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Rat Hippocampal Neurons in Culture: Potassium Conductances

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SUMMARY AND CONCLUSIONS

1. Two-electrode voltage-clamp methodology was used to analyze voltage-dependent ionic conductances in 81 rat hippocampal neurons grown in culture for 4–6 wk. Pyramidal and multipolar cells with 15- to 20-μm-diameter cell bodies were impaled with two independent KCl electrodes. The cells had resting potentials of −30 to −60 mV and an average input resistance of about 30 MΩ.

2. A depolarizing command applied to a cell maintained in normal medium invariably evoked a fast (2–10 ms) inward current that saturated the current-passing capacity of the system. This was blocked in a reversible manner by application of tetrodotoxin (TTX) (0.1–1.0 μM) near the recorded cell.

3. In the presence of TTX, a depolarizing command evoked a rapidly rising (3–5 ms), rapidly decaying (25 ms) transient outward current reminiscent of "I_A" reported in molluscan neurons. This was followed by a more slowly activating (~100 ms) outward current response of greater amplitude that decayed with a time constant of about 2–3 s. These properties resemble those associated with the K^+ conductance, I_K, underlying delayed rectification described in many excitable membranes.

4. I_K was blocked by extracellular application of tetraethylammonium (TEA) but was insensitive to 4-aminopyridine (4-AP) at concentrations that effectively eliminated I_A. I_A, in turn, was only marginally depressed by TEA.

5. Unlike I_K, I_A was completely inactivated when the membrane was held at potentials positive to −50 mV. Inactivation was completely removed by conditioning hyperpolarization at −90 mV. A brief hyperpolarizing pulse (10 ms) was sufficient to remove 95% of the inactivation. I_A activated on commands to potentials more positive than −50 mV.

6. The inversion potential of the ionic conductance underlying I_A and I_K was in the range of the K^+ equilibrium potential, E_K, as measured by the inversion of tail currents; and this potential was shifted in a depolarizing direction by elevated $[K^+]_o$. Thus, both current species reflect activation of membrane conductance to K^+ ions.

7. Hyperpolarizing commands from resting potentials revealed a time- and voltage-dependent slowly developing inward current in the majority of cells studied. This membrane current was observed in cells exhibiting "anomalous rectification" and was therefore labeled I_{AR}. It was activated at potentials negative to −70 mV with a time constant of 100–200 ms and was not inactivated. A return to resting potential revealed a tail current that disappeared at about E_K.

8. I_{AR} was blocked by extracellular Cs^+ and was enhanced by elevating $[K^+]_o$. It thus appears to be carried by inward movement of K^+ ions. Sodium might also be involved in this response, since replacement of Na^+ by choline abolished I_{AR}. The properties of I_{AR} were distinctly different from those of another K conductance, I_M, which was noticeably rare in the cells studied.

9. Functionally, the three K^+ conductances play important roles in regulating cellular excitability of hippocampal cells.

INTRODUCTION

Since the original description of two primary ionic conductances governing the elec-

trical excitability of the squid giant axon (26), a variety of other voltage-dependent conductances have been described in various invertebrate and vertebrate neurons (1, 3, 11). At least five distinct species of K^+ conductance have been identified. These are associated with one of the following: the classical delayed rectifier (I_K), a transient outward current (I_A) (15–17, 35, 36, 47), a Ca^{2+}- and voltage-dependent, slowly inactivating outward current (I_C) (33), a noninactivating outward current (I_M) (3, 11), and a voltage- and time-dependent inward current triggered at hyperpolarized potentials, the anomalous rectifier (27, 37), also known as I_Q (23). Some of these K^+ conductances are tissue and/or cell specific or have different properties in different preparations. All of these conductances have been characterized using the voltage-clamp technique, which is a prerequisite for studying their properties. However, the technique has been difficult to apply to central nervous system (CNS) neurons in vitro because of their limited accessibility, with the exception of spinal motoneurons (8, 9). The innovation of voltage-clamp techniques utilizing a single microelectrode (49) has permitted recent application of the method to sympathetic neurons and hippocampal cells in vitro (11, 18, 21, 23). The initial results demonstrate that some of the previously described conductance mechanisms are resident in central vertebrate neurons. However, many of these results have been obtained using single-microelectrode voltage-clamp techniques, which have time-resolution and voltage-control capabilities that are not as good as those obtained with the two-electrode clamp (45). We have applied the voltage-clamp technique, using two microelectrodes, to embryonic hippocampal neurons grown in dissociated cell culture to study some of the conductance mechanisms present in these cells (42). In this paper we characterize some properties of three K^+ conductances, each of which appears to be present in the majority of hippocampal cells grown in culture and each of which has properties characteristic of K^+ conductances described previously in other nerve cells.

