished observations; cf. ref. 28).

Ba²⁺ ions also inhibit the Ca²⁺-activated K⁺ current \( I_C \) previously described in hippocampal neurones (29). Unlike \( I_C \), however, \( I_{bac} \) was not inhibited by 100 μM Cd²⁺ (see also ref. 22), showing that \( I_{bac} \) did not depend on a prior influx of Ca²⁺ through Cd²⁺-sensitive Ca²⁺ channels.

GABA Can Imitate Baclofen. Fig. 4 shows a comparison of the effects of baclofen and GABA on a voltage-clamped hippocampal neurone. In contrast to baclofen, application of GABA (100 μM) at a clamping holding potential of –62 mV produced an inward current. This may be ascribed to a GA-BA₂₆-mediated increase in Cl⁻ conductance, the inward current resulting from the high intracellular Cl⁻ concentration attained with KCl electrodes (33–35). The mean (±SEM) reversal potential for \( I_{GABA} \) in six experiments was –46.7 ± 4.2 mV—i.e., on average 26.0 ± 5.0 mV positive to the reversal potential for \( I_{bac} \) in the same cells (Table 1). After 10
μM pitrazepin, a GABA_A antagonist (23), had been added to the perfusion fluid, GABA produced an outward current, with a reversal potential of −71.7 ± 1.2 mV—identical with that for \( I_{\text{bac}} \) (Table 1). Pitrazepin did not affect either the magnitude of \( I_{\text{bac}} \) or its reversal potential. In the presence of pitrazepin, \( I_{\text{GABA}} \) also showed the inward rectification previously noted for \( I_{\text{bac}} \); before pitrazepin \( I_{\text{GABA}} \) did not show rectification. Like \( I_{\text{bac}} \), \( I_{\text{GABA}} \) in the presence of pitrazepin was blocked by 1 mM Ba\(^{2+}\). These experiments suggest that GABA and baclofen activate the same K\(^+\) conductance in hippocampal cells under conditions in which the increased Cl\(^-\) conductance produced by GABA is suppressed.

**Baclofen Does Not Inhibit Ca\(^{2+}\) Current.** In Fig. 1 an inward current deflection may be noted after the initial transient outward current during the depolarizing command. This is generated by the slow inward Ca\(^{2+}\) current \( I_{\text{Ca}} \) previously described in hippocampal neurones (27, 36). \( I_{\text{Ca}} \) itself is largely masked by subsequent outward K\(^+\) currents during the depolarizing step, but its continued presence throughout the command is suggested by the inward tail current when the holding potential is restored. This inward tail may reflect both persistence of \( I_{\text{Ca}} \) (27) and a residual Ca\(^{2+}\)-activated Cl\(^-\) current (43). In baclofen solution, \( I_{\text{Ca}} \) is less apparent during the depolarizing command, but the repolarizing tail current is still present; this suggests that the reduction in \( I_{\text{Ca}} \) may be secondary to the increased outward current. To test the effect of baclofen on \( I_{\text{Ca}} \) itself, outward currents were inhibited by injecting Cs\(^+\) into the neurone by using a CsCl-filled microelectrode (36). This also inhibited the outward current generated by baclofen. Under these conditions depolarizing

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**Table 1. Reversal potentials for the ionic currents induced by GABA and baclofen before and after addition of pitrazepin**

<table>
<thead>
<tr>
<th>Holding potential, ( mV )</th>
<th>Control</th>
<th>GABA</th>
<th>Baclofen</th>
<th>Pitrazepin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−61</td>
<td>−53</td>
<td>−76</td>
<td>−74</td>
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<td>6</td>
<td>−62</td>
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<td>Mean</td>
<td>−46.7</td>
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<td>−71.7</td>
<td>−71.7</td>
</tr>
<tr>
<td>SEM</td>
<td>±4.2</td>
<td>±1.5</td>
<td>±1.2</td>
<td>±2.2</td>
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</tbody>
</table>

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**Fig. 4.** GABA imitates baclofen when GABA\(_A\)-mediated Cl\(^-\) conductance is suppressed. The records in A show currents induced by 10 μM baclofen and 100 μM GABA applied before and after adding 10 μM pitrazepin (23) to the perfusion fluid. Holding potential was −62 mV. Downward deflections are currents induced by 1-s 20-mV hyperpolarizing commands at 15-s intervals (cf. Fig. 1). Outward current upwards. Note that pitrazepin reversed the GABA-induced current from inward to outward but did not affect the baclofen-induced current. The graphs in B show the voltage dependence of GABA- and baclofen-induced currents recorded in the absence (●, ○) and presence (●, ○) of pitrazepin. Graphs were obtained from subtracted I/V curves as described for Fig. 2.
recorded in during 1-s mV hyperpolarizing previously commands rapid These currents of Fig. of the principal detected in these ward membrane potential for cells pal K+ with blocked the GABAA-mediated current a GABA hippocampal CA1 experiments of the expected "leak" currents showed by baclofen on mammalian primary afferent fibers, since baclofen failed to reduce the "Ca2+ component" of the action potential in sensory spinal ganglion cells impaled with Cs+-filled microelectrodes (20) in which \( I_{\text{bac}} \) would be suppressed.

