L-Type Ca\(^{2+}\) Channels Mediate the Slow Ca\(^{2+}\)-Dependent Afterhyperpolarization Current in Rat CA3 Pyramidal Cells In Vitro

MITSUO TANABE, BEAT H. GÄHWILER, AND URS GERBER
Brain Research Institute, University of Zurich, CH-8057 Zurich, Switzerland

Tanabe, Mitsuo, Beat H. Gähwiler, and Urs Gerber. L-type Ca\(^{2+}\) channels mediate the slow Ca\(^{2+}\)-dependent afterhyperpolarization current in rat CA3 pyramidal cells in vitro. J. Neurophysiol. 80: 2268–2273, 1998. Single-electrode voltage-clamp recordings were obtained from CA3 pyramidal cells in rat hippocampal organotypic slice cultures, and the slow Ca\(^{2+}\)-dependent K\(^+\) current or afterhyperpolarization current (I\(_{\text{AHP}}\)) was elicited with brief depolarizing voltage jumps. The slow I\(_{\text{AHP}}\) was suppressed by the selective L-type Ca\(^{2+}\) channel antagonists isradipine (2 μM) or nifedipine (10 μM). In contrast, neither ω-conotoxin MVIIA (1 μM) nor ω-agatoxin IVA (200 nM), N-type and P/Q-type Ca\(^{2+}\) channel antagonists, respectively, attenuated this slow outward current. The slow I\(_{\text{AHP}}\) was significantly reduced by thapsigargin (10 μM), a Ca\(^{2+}\) ATPase inhibitor that depletes intracellular Ca\(^{2+}\) stores, and by ryanodine (10–100 μM), which blocks Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular compartments. At this concentration thapsigargin did not modify high-threshold Ca\(^{2+}\) current, which was, however, blocked by isradipine. Thus, in hippocampal CA3 pyramidal cells, Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels is necessary to trigger the slow I\(_{\text{AHP}}\). Furthermore, intracellular Ca\(^{2+}\)-activated Ca\(^{2+}\) stores represent a critical component in the transduction pathway leading to the generation of the slow I\(_{\text{AHP}}\).

INTRODUCTION

The slow I\(_{\text{AHP}}\), a Ca\(^{2+}\)-dependent K\(^+\) current, modulates the firing pattern of many classes of neurons by inducing accommodation of action potential discharge. The physiological importance of this current is reflected in the multitude of neurotransmitter systems and second messenger pathways that evolved for its regulation (Nicol 1988). In hippocampal pyramidal cells the slow I\(_{\text{AHP}}\) plays a prominent role in determining neuronal activity patterns and is sensitive to regulation by the cholinergic, glutamatergic, and diverse amnergic afferents impinging on these cells. Earlier studies to characterize the slow I\(_{\text{AHP}}\) in hippocampal pyramidal cells have shown that this K\(^+\) current depends on influx of extracellular Ca\(^{2+}\) (Gustafson and Wigström 1981; Hablitz 1981; Hotson and Prince 1980; Schwatzkroin and Stafstrom 1980), is relatively insensitive to voltage and tetraethylammonium chloride (TEA) (Lancaster and Adams 1986), is not blocked by apamin, as opposed to the medium and slow I\(_{\text{AHP}}\) in most other cell types (Lancaster and Nicoll 1987; Storm 1989), and underlies accommodation of cell firing (Madison and Nicoll 1984). However, although a rise in intracellular Ca\(^{2+}\) clearly triggers the slow I\(_{\text{AHP}}\), the channels mediating this Ca\(^{2+}\) influx in hippocampal pyramidal cells were not yet identified. In various other types of neurons the Ca\(^{2+}\) channels underlying the induction of the apamin-insensitive slow I\(_{\text{AHP}}\) were recently described. For example, in rat sensorimotor cortical pyramidal neurons and in cholinergic nucleus basalis neurons, mainly N- and P-type Ca\(^{2+}\) channels are responsible for initiating this current (Pineda et al. 1996; Williams et al. 1997).