METHODS

Cell culture

The methods for cell culture are detailed in a preceding paper (36). Prior to each experiment the culture dish was washed thoroughly with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid-(HEPES) buffered balanced salt solution and placed on the modified stage of an inverted phase contrast microscope. The recording solution had an osmolarity of 310–320 mosM and contained (in mM): 138 NaCl, 5 KCl, 11 glucose, and 12.5 HEPES (pH 7.4). Phenol red (0.05 mM) was added to some solutions to monitor the stability of the pH. Tetrodotoxin (1.0 μM) was routinely added to the recording medium to suppress voltage-dependent Na^+ conductances. In some experiments 25 mM NaCl was replaced by 25 mM tetraethylammonium (TEA) to block I_K. Most of the experiments were performed with medium containing 4 mM Ca^{2+} and 4 mM Mg^{2+}. This ratio of divalent cations was found to yield stable long-term (>30 min) recording of cells with relatively high input resistance. Experiments were also done with lower cationic strength solutions and different Ca:Mg ratios, and the results with these were similar but of lower quality than those obtained with the 4:4 ratio. In a number of experiments, 5 mM Co^{2+} or 0.5 mM Cd^{2+} was added to block I_{Ca}. All the experiments were carried out at room temperature (21–25°C) on 4- to 6-wk-old pyramidal- and multipolar-shaped neurons (15- to 20-μm diameter cell bodies).

Electrophysiology

Neurons were impaled with two independent 3 M KCl microelectrodes (tip resistance, 40–100 MΩ). Membrane potential was controlled using a two-electrode voltage-clamp system (45) and membrane current was recorded with a virtual ground current-to-voltage converter. The electrodes were shielded and fluid level in the bath was kept low to reduce unwanted coupling capacitance between the electrodes. Under these conditions, the settling time for a 30- to 40-mV voltage command was consistently less than 1 ms (see Fig. 1*A*). Large capacitative currents were associated with the voltage commands, but these settled within 2–4 ms and were followed by measurable ionic currents. Measurements of "instantaneous" current responses were taken at least 5 ms after termination of the capacitative currents or at the peak of a transient outward current, which clearly followed the capacitative current. Current and voltage signals were displayed continuously on a Brush chart recorder and digitized and stored in a Nicolet digital oscilloscope for plotting on an X-Y recorder. Drugs were freshly prepared in the recording medium and applied near the cell under study by pressure from micropipettes with tip diameter of 1–2 μm. The pressure utilized was less than 1.0 lb/in^2 in order to minimize direct effects of pressure on electrical membrane properties. Occasional experiments were done with control pipettes containing the recording medium. Pressure artifacts, whenever present, were easily distinguishable from genuine drug effects. A com-

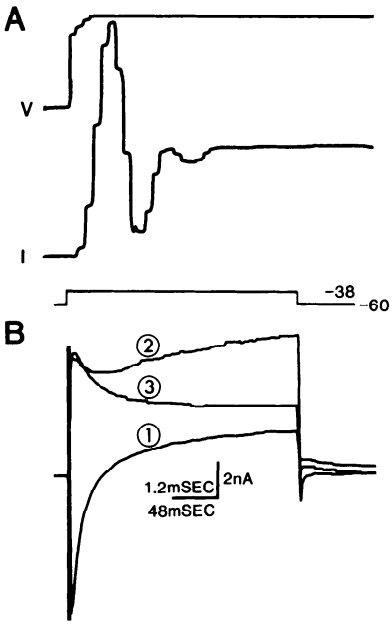

FIG. 1. Digitized records of a voltage-clamped cultured hippocampal cell. *A*: current response (bottom) to a voltage step (top) showing that the command potential reaches its final value (−38 mV) within 0.6 ms, while a brief capacitative current surge follows and dampens completely within several milliseconds. *B*: traces of currents generated in the same cell as shown in *A* are displayed at slower speed to reveal the entire duration of the membrane responses to the depolarizing command under control conditions (trace labeled 1), in the presence of 1 μM TTX (trace labeled 2), or TTX and 20 mM TEA (trace labeled 3). TTX blocks the rapid inward- (downward) going current response seen in trace 1, revealing a complex outward- (upward) going response (trace 2). In the presence of TTX and TEA (trace 3), the outward current response is transient and decays rapidly in an exponential manner. The voltage command has been traced from the digitized record. Experiments were done in the presence of 5 mM Co^{2+} to eliminate Ca^{2+} currents.

plete change of the recording medium was never attempted, since this would run the risk of losing the recording and would cause unwanted changes in the coupling capacitance between the electrodes.

