Properties of a Ca\textsuperscript{2+}-Activated K\textsuperscript{+} Conductance in Acutely Isolated Pyramidal-Like Neurons From the Rat Basolateral Amygdaloid Complex

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Meis, S. and H.-C. Pape. Properties of a Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductance in acutely isolated pyramidal-like neurons from the rat basolateral amygdaloid complex. J. Neurophysiol. 78: 1256–1262, 1997. A calcium (Ca\textsuperscript{2+})-activated potassium (K\textsuperscript{+}) conductance was studied in large pyramidal-like neurons acutely dissociated from the rat basolateral amygdaloid complex. Neurons were immunoreactive to anti-α\textsubscript{(913-926)}, a sequence-directed antibody directed against the pore-forming α-subunit of the BK\textsubscript{Ca} channel, also termed slo. Whole cell current-voltage (I-V) relationships obtained on application of slow (46.7 mV/s) voltage ramps from -110 to +100 mV were N shaped positive to -30 mV. Maximal current activation occurred at +9.8 ± 2.7 (SE) mV, with a mean current density of 404.8 ± 25.0 pA/pF. Substitution of extracellular Ca\textsuperscript{2+} with manganese (Mn\textsuperscript{2+}), or with magnesium (Mg\textsuperscript{2+}) and addition of 5 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, abolished the N-shaped I-V relationship with a reduction in maximal outward current to 15.3 ± 2.3% of the control value. The Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} current component, as revealed by voltage step protocols, activated at depolarizations positive to -30 mV with a slow time course (time constant 430.7 ± 78.6 ms). The current was reduced by 80.4 ± 4.6% through 1 mM tetraethyammonium chloride and by 66.8 ± 3.4% through 100 nM iberiotoxin, whereas apamin up to 1 μM had no effect. It is concluded that pyramidal-like neurons of the basolateral amygdaloid complex possess BK\textsubscript{Ca} channels and the corresponding macroscopic Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} conductance, activation of which will substantially contribute to the Ca\textsuperscript{2+}-dependent regulation of electrogenic behavior in these neurons.

INTRODUCTION

The amygdala is involved in multiple complex behaviors and cognitive functions such as learning, emotion, social cognition, and memory and, in addition, seems to be particularly susceptible to epileptic discharges and dysfunctions relating to various neurological disorders (Aggleton 1993). The basolateral division represents the major recipient of incoming sensory information to the amygdala and gives rise to a large number of intra-amygdaloid projections (Pitkänen et al. 1995; Savander et al. 1995). The vast majority of cells within the basolateral amygdaloid complex are spiny pyramidal-like neurons, among which projection cells providing the major excitatory output of the complex have been characterized morphologically and immunologically (McDonald 1992, 1996; Millhouse and DeOlmos 1983). Recent studies aimed at the investigation of the intrinsic electrophysiological properties of amygdaloid neurons with spiny, pyramidal-like morphology demonstrated a wide range of electrogenic behavior, including accommodating/nonaccommodating spike patterns, burst firing, and rhythmic-oscillatory activity (Pape and Paré 1995; Paré et al. 1995; Rainnie et al. 1993; Sugita et al. 1993; Washburn and Moises 1992a). Ca\textsuperscript{2+}-dependent mechanisms appear to be most important for the regulation of these firing patterns. For instance, the neurons are capable of producing high-threshold Ca\textsuperscript{2+} spikes (Washburn and Moises 1992b), and the resulting inward flux of Ca\textsuperscript{2+} is thought to control a number of secondary processes, such as a chloride conductance (Sugita et al. 1993) and a number of K\textsuperscript{+} conductances (Danobert and Pape 1996; Driesang and Pape 1997; Lang and Paré 1996; Womble and Moises 1993), which, in turn, shape the form of individual action potentials and afterpotentials as well as repetitive firing patterns and synaptic responses. Moreover, stimulation of metabotropic glutamate receptors in basolateral amygdaloid neurons was found to result in a membrane hyperpolarization, most likely due to activation of a large-conductance Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current (Holmes et al. 1996). Although the properties of Ca\textsuperscript{2+} conductances with high threshold of activation have been studied in detail in isolated amygdaloid neurons (Foehring and Scroggs 1994; Viana and Hille 1996), the properties of the Ca\textsuperscript{2+}-regulated K\textsuperscript{+} conductances remain largely unknown in these cells. A single study in which voltage-clamp techniques with sharp microelectrodes in a slice preparation were used demonstrated the existence of a biphasic K\textsuperscript{+} tail current following repetitive spike activity, which was sensitive to extracellular Ca\textsuperscript{2+} and was thus concluded to be mediated by two types of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} currents (Womble and Moises 1993). The more rapidly decaying component of tail current was proposed to reflect the existence of one particular type of Ca\textsuperscript{2+}-activated K\textsuperscript{+} current, termed I\textsubscript{C} (Womble and Moises 1993; reviewed by Storm 1993), although the underlying conductance has not yet been characterized in amygdaloid neurons.