On the basis of the delayed activation and prolonged decay of the slow I\(_{\text{AHP}}\), a number of mechanisms that might explain the slow action of Ca\(^{2+}\) was proposed. These include a redistribution of cytoplasmic Ca\(^{2+}\) by simple diffusion (Lancaster and Zucker 1994; Zhang et al. 1995), Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores (Sah and McLachlan 1991), and Ca\(^{2+}\)-induced activation of an intracellular signal transduction cascade (Lasser-Ross et al. 1997; Schwindt et al. 1992). Currently, however, the actual mechanism involved remains unclear.

The goal of this investigation was to establish which Ca\(^{2+}\) channels are involved in the generation of the slow I\(_{\text{AHP}}\) in hippocampal CA3 pyramidal cells and to clarify whether Ca\(^{2+}\)-induced Ca\(^{2+}\) release contributes to this current.

METHODS

Experiments were performed with organotypic hippocampal slice cultures. Hippocampi were removed aseptically from 6-day-old Wistar rats that were killed by cervical dislocation. Tissue slices of 400 μm thickness were prepared and cultured by means of the roller tube technique as described previously (Gähwiler 1981).

Electrophysiological recordings

After 15–30 days in vitro, the cultures were transferred to a recording chamber mounted onto the stage of an inverted microscope (Axiovert 35 M, Zeiss, Jena, Germany) and superfused with an external solution (at 32°C, pH 7.4) containing (in mM) 148.9 Na\(^+\), 2.7 K\(^+\), 146.2 Cl\(^-\), 2.8 Ca\(^{2+}\), 0.5 Mg\(^{2+}\), 11.6 HCO\(_3\)\(^-\), 0.4 H\(_2\)PO\(_4\)\(^-\), and 5.6 d-glucose. Single-electrode voltage-clamp recordings were made from CA3 pyramidal cells (Axoclamp 2 amplifier, Axon Instruments, Foster City, CA) with microelectrodes filled with 2 M potassium methylsulfate (KMeSO\(_4\)) and tip resistances of 50–80 MΩ. The switching frequency ranged between 1.5 and 2 kHz, which provided sufficient resolution to analyze the slow I\(_{\text{AHP}}\) signals, and headstage output was monitored continuously to ensure adequate settling time between samples. Input resistance was assessed with 500-ms hyperpolarizing voltage commands of 10 mV.

The slow I\(_{\text{AHP}}\) was induced with brief depolarizing voltage jumps (from a holding potential of between −55 and −60 mV to 0 mV.
for 50–100 ms, 0.04 Hz) in the presence of tetrodotoxin (TTX, 0.5 μM). When Ca2+ currents were elicited, 20-nm depolarizing voltage jumps were applied from a holding potential of −40 mV for 0.5–1 s (0.05 Hz) in the presence of TTX (0.5 μM) and TEA (10 mM) with microelectrodes filled with 2 M cesium chloride (CsCl). Excitatory postsynaptic potentials (EPSPs) were evoked in current-clamp mode at a membrane potential of approximately −80 mV by electrically stimulating the mossy fibers (0.05 Hz) in the presence of TTX and nifedipine (0.1 μM each), and a nonsaturating concentration of the non-NMDA receptor antagonist and 6-cyano-7-nitroquinocxaline-2,3-dione (CNQX, 10 μM).

Numerical data in the text are expressed as means ± SE. Student’s t-test was used to compare means when appropriate. P < 0.05 was considered significant.

Drugs and chemicals

(−)-Bicuculline methochloride, thapsigargin, and ryanodine were purchased from Sigma (St. Louis, MO). CNQX was from Tocris Neuramin (Bristol, UK). TTX was from Sankyo (Tokyo). ω-agatoxin IVA was from Latoxan (Rosans, France) or from the Peptide Institute (Osaka, Japan), and synthetic ω-conotoxin MVIIA was from Latoxan. Isradipine, CPP, and CGP 52 432 were kindly donated by Novartis (Basel). Drugs were directly applied via the superfusion solution. Peptide toxins used to block Ca2+ channels were bath-applied in the presence of cytochrome C (0.1 mg/ml) to reduce nonspecific binding.

