Metabotropic Glutamate Receptor Agonist ACPD Inhibits Some, but Not All, Muscarinic-Sensitive K⁺ Conductances in Basolateral Amygdaloid Neurons

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KEY WORDS Amygdala, K⁺ currents, Glutamate receptors, ACPD

ABSTRACT Muscarinic agonists produce membrane depolarization and losses of spike frequency accommodation and the slow afterhyperpolarization (AHP) when applied to neurons of the basolateral amygdala (BLA). Underlying these changes are the muscarinic-induced inhibitions of several K⁺ conductances, including the voltage-activated M-current (I₇), a slowly decaying Ca²⁺-activated current (IₐHP), a voltage-insensitive leak current (Iₑₐ₉), and the hyperpolarization-activated inward rectifier current (Iₐ₉). Similar depolarizations and losses of the slow AHP have been observed in other neuronal cell types following stimulation of metabotropic glutamate receptors. Therefore, we tested the effects of the metabotropic glutamate receptor agonist, 1-aminocyclopentane-1s,3a-dicarboxylic acid (ACPD), on pyramidal neurons impaled with a single microelectrode for current- and voltage-clamp recordings in a brain slice preparation of the rat BLA. Application of ACPD (20 or 100 μM) to BLA neurons inhibited I₇ and IₐHP, resulting in membrane depolarization and reductions in the amplitude and duration of the slow AHP. However, ACPD did not inhibit the muscarinic-sensitive current I₉, nor was Iₑ₉ blocked in the majority of neurons examined. These findings suggest the possibility that muscarinic cholinergic and metabotropic glutamatergic receptor agonists may activate separate intracellular transduction pathways which have convergent inhibitory effects onto I₇ and IₐHP in BLA pyramidal neurons.

INTRODUCTION

The basolateral nucleus of the amygdala (BLA) receives a substantial cholinergic innervation from neurons of the basal forebrain region (Carlson et al., 1985; Hellendall et al., 1986). Stimulation of this pathway or direct application of muscarinic agonists to BLA pyramidal neurons in vitro results in membrane depolarization and losses of spike frequency accommodation and the slowly decaying portion of the afterhyperpolarization (AHP), changes which serve to greatly enhance the level of neuronal excitability (Washburn and Moises, 1992). Similar responses to cholinergic stimulation have also been observed in neurons from several regions of the mammalian brain (Benardo and Prince, 1982; Cole and Nicoll, 1983; Halliwell and Adams, 1982; Madison et al., 1987; McCormick and Prince, 1986; Uchimura and North, 1990). Voltage-clamp analysis of BLA neurons reveals that the muscarinic-induced depolarization results from inhibitions of the voltage-activated M-current (I₇) and a voltage-insensitive K⁺ leak current (Iₑ₉) (Womble and Moises, 1992), while the losses of accommodation and the slow AHP are due to the muscarinic inhibition of a slowly decaying, Ca²⁺-activated K⁺ current (IₐHP) (Womble and Moises, 1993a). In addition to these currents, BLA neurons also possess another muscarinic-sensitive K⁺ current, the hyperpolarization-activated inward rectifier current (I₉) (Womble and Moises, 1993b). The mechanisms underlying muscarinic inhibition of K⁺ conductances in BLA pyramidal neurons is unknown. However, in hippocampal pyramidal neurons, muscarinic receptors are positively coupled to the phosphoinositide (PI) transduction pathway (Fisher and Bartus, 1985), activation of which is thought to mediate the muscarinic inhibitions of I₉, Iₑ₉, and IₐHP (Baraban et al., 1985; Dutar and Nicoll, 1988a,b; Malenka et al., 1986). The BLA also receives a substantial excitatory glutamatergic innervation (Rainnie et al., 1991). Recently, a new subtype of glutamate receptor, the metabotropic receptor, has been identified (Sugiyama et al., 1987). Activation of metabotropic glutamate receptors in-