RESULTS

Data were obtained from long-term stable recordings of 81 hippocampal neurons. On impalement with a second microelectrode, the resting membrane potential and input resistance deteriorated transiently and usually recovered within 5–10 min as sealing improved. After the recording had stabilized, the mean input resistance of the cells was 30.2 ± 1.5 (SE) MΩ when measured with 10- or 20-mV hyperpolarizing commands from a holding potential of −60 or −70 mV.

Depolarizing commands to potentials more positive than −50 mV consistently evoked a rapidly rising inward current that peaked within 5 ms. Its full amplitude and time course could not be measured accurately because of technical limitations involving the voltage clamp and the current-carrying capability of the system. Application of micromolar concentrations of TTX effectively eliminated this inward current response and routinely uncovered a rapidly rising outward current that peaked in 3–10 ms (Fig. 1*B*). Another, later component of the outward current response evoked during depolarizing commands was also detected in all cells studied (Fig. 1*B*). This had a slow time course and was activated at more depolarized potentials than the fast outward current. It was eliminated by 20–25 mM TEA, revealing a transient outward current with rapid kinetics. The relatively rapid kinetics of the early component resemble those associated with I_A in molluscan neurons (15–17, 35, 36). We will therefore refer to the early component of the outward current response recorded in cultural hippocampal neurons as I_A. The relatively slow kinetics of the later component and its sensitivity to TEA are two properties characteristic of the delayed rectifier K^+ conductance (I_K) described initially in the squid axon (26) and since identified in many other excitable membranes. It is distinctly different from the Ca-dependent K^+ conductance (I_C) in its resistance to Ca^{2+} conductance antagonists (see below). We will refer to the later component of the outward current response as I_K.

Hyperpolarizing commands to potentials negative to −80 mV revealed a slowly developing inward relaxation resulting in a greater steady state than instantaneous current response (Fig. 2*A*). This time-dependent inward current may underlie the anomalous rectifier described initially in hippocampal cells by Purpura et al. (37). A K^+ conductance activated over a similar range of membrane potential has recently been described in egg cells (22), retina (7), spinal cord (9), dorsal root ganglion (32), sympathetic ganglion (3), olfactory cortex (18), and hippocampal slices (23). We will refer to the current response observed over the hyperpolarized range of potential in hippocampal cells as I_{AR}.