RESULTS

Hippocampal pyramidal cells express voltage-gated Ca2+ channels belonging to the L-, N-, P-, and Q-types (Ahlijanian et al. 1990; Westenbroek et al. 1990, 1992, 1995). Essentially the same pattern of Ca2+-channel distribution was described in CA3 pyramidal cells in hippocampal slice cultures (Elliott et al. 1995). In a first series of experiments, specific Ca2+-channel antagonists were used to determine the channel types associated with the induction of IAH.

L-type Ca2+-channel blockers suppress slow IAH

Isradipine, a dihydropyridine (DHP) that selectively blocks L-type Ca2+ channels (Hof et al. 1984), significantly reduced the slow IAH in CA3 pyramidal cells. Figure 1A illustrates the typical time course of IAH inhibition in response to isradipine (2 μM) in a representative cell. In seven cells, isradipine superfused for 7 min depressed the slow IAH from 247.3 ± 48.6 pA to 45.8 ± 13.1 pA (a decrease of 82.3 ± 4.2%, P < 0.01). Attempted washout of the drug for >30 min did not lead to recovery. Representative traces of the slow IAH are depicted in Fig. 1B. An alternative DHP, nifedipine (10 μM for 5–10 min), similarly suppressed the slow IAH from 309.1 ± 37.4 pA to 178.9 ± 22.8 pA (a decrease of 42.1 ± 4.7%, n = 7, P < 0.01, not shown). The weaker action of nifedipine probably reflects the lower affinity of this compound for L-channels versus isradipine (Cognard et al. 1986; Ohya and Sperelakis 1990).

N- and P/Q-type Ca2+-channel blockers do not reduce slow IAH

In contrast to the potent inhibition by L-type Ca2+ channels blockers, the specific N-type Ca2+-channel antagonist ω-conotoxin MVIIA (CmTx) (Olivera et al. 1987) did not reduce the slow IAH. In fact, superfusion of CmTx (1 μM for 5 min) slightly, but significantly, increased the amplitude of slow IAH by 11.4% (from 326.6 ± 19.4 pA to 363.9 ± 23.8 pA, n = 5, P < 0.05, Fig. 2A). This observation, which may be explained by improved membrane space clamping as a result of closure of N-type Ca2+ channels, was not further investigated. To confirm that CmTx was indeed blocking Ca2+ channels, we examined its effects on pharmacologically isolated EPSPs evoked in CA3 cells by stimulating mossy fiber afferents with an electrode positioned in the dentate hilus area. As we previously reported for CA3 pyramidal cells in this preparation (Poncer et al. 1997), EPSPs were rapidly reduced in the presence of CmTx (by 55.0 ± 4.5%, n = 5, Fig. 2B).

Experiments were then performed with ω-agatoxin IVA (Aga IVA) at 200 nM, a concentration that blocks both P- and Q-type Ca2+ channels (Olivera et al. 1994; Zhang et al. 1993). Aga IVA had no effect on the slow IAH (a 2.7% increase P > 0.05, n = 4, Fig. 2C) but reduced evoked EPSPs (by 53.1 ± 7.7%, n = 3, Fig. 2D).

Thus Ca2+ influx via N- or P/Q-type Ca2+ channels does not mediate the slow IAH in hippocampal CA3 pyramidal cells.

Intracellular Ca2+ stores contribute to the generation of slow IAH

A characteristic feature of the slow IAH is its prolonged time course lasting several seconds and the slow onset and slow decay times. This suggests that Ca2+ influx does not directly activate the K+ channels conducting the IAH. It is reasonable to assume that Ca2+ release from intracellular
stores might contribute to the slow time course of this response in CA3 cells.