Therefore, in the present study, immunocytochemical techniques and whole cell patch-clamp techniques were combined to test for the presence of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} conductances in pyramidal-like cells acutely dissociated from the basolateral amygdaloid complex of rats. Particular attention was paid to the expression of one family of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels, termed BK\textsubscript{Ca}, because of their high unitary conductance, and the corresponding macroscopic current that has been shown to be I\textsubscript{C} in other types of cells (as reviewed by Sah 1996).

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Methods

Preparation

Cells were acutely dissociated with the use of minimal enzymatic treatment as described earlier (Budde et al. 1992; Kay and Wong 1986; Meis et al. 1996). In brief, Long Evans rats of either sex (postnatal day 12–16) were anesthetized with halothane and killed by decapitation. The brain was rapidly removed and placed in ice-cold oxygenated physiological saline containing (in mM) 2.4 KCl, 10 MgSO₄, 0.5 CaCl₂, 20 piperazine-N,N′-bis (ethanesulphonic acid) (PIPES), 10 glucose, and 195 sucrose, pH 7.35. Coronal slices (400 μm thick) were cut from a block of tissue containing the amygdala on a vibratome (Model 1000, Ted Pella, Redding, CA). The basolateral amygdaloid complex was dissected free under stereo microscopic control from the amygdalostriatal transition area, caudate putamen and central amygdaloid nucleus, external capsule, and piriform cortex by three cuts (Fig. 1A). Tissue pieces were incubated in a stirring chamber at 30 °C in oxygenated medium of the following composition: 132.5 mM NaCl, 5 mM KCl, 3 mM MgCl₂, 0.25 mM CaCl₂, 20 mM PIPES, 25 mM dextrose, 1 mg/ml trypsin (Sigma type XI), and 0.5 mg/ml bovine serum albumin, pH adjusted to 7.35 with NaOH. After 22–30 min of incubation in enzyme, tissue was repeatedly rinsed with enzyme-free solution and kept at 23 °C until further use. Neurons were mechanically dissociated by trituration of a single slice with fire-polished Pasteur pipettes and transferred into a recording chamber.

Recording techniques

Tight-seal whole cell recordings were obtained according to Hamill et al. 1981 with the use of a patch-clamp amplifier (EPC-7, List Medical Systems, Darmstadt, Germany). Patch pipettes were pulled from borosilicate glass (GC150TF-10, Clark Electromedical Instruments, Pangbourne, UK). Typical electrode resistances were 3–5 MΩ in the bath, with access resistances in the range of 4–8 MΩ. Records were low-pass filtered at 2.5 kHz (8-pole Bessel filter). Voltage-clamp experiments were performed with the use of pClamp software operating via a Labmaster DMA interface (Axon Instruments, Foster City, CA) on an IBM computer. Leak current interferences were estimated as the ohmic component of the membrane current during the prepulse to −110 mV and were compensated off-line by subtracting the scaled values from the current traces. Series resistance compensation >50% was routinely utilized. Experiments were conducted at 22–25°C. Neurons were continuously superfused (0.1–1 ml/min) with “extracellular” solution containing (in mM) 136 NaCl, 2 KCl, 1 MgCl₂, 2 CaCl₂, 10 N-(2-hydroxyethyl)-piperazine-N′-2-ethane sulfonic acid (HEPES), 15 dextrose, and 15 d-mannitol, pH adjusted to 7.35 with NaOH. Solution exchange was achieved by a multibarreled laminar-flow perfusion system (0.1 ml/min) placed in close proximity to the recorded neuron. Ca²⁺ currents were isolated with the use of an extracellular solution composed of (in mM) 115 NaCl, 2 KCl, 1 MgCl₂, 3 CaCl₂, 1 CsCl, 10 HEPES, 20 dextrose, 10 d-mannitol, 20 tetraethylammonium chloride (TEA), 6 4-aminopyridine, and 0.0015 tetrodotoxin (TTX), pH adjusted to 7.35 with NaOH. The pipette (“intracellular”) solution contained 105 mM CsCl, 1 mM KCl, 10 mM NaCl, 2 mM MgCl₂, 0.1 mM CaCl₂, 10 mM HEPES, 1.1 mM ethylene glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 20 mM TEA, 5 mM Na₂-ATP, 0.5 mM Na₂-guanosine 5′-triphosphate (GTP), 15 mM phosphocreatine, and 50 U/ml creatine-phosphokinase, pH adjusted to 7.3 with KOH. K⁺ currents were estimated at 13.5 nM with the use of MAXC software.
(Bers et al. 1994). Ca$^{2+}$ influx through voltage-activated Ca$^{2+}$ channels was blocked by substituting Ca$^{2+}$ with an equimolar amount of Mn$^{2+}$ or Mg$^{2+}$, the latter solution with 5 mM EGTA added to obtain a virtually Ca$^{2+}$-free solution. All substances were obtained from Sigma (Diepenhoefen, Germany), except for iberotoxine, which was purchased from RBI (Natick, MA). Data are presented as means ± SE.