In some cells (e.g., Figs. 2*A*, 17) a hyper-

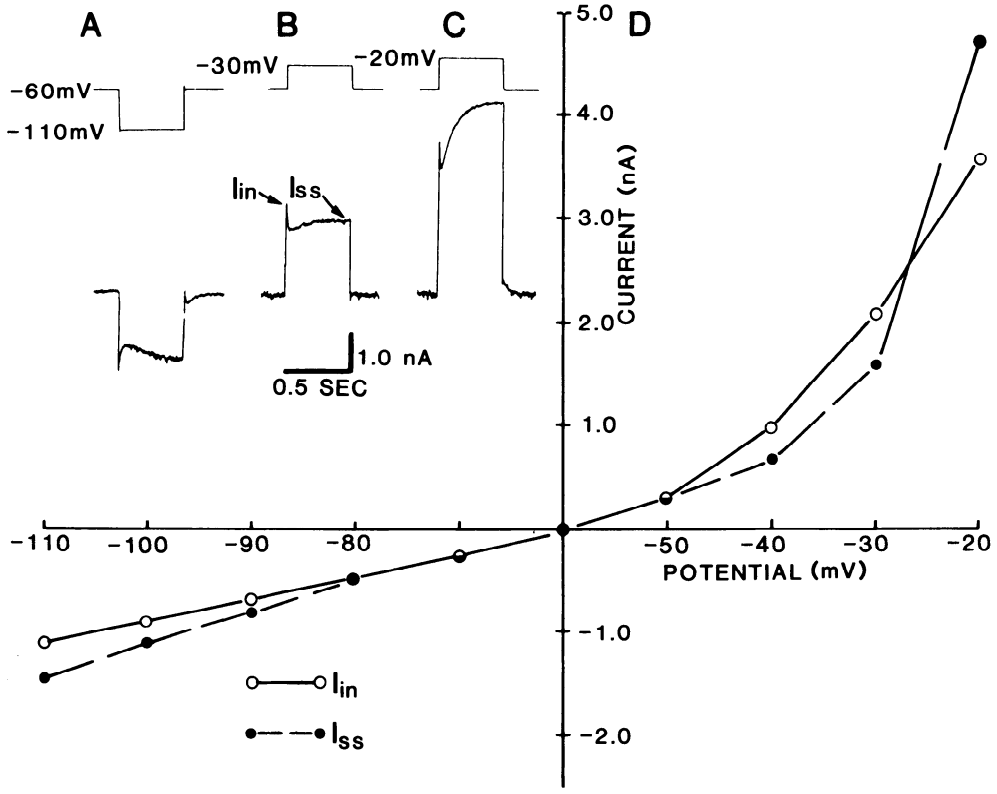

FIG. 2. Current responses to a series of depolarizing and hyperpolarizing commands from a holding potential of −60 mV with the cell recorded in medium containing 1 μM TTX and 5 mM Co^{2+}. *A*: a hyperpolarizing command to −110 mV produces both an initial transient phase and a slowly rising, sustained component to the inward current response. The latter current probably corresponds to the anomalous rectifier (I_{AR}). The transient inward current is only found in some cells and has not been analyzed in the present study. *B*: a depolarizing command to −30 mV generates both an initial transient phase and a delayed, sustained component to the outward current response. Note that a step from −30 mV back to −60 mV does not produce the transient inward current seen in *A*, indicative of the fact that this current is not simply unclamped capacitative transient. *C*: a depolarizing step to −20 mV reveals that the amplitude of the sustained component of the current response exceeds that of the transient phase. *D*: current-voltage plots of instantaneous current deflections (I_{in}) and steady-state current (I_{ss}) measured at the end of the 500-ms voltage commands. Note that I_{in}, corresponding to a previously reported species of K^+ conductance known as I_A, has a lower threshold for activation than the delayed, sustained component, which corresponds to the delayed rectifier, identified as I_K. However, the latter is associated with larger currents at more depolarized potentials. It is clear that over the range of membrane potential encompassing the usual spectrum of resting potentials in hippocampal neurons (−50 to −80 mV), there are no time-variant currents ($I_{in} = I_{ss}$), indicating that resting membrane potential is derived from ionic mechanisms that are relatively stable.

polarizing command resulted in a rapidly activating and rapidly inactivating inward current generated at potentials negative to −80 mV. This might be another distinct current or a capacitative transient. We have not yet characterized this current response in detail to distinguish between these possibilities.

Isolation of I_K and I_A

I_A usually became detectable when the membrane was stepped to potentials positive to −50 mV, while I_K became evident only at potentials positive to −40 mV. This can be seen in Fig. 2 where I_{in}, reflecting I_A, and I_{ss}, reflecting I_K, depart from the linearity of the current-voltage (*I-V*) curve observed over the range −80 to −50 mV. It was not possible to record the membrane current evoked at positive potentials because the current response saturated the current-carrying capacity of the system (~15 nA). Thus, by necessity, our observations have been made over approximately

a 100-mV range of membrane potential, from about −110 to about −10 mV. I_{in} usually departed from the linear slope of the *I-V* curve between −50 and −40 mV, whereas I_{ss} departed between −40 and −30 mV. The latter current response (I_K) was typically greater in amplitude than the early response (I_A) at potentials positive to −30 mV.

Both outward current responses could be inactivated by conditioning the cell at the appropriate potentials, although the potential range over which inactivation occurred differed and the rates at which inactivation proceeded were quite dissimilar (see Fig. 3). I_K inactivated at a much slower rate than I_A. A conditioning hyperpolarizing pulse could remove 50% of the inactivation of I_K if it were applied up to 3 s before a depolarizing step of sufficient amplitude. This contrasts with I_A, in which removal of inactivation could be achieved only with very short (20 ms) conditioning-test intervals (see below). These differences allowed us to study some of the properties of I_K and I_A in relative isolation.

I_K: electrophysiological properties

Experiments on the properties of I_K were done with 12 voltage-clamped hippocampal cells. Of these, four cells were recorded in a medium containing (in mM): 3 Ca^{2+}, 9 Mg^{2+}, and 5 Co^{2+} (three cells, e.g., Fig. 2) or 0.5 Cd^{2+} (one cell). Eight cells were recorded in the standard recording medium, containing 4 mM Ca^{2+} and 4 mM Mg^{2+}. There were no obvious differences between the two groups of cells, indicating that it is unlikely that I_K observed in these cells is contaminated much if at all by the Ca^{2+}-dependent K^+ current, I_C. Indeed, experiments on the characteristics of I_C (unpublished observations) revealed that it is associated with much less conductance than I_K when evoked over the same potential range. The experiments with I_K were not done in the presence of 4-AP (to eliminate I_A) because most of the measurements were taken when I_A was already inactivated. Also, high concentrations of 4-AP could affect I_K.