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creases PI hydrolysis in several cell types, leading to production of the second messengers diacylglycerol and inositol 1,4,5-trisphosphate (IP$_3$) and mobilization of intracellular Ca$^{2+}$ stores (Desai and Conn, 1990; Furuya et al., 1989; Sladeczek et al., 1985; Sugiyama et al., 1987). Stimulation of these receptors on hippocampal pyramidal neurons mimics several of the actions produced by muscarinic agonists, including membrane depolarization and reductions in accommodation and the slow AHP (Desai and Conn, 1991; Stratton et al., 1989). Several studies have shown that these changes result, at least in part, from the metabotropic glutamatergic-induced inhibitions of $I_{M}$ and $I_{AHP}$ (Baskys, 1992; Charpak et al., 1990; Ito et al., 1992). The effect of metabotropic receptor activation on $I_{Leak}$ has not been examined. Although the intracellular steps linking stimulation of PI hydrolysis to the inhibition of $I_{M}$ or $I_{AHP}$ remain unclear, these findings suggest the possibility that a common intracellular transduction pathway may be activated by muscarinic and metabotropic glutamatergic agonists. The presence of metabotropic glutamate receptors which are functionally coupled to increased PI hydrolysis have been demonstrated in the rat amygdala (Akiyama et al., 1992; Martin et al., 1992). Therefore, we have voltage-clamped BLA pyramidal neurons in a slice preparation of the basal forebrain to examine the possibility that application of the metabotropic receptor agonist, 1-aminocyclopentane-1s,3R-dicarboxylic acid (ACPD) (Desai and Conn, 1990; Palmer et al., 1989), might produce inhibition of the muscarinic-sensitive currents $I_{M}$, $I_{Leak}$, $I_{AHP}$, or $I_{IR}$. Our findings demonstrate that ACPD inhibited $I_{M}$ and $I_{AHP}$, resulting in membrane depolarization accompanied by reductions of spike frequency accommodation and the slow AHP. However, $I_{Leak}$ and $I_{IR}$ were unaffected by ACPD treatment. Some of these results have previously been reported in abstract form (Womble et al., 1992).

MATERIALS AND METHODS

Horizontal slices of the ventral forebrain (500 μm) were prepared from adult male rats and maintained in a recording chamber perfused with physiological saline saturated with 95% O$_2$-5% CO$_2$ at room temperature. The saline consisted of (in mM) NaCl, 124; KCl, 3.5; CaCl$_2$, 3.0; MgSO$_4$, 1.5; Na$_2$PO$_4$, 1.0; NaHCO$_3$, 26.2; glucose, 11.0, pH 7.3. In various experiments, 2.7 M KCl/0.4 M potassium acetate (30–100 μM) for current- and voltage-clamp recording using an Axoclamp 2A sample and hold amplifier, as previously described (Womble and Moises, 1992, 1993a,b). The data were digitized using pClamp software (Axon Instruments) and analyzed offline with DAOS software (Laboratory Software Associates, Victoria, Australia), equipped with a cursor-based, least-squares exponential curve fitting routine. Fitted curves were extrapolated back to the preceding voltage change and used for estimation of the instantaneous and steady-state current values. Statistical differences were determined using the paired t-test.

RESULTS

Stable intracellular recordings were obtained from 16 BLA pyramidal neurons, with an average resting membrane potential of $-66.1 \pm 1.3$ mV (± S.E.M.), similar to previous descriptions of these cells (Washburn and Moises, 1992; Womble and Moises, 1992, 1993a,b). Injection of a prolonged (500 ms) depolarizing current pulse through the recording electrode caused the cell to fire a burst of action potentials whose rate of firing accommodated with time (Fig. 1A). Termination of the current pulse was followed by a long-lasting, biphasic AHP, consisting of an initial, rapidly decaying component (medium AHP) and a prolonged, slowly decaying component (slow AHP) (Fig. 1B), as previously described (Washburn and Moises, 1992; Womble and Moises, 1993a). Rapid switching of the amplifier to voltage-clamp mode upon termination of the depolarizing current pulse (hybrid-clamp) revealed the presence of a biphasic outward tail current that corresponded in time with the AHP (Fig. 1C). Similar biphasic tail currents were also observed in voltage-clamped BLA neurons following a 500 ms depolarizing voltage step to $-30$ mV from a $-60$ mV holding potential (not shown). We have previously shown that the later, slowly decaying component of the AHP tail current consists of a slowly decaying Ca$^{2+}$-activated K$^+$ current ($I_{AHP}$) (Lancaster and Adams, 1986; Madison et al., 1987), which is responsible for spike frequency accommodation and production of the slow AHP in BLA neurons (Womble and Moises, 1993a). In the present study, $I_{AHP}$ was measured by extrapolating a single exponential curve fitted to the slowly decaying portion of the AHP tail current back to the end of the previous depolarizing step. This analysis yielded for $I_{AHP}$ an average peak amplitude of $54 \pm 9$ pA and decay tau of $2,139 \pm 407$ ms ($n = 15$), values which are similar to those previously reported for BLA neurons (Womble and Moises, 1993a).