**Immunohistochemical staining**

The immunoreactivity of isolated cells was tested with the use of an affinity-purified antibody directed against the α-subunit of BK$_{Ca}$ channels, anti-α(913-926), (Knaus et al. 1996), which was kindly provided by O. Pongs, Hamburg, Germany. Cells were plated on poly-l-lysine-coated glass slides and allowed to settle for ~1 h at room temperature. Cells on slides were then transferred into paraformaldehide (rising concentration 0.4–4%) in phosphate-buffered saline (PBS; 0.4%, 1.2%, 3% for 5 min, 4% for 30 min), rinsed with PBS, and stored at 4°C overnight. After wash in PBS, cells were incubated for 1 h in 2% normal goat serum and 0.4% Triton octophenol poly(ethyleneglycolether) (X-100) in PBS (Sigma, G-9022) to avoid unspacific binding, followed by incubation in anti-α(913-926), (dilution 1:200–1:100; 18 h at 4°C), biotinylated goat anti-rabbit immunoglobulin (Vector Labs, BA 1000; 1:200; 1.5 h), and avidin-biotin-complex horseradish peroxidase (Vector Labs, PK 4000; 1:100; 1.5 h). Between steps, cells were repeatedly washed with PBS (3 times, 10 min). Addition of 3,3′-diaminobenzidine (0.6 mg/ml) and H$_2$O$_2$ (0.01%) for ~15–20 min and subsequent rinsing with PBS completed the reaction. Cells were then dehydrated and coverslipped in DePeX (Serva, Heidelberg, Germany). Immunoreagents were diluted in PBS including 0.4% Triton X-100. Anti-α(913-926) was further supplemented with 2% bovine serum albumin (Sigma) and 2% normal goat serum. In control cells, nonspecific immunoreactivity was assessed by incubation without the primary antibody. These negative controls showed no staining.

**RESULTS**

**Morphology and immunoreactivity of isolated amygdaloid neurons**

The data of the present study were derived from 85 acutely isolated basolateral amygdaloid neurons that possessed a large cell body (maximal diameter ~23 µm) giving rise to a prominent apical and multiple smaller dendrites (Fig. 1B), thereby resembling pyramidal-like class I neurons (McDonald 1982). All isolated pyramid-like amygdaloid cells that were tested (n = 1,199) were immunoreactive to anti-α(913-926), a sequence-directed antibody directed against the pore-forming α-subunit of the BK$_{Ca}$ channel, a member of the s10 family of K$^{+}$ channels (Knaus et al. 1996).