The ionic mechanism of I_K was studied by examining the effect of changes in $[K^+]_o$ on the reversal potential of the tail current. A four-step protocol was applied, involving a conditioning hyperpolarization to remove inactivation, then a brief step to −50 mV, sufficient to inactivate I_A yet too brief to cause

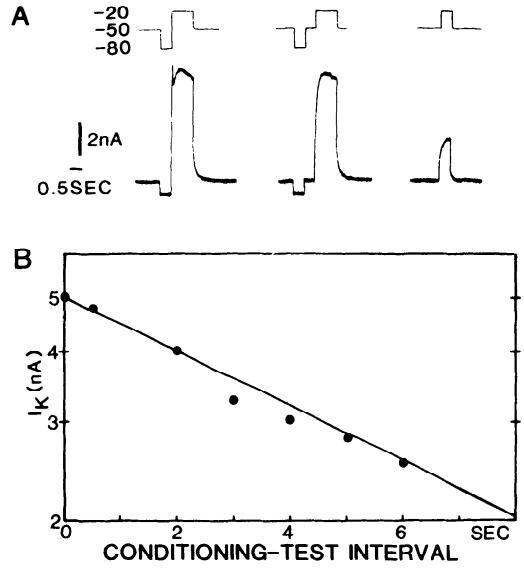

FIG. 3. Effects of conditioning hyperpolarization on the magnitude of I_K. With the cell conditioned first at −50 mV and then stepped to −20 mV, the outward current response consists of only the delayed, sustained component, I_K, at reduced amplitude (*A*). A 0.5-s hyperpolarizing command to −80 mV removes much of the inactivation present at −50 mV. The time course of inactivation that develops at −50 mV has been studied by varying the duration of the intermediate conditions of step at −50 mV. The transient outward current response, I_A, is evident when the intermediate step to −50 mV is brief but is absent when the intermediate step is prolonged. *B*: inactivation of I_K proceeds in an exponential manner as the interval between hyperpolarizing conditioning step and the depolarizing test step is increased. The medium in this experiment contained 3 mM Ca^{2+}, 9 mM Mg^{2+}, and 5 mM Co^{2+} to suppress activation of a Ca^{2+}-dependent K^+ current. Under similar conditions (see Fig. 12), I_A would not be seen past the origin (0 s) as it would be completely inactivated at the second data point (500 ms).

significant inactivation of I_K, followed by a 500-ms step adequate to activate I_K markedly, and then a series of steps over the −40- to −110-mV range of potential to assess the reversal potential of the tail current. Although the tail current became smaller when longer depolarizing commands (>5 s) were used, we did not explore the possibility that a shift in the reversal potential could account for this phenomenon. In normal, 5 mM K^+-containing medium, the reversal potential of I_K tail ranged between −70 and −80 mV. Elevation in $[K^+]_o$ by close application of 10-fold the normal concentration of K_o^+ consistently shifted the reversal potential in a depolarizing