Within minutes of changing the bath perfusate to medium containing ACPD (20 or 100 μM), BLA pyramidal neurons depolarized by an average of $0.8 \pm 0.9$ mV ($P < 0.01; n = 16$), from their normal resting level of $-66.1$ mV to an average of $-63.1 \pm 1.7$ mV. The depo-
ACPD INHIBITION OF K⁺ CONDUCTANCES

Fig. 1. Effects of ACPD on accommodation and the slow AHP. A: Injection of a 500 ms depolarizing current pulse (+0.2 nA) elicited a series of action potentials in control medium whose rate of firing declined with time before firing ceased. This accommodation response was decreased during perfusion of 20 μM ACPD, an effect that was reversed after washing for 30 min with control saline. B: Termination of a 500 ms depolarizing current pulse was followed in control medium by a long-lasting, biphasic AHP. Application of 20 μM ACPD inhibited the slowly decaying portion of the AHP, an effect reversed by washing with control saline for 30 min. C: Hybrid-clamp records obtained from the same neuron as B. The cell was depolarized in current-clamp mode by a depolarizing voltage step to −30 mV from a holding potential of −60 mV (not shown). Overall, ACPD (20 or 100 μM) reduced Imp amplitude by 63%, from 54 ± 9 pA to 20 ± 6 pA (n = 15; P < 0.005). The inhibitory action of ACPD on Imp was not prevented in the presence of the muscarinic receptor antagonist atropine (2 μM; n = 4) or a combination of the ionotropic glutamate receptor antagonists CNQX (20 μM) and APV (50 μM) (n = 4) (Fig. 1).

The voltage- and muscarinic-sensitive M-current (Brown and Adams, 1980; Halliwell and Adams, 1982; Womble and Moises, 1992) was identified in voltage-clamped BLA neurons as a slow inward current relaxation elicited during a 1 s hyperpolarizing voltage step to −55 mV from a holding potential of −40 mV (Fig. 2). The current relaxation followed a single exponential time course, with an average decay tau of 220 ± 39 ms and peak amplitude of 82 ± 11 pA (n = 12), in agreement with our previous measurements of IM in BLA neurons (Womble and Moises, 1992). The inward current relaxation observed during the voltage step to −55 mV was not due to decay of IAHP, since it was unchanged after blockade of IAHP by bath application of

larization produced by 20 μM or 100 μM ACPD was similar in magnitude, suggesting that the lower concentration produced a maximal effect. However, the 3.0 mV resting membrane depolarization produced by ACPD was less than half of the depolarization produced in BLA neurons by the cholinergic agonist carbachol (6.5 ± 1.0 mV, n = 13, P < 0.05; data from Womble and Moises, 1992). This provided us with the first indication that ACPD does not fully replicate the effects of muscarinic receptor stimulation in BLA neurons.

In three neurons examined in the absence of TTX, application of ACPD decreased the accommodation response normally observed during a 500 ms depolarizing current pulse (Fig. 1A) and reduced the amplitude and duration of the subsequent slow AHP (Fig. 1B). Recordings obtained from these cells using the hybrid-clamp protocol revealed a concomitant inhibition of the underlying IAHP (Fig. 1C). The inhibitory effects of ACPD on accommodation, the slow AHP and IAHP were reversed within 30 min of switching to normal ACSF bathing solution (Fig. 1). Similar reductions in IAHP amplitude were observed in 12 other neurons in the presence of TTX in which IAHP was activated in voltage-clamp mode by a depolarizing voltage step to −30 mV from a holding potential of −60 mV (not shown). Overall, ACPD (20 or 100 μM) reduced IAHP amplitude by 63%, from 54 ± 9 pA to 20 ± 6 pA (n = 15; P < 0.005). The inhibitory action of ACPD on IAHP was not prevented in the presence of the muscarinic receptor antagonist atropine (2 μM; n = 4) or a combination of the ionotropic glutamate receptor antagonists CNQX (20 μM) and APV (50 μM) (n = 4) (Fig. 1).