**Electrophysiological properties of a Ca$^{2+}$-activated K$^{+}$ conductance**

The properties of the underlying Ca$^{2+}$-activated K$^{+}$ conductance were investigated through electrophysiological and pharmacological experiments. Whole cell outward currents were evoked by depolarizing voltage commands between −60 and +45 mV in +15-mV increments following a hyperpolarizing prepulse to −110 mV for 1 s (Fig. 2A). Typically, membrane currents were composed of rapidly activating/inactivating outward currents followed by a late, sustained component at membrane potentials positive to approximately −30 mV. The reduction of Ca$^{2+}$ inward fluxes by removal of Ca$^{2+}$ and addition of EGTA (5 mM) to the bathing solution or by substitution of Mn$^{2+}$ for extracellular Ca$^{2+}$ resulted in a strong and reversible reduction of the sustained current component. The subtraction currents obtained from records before and during removal of Ca$^{2+}$ demonstrated that an outward current component was eliminated that activated at depolarizations positive to −30 mV with a slow time course (time constant 430.7 ± 78.6 ms, mean ± SE, voltage step to +30 mV, n = 3) and that did not decline during maintained depolarizations 1,800 ms in duration (Fig. 2A). Isolated amygdaloid neurons were observed to rapidly deteriorate after excessive hyperpolarizations beyond −110 mV. To reliably estimate the reversal potential of the current under study, the external K$^{+}$ concentration was elevated to 10 mM, resulting in a shift of the calculated K$^{+}$ equilibrium potential (E$_K$) to −67 mV. Tail currents recorded under these conditions reversed at −60.6 ± 2.4 mV (n = 5), i.e., close to E$_K$ (data not shown). Slow voltage ramps (46.7 mV/s) applied from −110 to +100 mV revealed an N-shaped current-voltage (I-V) relationship positive to −30 mV, with maximal current amplitude of 5868.5 ± 387.3 pA (n = 63; Fig. 2B). Dividing maximal current amplitude by input capacity of the cell yielded a mean current density of 404.8 ± 25.0 pA/pF (n = 63). Maximal current activation occurred at +9.8 mV ± 2.7 mV (n = 12), as was calculated from membrane currents with maximal amplitudes <3 nA (average of 2162.9 ± 135.7 pA, n = 12) and series resistance compensation of 90% to minimize errors due to series resistance. Superfusion of Ca$^{2+}$-free or Mn$^{2+}$-containing solutions strongly reduced the total outward current and abolished the N-shaped I-V relationship. At the maximum of the N-shaped current-voltage relationship, the current was reduced to 15.3 ± 2.3% (n = 33) of the control value, indicating that it was largely secondary to an influx of Ca$^{2+}$. Indeed, voltage-dependent Ca$^{2+}$ inward currents activated through voltage steps to between −90 and +40 mV in the presence of TTX and blockade of K$^{+}$ currents possessed an I-V relationship largely resembling that of the Ca$^{2+}$-sensitive outward current, with activation threshold and maximal current amplitude (−633.6 ± 94.8 pA; n = 12) occurring at around −50 and 0 mV, respectively (Fig. 2C).

**Pharmacological properties of the Ca$^{2+}$-activated K$^{+}$ conductance**

Despite the inclusion of an ATP-regenerating system, containing phosphocreatine and creatine phosphokinase (Forscher and Oxford 1985) in the pipette solution, the Ca$^{2+}$-dependent K$^{+}$ current showed rundown. To estimate the magnitude of the Ca$^{2+}$-dependent K$^{+}$ conductance at various times before/during action of pharmacological substances during an ongoing experiment, slow voltage ramps (46.7 mV/s) were applied and the maximal current of the N-shaped I-V relationship was plotted.
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FIG. 2. Electrophysiological properties of Ca$^{2+}$-dependent conductances. A: families of outward currents evoked by depolarizing steps in the range of $-60$ to $+45$ mV for 1,800 ms after a conditioning prepulse to $-110$ mV for 1 s (holding potential at $-70$ mV). Substitution of magnesium for Ca$^{2+}$ and addition of 5 mM ethyleneglycol-bis(β-aminoethylether)-N,N',N''-tetraacetic acid (EGTA; 0 Ca$^{2+}$) or by manganese (Mn$^{2+}$) results in a reversible blockade of a slowly activating current component, as seen in the subtraction currents (difference) obtained from records before and after omission of Ca$^{2+}$.

B: slow voltage ramps (46.7 mV/s) between $-110$ and $+100$ mV reveal current-voltage (I-V) relationships with N-shaped profiles, which were abolished on removal of extracellular Ca$^{2+}$ (Mn$^{2+}$; 0 Ca$^{2+}$). Subtraction currents (differences) demonstrate activation threshold and maximal amplitude of the Ca$^{2+}$-sensitive outward current at around $-30$ and $+10$ mV, respectively.