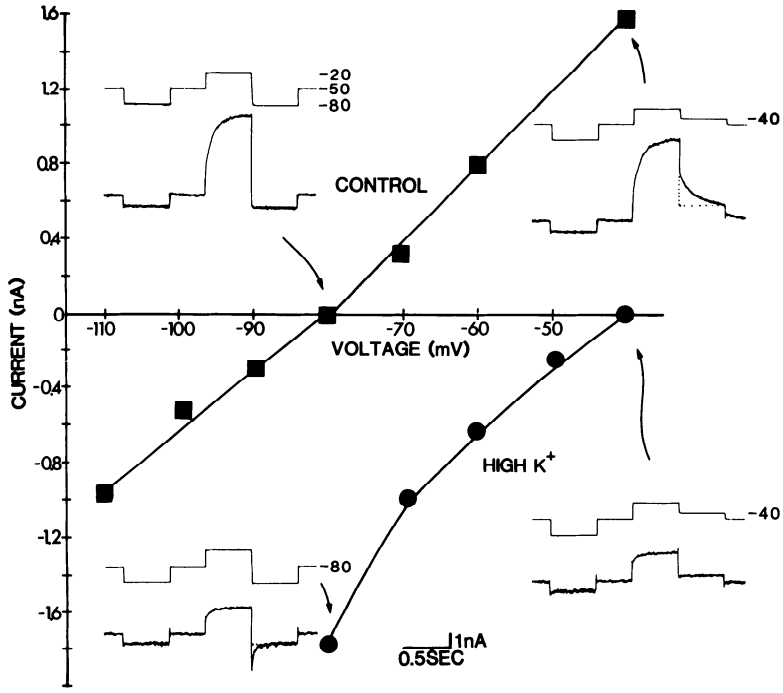

FIG. 4. Reversal potential of I_K in the cell illustrated in Fig. 3. A four-step paradigm was used and the magnitude of the tail current measured at various potentials following a depolarizing command to −20 mV. The two specimen records above the voltage axis are from the control condition where $[K^+]_o$ = 5 mM. The inversion potential is −80 mV at this $[K^+]_o$ (left). The two sets of traces below the voltage axis come from the part of the experiment conducted during application of medium containing 50 mM K^+. Note the reduction in the magnitude of I_K and the marked change in the tail currents evoked at the same voltage steps as in the control condition.

direction (Fig. 4). Since we did not measure the change in $[K^+]_o$ at the cell's surface, the 10-fold greater concentration of $[K^+]_o$ in the pipette should be considered an upper limit and unlikely to be the concentration effective over the entire surface of the cell. In Fig. 4 the reversal potential of the tail current response changes by 40 mV in a depolarizing direction, as would be expected if the conductance activated during the voltage command to −20 mV under these conditions primarily involves K^+ ions.

The maximal conductance, G_K, was estimated by extrapolating the exponential decay to zero time (Fig. 5*B*) and then dividing this value by the driving force. The latter was taken as the difference between the command potential and the K^+ equilibrium potential, which varied between −70 and −80 mV. We used −75 mV for cells where I_K tail reversal potential was not measured directly to estimate G_K. The calculated values for G_K ranged between 0.3 and 0.5 μS in five cells measured (mean, 0.35 μS), depending on the quality of recording and the ability to control the membrane potential.

The time required to reach the peak outward current response was about 500 ms, with the time constant of activation being typically less than 200 ms. I_K decay was likewise relatively slow, ranging in six cells from about 1.8 to about 4.0 s (the mean being 2.8 s) at command potentials equal to or less than −20 mV (Fig. 5*B*). The slow decay of I_K was also reflected in the tail currents (Fig. 4), which decayed exponentially with a single time constant. This was evident only for the outward tails developed at potentials < −50 mV. When the tail current was inward going, the decay was completed within a much shorter period (<100 ms compared to >500 ms at more depolarized potentials).

The activation and inactivation parameters could not be fully analyzed because currents

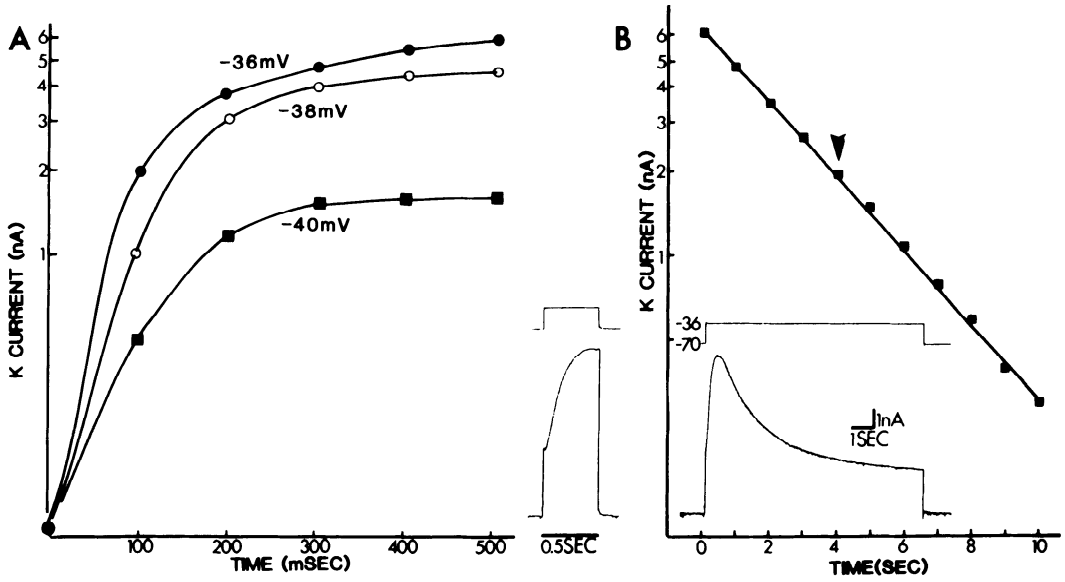

FIG. 5. Activation (*A*) and decay (*B*) times for I_K evoked by stepping the membrane from −70 mV in the depolarizing direction. Current responses to three voltage steps are plotted. The specimen record is in response to a voltage step to −36 mV. The activation time constant is ~180 ms. *B*: time constant of I_K decay was measured by stepping to −36 mV for 10 s. I_K decays with a single exponential, and in this illustration the time constant (arrowhead) is about 4 s.