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200 μM Cd²⁺ (n = 2) or 2 mM Co²⁺ (n = 1), or when 100 μM cyclic-AMP was included in the recording electrode (n = 3) (Womble and Moises, 1993a). In these experiments, following blockade of the slow AHP and Iₐhp, by the calcium channel blockers or intracellular perfusion of cyclic AMP, hyperpolarizing voltage steps to −55 mV from a holding potential of −40 mV revealed M-currents with an average decay tau of 191 ± 22 ms and amplitude of 107 ± 22 pA (n = 6) (data not shown).

Application of ACPD (20 μM or 100 μM) blocked the M-current, as evidenced by the loss of the inward current relaxation during the hyperpolarizing voltage step (Fig. 2), an effect that was reversed by washing for 30 min with normal bathing medium. Overall, ACPD reduced the amplitude of the M-current relaxation by 61%, from 82 ± 11 pA to 32 ± 9 pA (n = 12; P < 0.005). The inhibitory action of ACPD on Iₘ was not prevented in the presence of atropine (2 μM; n = 4) or a combination of CNQX (20 μM) and APV (50 μM) (n = 2) (data not shown).

Muscarnic agonists produce an inward shift in the level of steady-state current recorded at a holding potential of −40 mV in hippocampal and BLA pyramidal neurons, due to the inhibition of Iₘ and a voltage-insensitive K⁺ leak current (Iₐk) (Benson et al., 1988; Madison et al., 1987; Womble and Moises, 1992). Since perfusion of ACPD blocked Iₘ and produced a similar inward shift in holding current (Fig. 2), we examined the possibility that ACPD might also block Iₐk in BLA neurons. To test this, cells were voltage-clamped at a holding potential of −70 mV, where Iₘ is largely inactive (Womble and Moises, 1992), and a series of hyperpolarizing voltage steps was applied to command potentials of −75 to −140 mV (Fig. 3A). Current-voltage (I-V) relationships were constructed by plotting the instantaneous current response derived from exponential curves fitted to the current elicited during a hyperpolarizing voltage step as a function of the membrane potential obtained during the step (Fig. 3B). These plots were linear over the tested range of voltages, reflecting the presence of the voltage-insensitive leak conductance. In the majority of cells tested (64%; 9 of 14), application of 20 or 100 μM ACPD had no effect on this I-V relationship, indicating that Iₐk was unaffected by the drug in these neurons. In the remaining five neurons, ACPD appeared to produce a small decrease in conductance, suggesting a possible reduction in Iₐk.

However, several other lines of evidence support the conclusion that Iₐk was not blocked by ACPD. The level of steady-state current measured at a holding potential of −70 mV was not significantly altered by ACPD (control level of 80 ± 20 pA vs. 57 ± 30 pA in the presence of ACPD; n = 14; P > 0.5). Similarly, membrane conductance measurements obtained from the instantaneous current response recorded at the onset of a voltage step from −70 mV to −77 mV were unchanged by ACPD (9.2 ± 0.8 nS in controls vs. 8.7 ± 0.6 nS with ACPD; n = 14). These findings stand in contrast to the muscarinic inhibition of Iₐk in BLA neurons, where carbachol (40 μM) produced an average inward current shift of 53 ± 9 pA (n = 15) in steady-state current level recorded at a holding potential of −70 mV (Womble and Moises, 1992) and an average decrease in instantaneous membrane conductance measured during a voltage step from −70 mV to −77 mV of 2.4 ± 0.9 nS (n = 8; data not shown).

Two hyperpolarization-activated currents, the H-current (Iₜ) (Halliwell and Adams, 1982; Mayer and Westbrook, 1983) and the inward rectifier current (Iᵢᵣ) (Constanti and Galvan, 1983; Uchimura and North, 1990), have also been observed in BLA pyramidal neurons (Womble and Moises, 1993b). These currents differ in their rates and voltage ranges of activation, as illustrated in Figure 3. Stepping a BLA neuron from a holding potential of −70 mV to −99 mV for 1 s activated only Iᵢᵣ, producing a slowly developing inward current...
that followed a single exponential time course (Fig. 3A).
With a larger voltage step to a command potential of
-120 mV, the H-current component was preceded by
activation of the rapidly developing $I_{IR}$. Plotting the
total current evoked at -120 mV on a semi-logarithmic
scale (open symbols, Fig. 3C), enabled us to separate
the $I_{IR}$ and $I_{Ih}$ current components by exponential curve
peeling. During a step from -70 mV to -120 mV, the $I_{Ih}$
component had an average amplitude of 199 ± 30 pA
and activation tau of 188 ± 47 ms (n = 9), while the
amplitude of the $I_{IR}$ component was 125 ± 16 pA, with
an activation tau of 49 ± 9 ms (n = 8), in agreement
with our previous measurements of these current components
in BLA neurons (Womble and Moises, 1993b).