C: Ca$^{2+}$ inward currents obtained on voltage steps from $-110$ mV to between $-90$ and $-40$ mV for 180 ms (holding potential $-70$ mV) and I-V relationships averaged from recordings in 12 cells (bars: means ± SE).

against time, Ca$^{2+}$ sensitivity of the current was corroborated through superfusion of Ca$^{2+}$-free solution in every experiment, and the effect of a given substance on the Ca$^{2+}$-dependent K$^+$ conductance was estimated from the difference current immediately before and during maximal action of the drug relative to the Ca$^{2+}$-insensitive component. Typical experiments are illustrated in Fig. 3. Application of the SK$_{Ca}$ channel blocker apamin (Hughes et al. 1982; Sah 1996) at concentrations of up to 1 μM had no significant effect on the Ca$^{2+}$-dependent outward current ($n = 12$; Fig. 3A). By contrast, iberiotoxin, a selective blocker of BK$_{Ca}$ channels (Galvez et al. 1990), reduced this current component by 66.8 ± 3.4% at 100 nM ($n = 11$; Fig. 3B). The blocking effect of iberiotoxin was not reversible. Moreover, TEA at 1 mM blocked 80.4 ± 4.6% of the Ca$^{2+}$-sensitive outward current ($n = 6$; data not shown).

DISCUSSION

Given the clinical importance of the amygdala and the wide range of electrogenic activity observed in amygdaloid neurons, the properties of the underlying ionic conductances are of great interest. In the present study, evidence is pre-
FIG. 3. Basic pharmacological properties of the Ca$^{2+}$-dependent K$^+$ conductance. Diagrams: maximal current amplitudes of N-shaped I-V relationships (obtained from slow voltage ramp protocols as in Fig. 2) plotted against time. Slow voltage ramps were repeated every 20–30 s. Insets: single I-V relationships at times indicated. Note the rundown of current amplitudes. Lines were fitted by linear regression. 

A: application of 1 μM apamin has no measurable effect on maximal current amplitudes, whereas removal of extracellular Ca$^{2+}$ and addition of 5 mM EGTA (0 Ca$^{2+}$) reversibly abolish the maximum of the N-shaped I-V relationship, indicating a major contribution of the Ca$^{2+}$-dependent K$^+$ conductance. 

B: maximal membrane current is significantly and irreversibly reduced by 100 nM iberiotoxin, and omission of extracellular Ca$^{2+}$ (0 Ca$^{2+}$) reversibly suppresses the remaining current.

sentenced that pyramidal-like, presumed projection cells of the basolateral amygdaloid complex possess a Ca$^{2+}$-dependent K$^+$ conductance with pharmacological and immunologic properties indicative of BK$_{Ca}$ channels.

Ca$^{2+}$ dependence of the K$^+$ conductance

Ca$^{2+}$ dependence was investigated through substitution experiments including Mn$^{2+}$ or Mg$^{2+}$. These ions block Ca$^{2+}$ influx via voltage-dependent Ca$^{2+}$ channels (Miller 1987) with very little effect on the kinetics of Ca$^{2+}$-independent types of K$^+$ currents (Arhem 1980; Baker et al. 1973; Gilly and Armstrong 1982). The slowly developing outward currents on step depolarizations positive to 0 mV, which were obtained as difference currents from records before and during substitution of Ca$^{2+}$, can thus be assumed to largely reflect the Ca$^{2+}$-dependent current. The reversal potential of tail currents close to the presumed $E_K$ indicates K$^+$ ions as the main charge carriers. The positive deviation from $E_K$ may result from contaminating effects of high-voltage-activated Ca$^{2+}$ currents (Foehring and Scruggs 1994) or a Ca$^{2+}$-dependent Cl$^-$ conductance known to exist in amygdaloid neu-
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Ron (Sugita et al. 1993), although there was no evidence for the existence of a Ca$^{2+}$-dependent Cl$^-$ current in the population of acutely isolated amygdaloid neurons (data not shown). Slow voltage ramps demonstrated N-shaped I-V relationships, reflecting the increase in membrane conductance through activation of the Ca$^{2+}$-dependent K$^+$ current and increase in driving force for K$^+$ followed by a decrease in conductance when approaching the Ca$^{2+}$ equilibrium potential (Meech and Standen 1975). The N-shaped I-V relationship was largely reduced in Ca$^{2+}$-free media, with a small region of reduced positive slope conductance remaining in a potential region at around 0 mV. This may reflect an incomplete block of Ca$^{2+}$ inward current or a local maximum in the I-V relationship of voltage-dependent K$^+$ currents (Aldrich et al. 1979) caused by blocking effects of internal Na$^+$ (French and Wells 1977). The voltage dependence of the Ca$^{2+}$-dependent K$^+$ current well reflected that of the Ca$^{2+}$ current, thereby indicating a strong requirement for Ca$^{2+}$ in the gating process of the K$^+$ conductance.