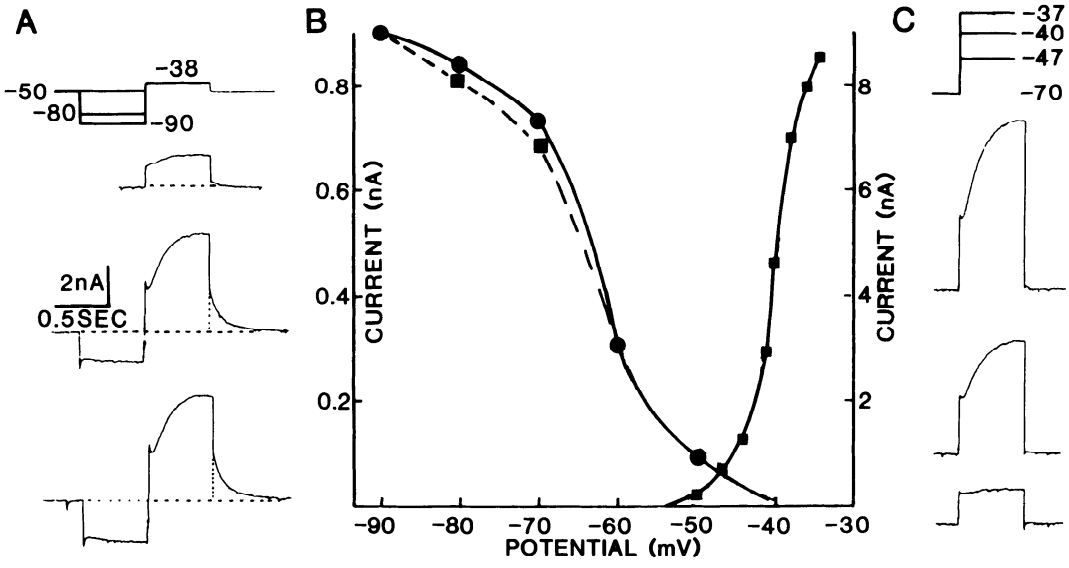

FIG. 6. Activation and inactivation properties of I_K. *A*: membrane potential was held at −50 mV and stepped to −38 mV to evoke an outward current response (top current record). Conditioning hyperpolarization for 0.5 s causes an increase in the outward current response to the depolarizing command. The voltage commands are schematized and are not actual records. The current response and its associated tail are plotted on the left-hand side of *B*. A close correspondence between magnitudes of the tail currents (filled circles, continuous line) and amplitudes of outward current responses (squares, dashed line) is evident. The left-hand scale is for the tail currents (nA). *C*: specimen records of current responses are shown for depolarizing voltage steps to −37, −40, and −47 mV from a holding potential of −70 mV. The peak amplitudes of the slowly rising current responses are plotted on the right-hand side of *B*. The inactivation and activation curves cross between −45 and −50 mV.