In subsequent experiments we have observed that the inward currents produced by baclofen and GABA (in the presence of pitrazepin) at membrane potentials negative to their reversal potential were also blocked in a voltage-dependent manner by 1 mM external Cs+ (unpublished observations). Since other inwardly rectifying K+ currents show voltage-dependent block by Cs+ (40–42) this supports our view that both compounds activate the same species of inward-rectifying K+ conductance.

This work was partly supported by a grant from the U.K. Medical Research Council to D.A.B.


**DISCUSSION**

The principal effect of baclofen on hippocampal neurones detected in these experiments is the generation of an outward membrane current \( I_{\text{bac}} \). This we attribute to an increased K+ conductance for three reasons. (i) The reversal potential for \( I_{\text{bac}} \) shows the expected dependence on external K+ concentration; (ii) \( I_{\text{bac}} \) is inhibited by external Ba2⁺; and (iii) it is also inhibited by internal Cs⁺. This current is presumably responsible for the hyperpolarization of hippocampal cells previously reported (21–23). The inward rectification would explain why this hyperpolarization appeared to diminish when the cells were depolarized (22). A comparable current probably underlies the hyperpolarizing effect of baclofen on *Onchidium* neurones (38).

This effect of baclofen probably results from an action on GABA receptors since it could be replicated by GABA when the GABA-A-mediated increase in Cl⁻ conductance was blocked with pitrazepin. Newberry and Nicoll (39) have also detected a shift in the reversal potential for the effect of GABA on hippocampal CA1 neurones to more hyperpolarized levels in the presence of the GABA-A antagonist bicuculline. In the absence of a selective GABA-B antagonist we cannot be certain that this reflects an effect of GABA and baclofen on the same receptor, as distinct from independent activation of the same conductance mechanism via different receptors, although ligand-binding studies (20) support a common receptor interaction.

At rest potentials between −50 and −70 mV the increased K+ conductance produced by GABA via GABA-A receptors was much less than the increased Cl⁻ conductance associated with GABA-A-receptor activation, so that in the absence of GABA antagonists the latter dominates the overall response to exogenous GABA. Thus, inhibition of the K⁺ component with Ba²⁺ did not noticeably change the reversal potential for GABA in the absence of pitrazepin. The increased K⁺ conductance becomes greater at membrane potentials beyond −80 mV, but it is difficult to imagine its significance during simultaneous activation of GABA-A receptors. Activation of this K⁺ current might be of more importance to the effects of synaptically released GABA if, as suggested by Newberry and Nicoll (39), there is a temporal dissociation between the activation of GABA-A and GABA-B receptors.

We could detect no effect of baclofen on \( I_{\text{ca}} \) in hippocampal neurones under conditions in which the K⁺ conductance was blocked. We therefore suggest that the inhibition of transmitter release by baclofen postulated to occur in the terminals of CA1 cell axons (13–15) is more likely to result from the indirect effects of the increased K⁺ conductance on Ca²⁺ influx than from a direct effect on Ca²⁺ conductance per se. This may also be true for the effect of baclofen on mammalian primary afferent fibers, since baclofen failed to reduce the "Ca²⁺ component" of the action potential in sensory spinal ganglion cells impaled with Cs⁺-filled microelectrodes (20) in which \( I_{\text{bac}} \) would be suppressed.

In subsequent experiments we have observed that the inward currents produced by baclofen and GABA (in the presence of pitrazepin) at membrane potentials negative to their reversal potential were also blocked in a voltage-dependent manner by 1 mM external Cs⁺ (unpublished observations). Since other inwardly rectifying K⁺ currents show voltage-dependent block by Cs⁺ (40–42) this supports our view that both compounds activate the same species of inward-rectifying K⁺ conductance.

<table>
<thead>
<tr>
<th>Control</th>
<th>+ Baclofen</th>
<th>Wash</th>
</tr>
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<tbody>
<tr>
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<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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<td>20 mV</td>
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**Fig. 5.** Lack of effect of baclofen (10 μM) on inward currents recorded in a cell impaled with a 2 M CsCl-filled microelectrode during 1-s step depolarization of +20 mV from a holding potential of 40 mV. The inward currents show fast transient and slow sustained responses (cf. ref. 37), the former being incompletely clamped. The lower records show corresponding "leak" currents induced by 20-mV hyperpolarizing commands.