We examined this possibility by depleting intracellular Ca\(^{2+}\) stores with thapsigargin, a Ca\(^{2+}\) ATPase inhibitor that prevents Ca\(^{2+}\) uptake into intracellular compartments. In six of seven cells, bath-application of thapsigargin (10 \(\mu\)M for 28–30 min) reduced the slow \(I_{\text{AHP}}\) from 225.7 ± 34.3 pA to 83.9 ± 21.1 pA \((P < 0.05)\). In one remaining cell, the slow \(I_{\text{AHP}}\) was not affected by thapsigargin. The time course of the response to thapsigargin and representative traces from a typical cell are illustrated in Fig. 3A.

Two functionally separate releasable pools of intracellular Ca\(^{2+}\) can be distinguished in neurons based on the ligands Ca\(^{2+}\)- or inositol 1,4,5-trisphosphate (IP\(_3\)), which mediate the respective liberation of the sequestered Ca\(^{2+}\) through channels spanning the membranes of the endoplasmic reticulum (Henzl and MacDermott 1992). Hippocampal CA3 pyramidal cells primarily express the Ca\(^{2+}\)-activated Ca\(^{2+}\) release channels that are sensitive to ryanodine (Pauda et al. 1991; Sharp et al. 1993). Furthermore, a recent study in cerebellar neurons demonstrated that these ryanodine receptors can functionally couple with L-type Ca\(^{2+}\) channels (Chavis et al. 1996). We therefore tested whether Ca\(^{2+}\)-activated Ca\(^{2+}\) release contributes to the generation of the slow \(I_{\text{AHP}}\) induced by Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels. Superfusion of ryanodine (10–100 \(\mu\)M for 4–20 min) to inhibit Ca\(^{2+}\)-activated Ca\(^{2+}\) release reduced the slow \(I_{\text{AHP}}\) from 219.7 ± 40.9 pA to 100.3 ± 15.7 pA \((n = 8, P < 0.05)\). This action was only partially reversible after 30 min of wash. The time course of the ryanodine effect and representative traces from one cell are shown in Fig. 3B. This result indicates that the Ca\(^{2+}\) signal mediated by L channels causes the release of intracellular Ca\(^{2+}\) stores that contribute to the activation of the K\(^+\) conductance underlying the slow \(I_{\text{AHP}}\).

It has been shown that thapsigargin, in addition to depleting intracellular Ca\(^{2+}\) pools, can also inhibit L-type Ca\(^{2+}\) current (Buryi et al. 1995; Nelson et al. 1994). Thus the observed inhibition of slow \(I_{\text{AHP}}\) in response to thapsigargin may result from a direct inhibition of L-type Ca\(^{2+}\) channels rather than the emptying of Ca\(^{2+}\) stores. Under our experimental conditions, however, this did not appear to be the case. Cells were clamped at −40 mV and stepped to −20 mV to elicit Ca\(^{2+}\) current with a strong L-type component (Gähwiler and Brown 1987). Thapsigargin (10 \(\mu\)M for 25 min) did not significantly decrease Ca\(^{2+}\) current (a reduction of 16.6 ± 3.5\%, \(P > 0.05, n = 3\), Fig. 4A). On the other hand, isradipine (2 \(\mu\)M for 7 min) reduced Ca\(^{2+}\) current by 80.1 ± 7.0\% \((n = 5, P < 0.05, \text{Fig. 4B})\).

**DISCUSSION**

The principal finding of this study is that Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels initiates the generation of the slow \(I_{\text{AHP}}\) in hippocampal CA3 pyramidal cells. Furthermore, it appears that Ca\(^{2+}\) release from intracellular stores is required to induce the slow \(I_{\text{AHP}}\). This suggests that L-type Ca\(^{2+}\) channels can functionally couple to Ca\(^{2+}\)-activated Ca\(^{2+}\) stores, which may account in part for the prolonged time course of the slow \(I_{\text{AHP}}\).

**Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels triggers the slow \(I_{\text{AHP}}\)**

Our results obtained with DHP Ca\(^{2+}\)-channel antagonists are consistent with an involvement of L-type Ca\(^{2+}\) channels in the generation of the slow \(I_{\text{AHP}}\) in the hippocampus. A number of previous studies demonstrating the colocalization of L-type Ca\(^{2+}\) channels with the K\(^+\) channels thought to underlie the slow \(I_{\text{AHP}}\) in hippocampal pyramidal cells provide support for this conclusion.

L-type Ca\(^{2+}\) channels are expressed mainly in the cell body and proximal dendrites of CA3 pyramidal cells (Ahijanian et al. 1990; Westenbroek et al. 1990), and their activa-
tion results in a pronounced somatic Ca\textsuperscript{2+} transient (Elliott et al. 1995). Such Ca\textsuperscript{2+} transients are closely associated in time with the induction of slow $I_{\text{AHP}}$ (Knöpfel and Gähwiler 1992; Lasser-Ross et al. 1997). In contrast, N and P/Q channels in these cells are expressed primarily in axon terminals where they mediate neurotransmitter release. Although there is some expression of these channels on the dendrites, they do not contribute significantly to Ca\textsuperscript{2+} transients induced by stimulating the cell body (Elliott et al. 1995).

The channels that conduct the slow $I_{\text{AHP}}$ in hippocampal pyramidal cells were identified as Ca\textsuperscript{2+}-dependent small conductance potassium channels referred to as SK channels.

![Diagram A](image1.png)

**FIG. 3.** Ca\textsuperscript{2+} release from intracellular Ca\textsuperscript{2+} stores contributes to the generation of slow $I_{\text{AHP}}$. A: bath-application of thapsigargin (10 μM) decreases the amplitude of slow $I_{\text{AHP}}$. Each point represents the mean amplitude of 4 consecutive slow $I_{\text{AHP}}$ s in a typical cell. B: bath-application of ryanodine (10 μM) results in a similar attenuation of slow $I_{\text{AHP}}$, although this effect is only partially reversible.

![Diagram B](image2.png)

**FIG. 4.** Distinct actions of thapsigargin and isradipine on Ca\textsuperscript{2+} currents. Suppression of the slow $I_{\text{AHP}}$ by thapsigargin is not due to its effect on the Ca\textsuperscript{2+} current. Cells were clamped at $-40 \text{ mV}$, and Ca\textsuperscript{2+} currents were elicited by voltage steps to $-20 \text{ mV}$ (for 0.5–1 s, 0.05 Hz) in the presence of TTX (0.5 μM) and tetraethylammonium chloride (10 mM) with microwires filled with 2 M CsCl. A: thapsigargin (10 μM) produced no significant change in the Ca\textsuperscript{2+} current. B: isradipine (2 μM) suppressed Ca\textsuperscript{2+} currents. Each sample trace represents the average of 4 consecutive Ca\textsuperscript{2+} currents.
with an estimated conductance of 2–5 pS (Sah and Isaacson 1995). Similarly to the L-type Ca\(^{2+}\) channels, these SK channels appear to be concentrated on the proximal dendrites (Sah and Bekkers 1996). Moreover, cell-attached, patch-clamp recordings in hippocampal pyramidal cells have shown that L-type Ca\(^{2+}\) channels colocalize exclusively with SK channels, whereas patches with N-type Ca\(^{2+}\) channels are associated with large conductance Ca\(^{2+}\)-dependent K\(^+\) channels (Tavalin and Marrion 1997).

Further evidence that activation of SK channels does indeed underlie the slow \(I_{\text{AMP}}\) was recently obtained with the cloning of a family of these K\(^+\) channels. One of these, the human SK1 channel (hSK1) with a single channel conductance of 9.9 pS displays all the properties associated with the slow \(I_{\text{AMP}}\) characterized in hippocampal pyramidal cells, including apamin insensitivity and voltage independence (Köhler et al. 1996).