We have previously shown that $I_{IR}$ but not $I_{Ih}$ is
inhibited following muscarinic receptor activation
(Womble and Moises, 1993b). However, as shown in
Figure 3, ACPD (20 μM) did not alter the time courses of
current activation or the amplitudes of either $I_{Ih}$ or
$I_{IR}$. Overall, ACPD (20 or 100 μM) did not reduce the
amplitudes of either $I_{Ih}$ (199 ± 30 pA versus 177 ± 32
pA with ACPD; n = 9) or $I_{IR}$ (125 ± 16 pA vs. 125 ± 12
pA with ACPD; n = 8) when measured during a step
from -70 mV to -120 mV.

**DISCUSSION**

Muscarinic receptor activation produces several changes in BLA pyramidal neurons, including pro-
longed membrane depolarization and losses of spike frequency accommodation and the slow AHP (Wash-
burn and Moises, 1992). These effects have been shown to result from the muscarinic inhibition of several $K^+$
conductances, including $I_{M}$, $I_{Leak}$, $I_{AHP}$, and $I_{IR}$ (Wom-
bble and Moises, 1992, 1993a,b). The present data dem-
onstrate that the metabotropic glutamate receptor agonist, 1s,3a-ACPD, mimicked several of these musca-
rinic effects. We found that ACPD inhibited $I_{AHP}$ in
BLA pyramidal neurons and thus reduced the spike
frequency accommodation response normally observed
during a current-induced burst of action potentials, as
well as the slowly decaying portion of the AHP which
followed. This agonist also blocked the M-current, pro-
ducing a sustained depolarization of the cell. However,
unlike the actions of muscarinic agonists, ACPD did not
appear to inhibit $I_{Leak}$ or $I_{IR}$. The lack of effect on $I_{Leak}$
may account for the finding that the depolarization pro-
duced in BLA neurons by a saturating concentration of
ACPD was significantly less than that produced by car-
bachol (Womble and Moises, 1992).

Cloning and expression studies have identified a
family of metabotropic receptor subtypes (Aramori and
Nakanishi, 1992; Houamed et al., 1991; Masu et al.,
1991; Tanabe et al., 1992). These receptors have been
shown to be linked to the stimulation of PI hydrolysis
and generation of diacylglycerol and IP3 (Desai and
Conn, 1990; Funaya et al., 1989; Sladecek et al., 1985;
Sugiyama et al., 1987), although effects on cyclic-AMP,
arachidonic acid and phospholipase D metabolism have
also been observed (Bos and Conn, 1992; Dumuis
et al., 1990; Schoepf et al., 1992; Winder and Conn,
1992). In hippocampal pyramidal neurons, activation of
PI turnover has been implicated in the inhibitions of
$I_{AHP}$ and $I_{M}$ induced by application of either muscarinic
receptor agonists (Baraban et al., 1985; Dutar and
Nicoll, 1988a,b; Malenka et al., 1986) or metabotropic glutamate receptor agonists (Baskys, 1992; Charpach et al., 1990). However, more recent evidence suggests that the direct excitatory effects of ACPD on hippocampal pyramidal neurons may be mediated by a separate class of metabotropic receptors which are positively linked to phospholipase D activation (Boss and Conn, 1992; Desai et al., 1992). Our results demonstrate that, like muscarinic cholinergic receptors, the metabotropic glutamate receptors of BLA pyramidal neurons which are activated by ACPD are functionally coupled to the inhibition of IAHP and IM. However, metabotropic receptor activation did not result in blockade of the muscarinic-sensitive currents IR, nor was LIs inhibited in the majority of BLA neurons examined. One possible interpretation of these results is that activation of the PI transduction system following muscarinic receptor activation results in the blockade of all four muscarinic-sensitive K+ currents, while activation of the phospholipase D system by metabotropic glutamatergic agonists produces inhibitions of only IM and IAHP. Thus, there may be a partial convergence of action between two intracellular transduction systems in pyramidal neurons of the basolateral amygdala.

ACKNOWLEDGMENTS

This work was supported by DA03365 and AG10667 to H.C.M.

REFERENCES