Pharmacological and immunocytochemical evidence for the existence of BK$_{Ca}$ channels

Ca$^{2+}$-dependent K$^+$ channels are generally divided into two distinct families: BK$_{Ca}$ and SK$_{Ca}$ channels (for review see Kaczorowski et al. 1996; McManus 1991; Sah 1996). BK$_{Ca}$ channels possess a large single-channel conductance, are voltage sensitive because of voltage-dependent Ca$^{2+}$ binding, and are blocked by submillimolar concentrations of TEA (dissociation constant values ≤ 1 mM) (Adams et al. 1982; Blatz and Magleby 1987; Reinhart et al. 1989) and in most cases by the peptidyl toxins charybdotoxin or the more selective drug iberiotoxin (Galvez et al. 1990; Sah 1996). SK$_{Ca}$ channels have smaller unitary conductance, are voltage insensitive, are not blocked by TEA in the submillimolar concentration range, are insensitive to charybdotoxin and are sensitive to apamin (McManus 1991; Sah 1996). The pharmacological profile of the Ca$^{2+}$-dependent K$^+$ current in amygdaloid neurons, i.e., reduction by ~80% through 1 mM TEA and sensitivity to iberiotoxin but not apamin, strongly indicated mediation through BK$_{Ca}$ channels.

The BK$_{Ca}$ channel consists of two distinct subunits, α and β. The α-subunit is a member of the slo Ca$^{2+}$-activated K$^+$ channel gene family and forms the ion conduction pore. The β-subunit is a structurally unique, membrane-spanning protein that has pronounced effects on channel gating and pharmacology (Kaczorowski et al. 1996). slo was found to be widely expressed in rat brain, with particular enrichment in terminal areas of major projection tracts and in cerebellar Purkinje cells (Knaus et al. 1996). The immunocytochemical data of the present study extend these observations in showing that slo is expressed by pyramidal-like neurons of the basolateral amygdaloid complex. In addition, immunocytochemical experiments in slices demonstrated a wide distribution of slo in the amygdala and positive reactions of pyramidal-like and nonpyramidal cells (data not shown). In any case, the immunocytochemical data confirm the existence of BK$_{Ca}$ channels in pyramidal-like neurons of the basolateral amygdaloid complex that was inferred from the electrophysiological and pharmacological properties of the corresponding whole cell current.

Possible functional significance of the BK$_{Ca}$ conductance in amygdaloid neurons

BK$_{Ca}$ channels are voltage sensitive and require a relatively high activity of Ca$^{2+}$ (1–10 μM) for activation at membrane potentials near the resting level (Reinhart et al. 1989). Recruitment of a significant fraction of these channels may therefore depend on the Ca$^{2+}$ influx during action potentials (Llinás et al. 1992) and/or a close proximity to voltage-gated Ca$^{2+}$ channels (Gola and Crest 1993; Robin-taille et al. 1993). Observations in neurons from various regions of the brain indeed indicated a contribution of the corresponding macroscopic current $I_C$ to spike repolarization and generation of a fast component of the hyperpolarizing afterpotential (see Sah 1996). In mature neocortical pyramidal cells, BK$_{Ca}$ channel density in apical dendrites was found to be less than one-half of that in the somatic region (Kang et al. 1996). In acutely isolated amygdaloid neurons, which lack most of their dendritic tree, BK$_{Ca}$ channels were still detectable immunocytochemically and on activation of the corresponding macroscopic current. These results confirm a somatic/perisomatic localization of the channels, although the additional existence in the dendritic membrane cannot be excluded. Although the exact role of BK$_{Ca}$ channels in pyramidal cells of the amygdala remains to be delineated, the findings of the present study and the existence of multiple components of somatic/perisomatic Ca$^{2+}$ conductances with activation threshold at around ~50 mV (Foehring and Scroggs 1994; Viana and Hille 1996) suggest a structural and functional coupling of the underlying BK$_{Ca}$ and Ca$^{2+}$ channels that in turn could ensure a regulation of repetitive spike firing and prevent cell damage on strong depolarizing influences. Observations in the slice preparation of the basolateral amygdaloid complex indicating a contribution of Ca$^{2+}$-dependent K$^+$ conductances to the patterning of spike trains, high-threshold oscillations, and epileptiform paroxysmal depolarization shifts (Danobert and Pape 1996; Driesang and Pape 1997) are in line with this hypothesis. Moreover, activation of metabotropic glutamate receptors has been found to activate an outward K$^+$ current with properties indicative of BK$_{Ca}$ channels in basolateral amygdaloid neurons (Holmes et al. 1996), indicating that these channels are also involved in the synaptic regulation of cellular activities in the amygdala.

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