It was reported that DHP antagonists also inhibit certain K\(^+\) currents. For example, DHPs rapidly inactivated Shaker K\(^+\) channels expressed in \textit{Xenopus} oocytes (Avdonin et al. 1997). In cerebellar granule cells, DHPs were found to block one-third of the large conductance Ca\(^{2+}\)-dependent K\(^+\) channels (Fagni et al. 1994). In contrast, SK channels do not appear to be sensitive to DHPs. In both cortical pyramidal neurons (Foehring and Waters 1995) as well as cholinergic nucleus basalis neurons (Williams et al. 1997), DHPs had no effect on the apamin-insensitive slow \(I_{\text{AMP}}\), which is mediated primarily by Ca\(^{2+}\) influx through N-type channels in these cells.

**A Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism is involved in generating slow \(I_{\text{AMP}}\)**

Releasable intracellular Ca\(^{2+}\) stores in brain cells perform important functions, not only in Ca\(^{2+}\) homeostasis but also in the modulation of diverse neuronal responses. Neurons express two main types of intracellular Ca\(^{2+}\) channels, which are activated, respectively, by IP\(_3\) or by Ca\(^{2+}\) itself (Henzi and MacDermott 1992). In CA3 pyramidal cells Ca\(^{2+}\)-release is mediated mainly by Ca\(^{2+}\) binding to ryanodine receptors, whereas in CA1 pyramidal cells Ca\(^{2+}\) stores are regulated by IP\(_3\) receptors (Pauda et al. 1991; Sharp et al. 1993). In keeping with this expression pattern, our data indicate that ryanodine-sensitive Ca\(^{2+}\) stores are involved in the activation of the slow \(I_{\text{AMP}}\) in CA3 pyramidal cells. Similarly, Ca\(^{2+}\)-activated Ca\(^{2+}\) stores were found to be necessary for the induction of an apamin-insensitive slow AHP in vagal motor neurons (Sah and McLachlan 1991) and in locus ceruleus neurons (Osmanovic and Shefner 1993). In these studies, however, the voltage-gated Ca\(^{2+}\) channels mediating Ca\(^{2+}\) influx were not characterized.

A close functional association between L-type Ca\(^{2+}\) channels and ryanodine-sensitive Ca\(^{2+}\) stores was demonstrated in a variety of cell types. The best-studied example is the coupling between L-type channels and ryanodine receptors that constitutes the key element of the excitation-contraction coupling machinery in skeletal muscle (Ashcroft 1991). Functional coupling between L-type Ca\(^{2+}\) channels and ryanodine receptors was also described in neurons, where this may play a role in regulating electrical properties and in synaptic plasticity (Chavis et al. 1996). On the basis of our results, we hypothesize that in CA3 pyramidal cells Ca\(^{2+}\)-activated Ca\(^{2+}\) stores may represent one component in an intracellular protein scaffold involved in the delivery of Ca\(^{2+}\) from the L-type channel to the appropriate binding sites on the SK channel. This arrangement may limit the diffusion of incoming Ca\(^{2+}\), thereby enhancing the specificity of this response. Furthermore, Ca\(^{2+}\) release from intracellular stores may serve to amplify the primary Ca\(^{2+}\) signal.

We thank L. Heeb, R. Kagi, H. Kasper, L. Rietschin, and R. Schob for excellent technical assistance. This research was supported by Sankyo Ltd., the Prof. Dr. Max Cloetta Foundation, and the Swiss National Science Foundation (31–45347.95).

Address for reprint requests: U. Gerber, Brain Research Institute, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland.

Received 8 May 1998; accepted in final form 14 July 1998.

**REFERENCES**


L-TYPE Ca^{2+} CHANNELS AND AFTERHYPERPOLARIZATION CURRENT