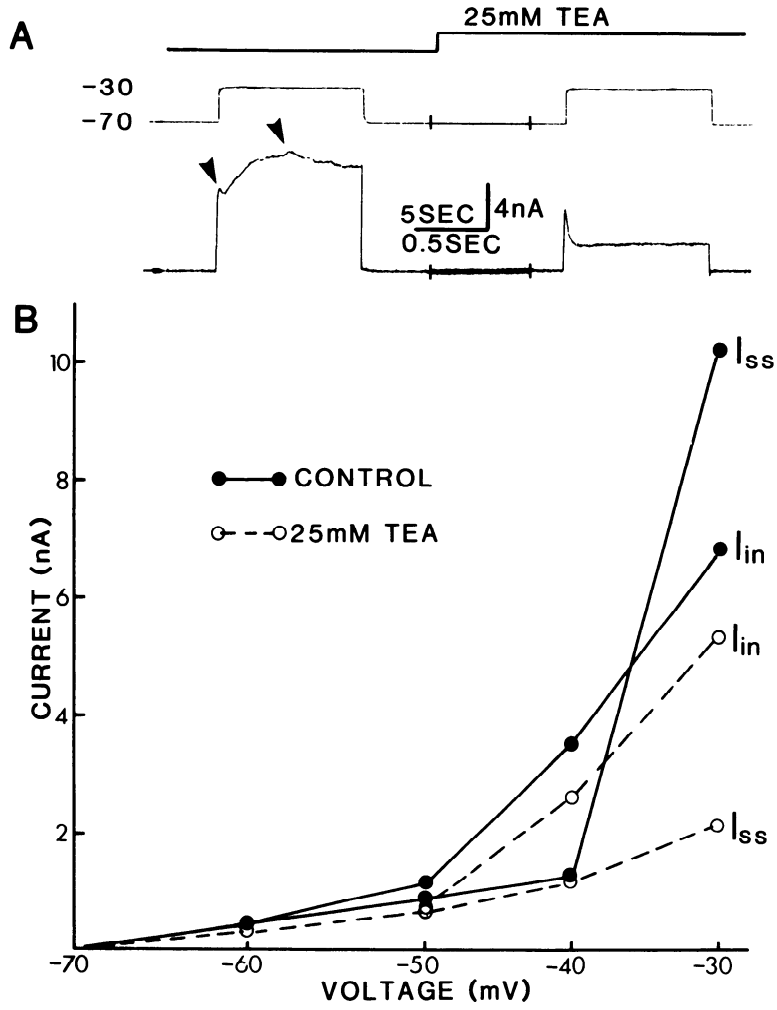

FIG. 7. Effects of TEA on outward current responses in a hippocampal cell. *A*: a continuous pen-recorder trace illustrating a membrane current response to a depolarizing command from a holding potential of −70 mV before and during extracellular application of TEA. The chart recorder was operated at 0.1 speed between the two commands. All the slowly developing, sustained current response (I_K; second arrowhead) is blocked by 25 mM TEA. The amplitude of the transient outward current (I_A; first arrowhead) is only slightly depressed. It is difficult to estimate the exact effect of TEA on I_A because I_A overlaps temporally with I_K, which is considerably greater in magnitude. *B*: current-voltage plots of the transient or instantaneous phase (I_{in}) and the slowly developing, sustained or steady-state component of the outward current response (I_{ss}), corresponding to I_A and I_K, respectively, before and during application of TEA. The control plots show that I_{in} is greater than I_{ss} at −40 mV, while I_{ss} is much greater than I_{in} at −30 mV. Plots in TEA show that at −30 mV, I_{in} becomes relatively greater than I_{ss} because I_{ss} is significantly more sensitive to TEA than I_{in}.

generated at potentials more positive than −10 mV usually saturated the system. Inactivation was assessed by using a multistep paradigm involving conditioning at different holding potentials, activating I_K by stepping to −38 mV and then stepping back to −50 mV, where the amplitude of the tail current could easily be measured. Both the amplitude of the tail current and the amplitude of the outward current response developed at the end of the depolarizing command were quite sensitive to the level of conditioning potential (Fig. 6). Since significant inactivation occurred at potentials more depolarized than −70 mV, activation was studied by stepping to more depolarized levels from holding potentials equal to or more hyperpolarized than −70 mV. Curves for inactivation and activation over-

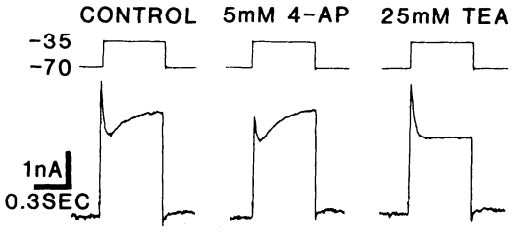

FIG. 8. Relative sensitivity of I_A and I_K to 4-AP and TEA. The three records are from the same cell, with sufficient time being allowed between successive drug applications for full recovery. The command to −35 mV was used so that only a low-amplitude I_K would be evoked. It is evident that 5 mM 4-AP and 25 mM TEA selectively depress the transient phase (I_A) and the delayed component of the current response (I_K), respectively.

lapped in the range −45 to −50 mV. Activation was detected at about −45 mV when evoked from a holding potential of −70 mV. Inactivation was significant at −60 mV and complete at −42 mV.

I_K: *pharmacology*

The sensitivity of I_K to TEA, known to interact with K^+ conductances in other membranes (22), was studied in nine cells. TEA caused a dose-dependent, reversible block of I_K (Fig. 7). Prior activation of the current was not required since a complete blockade of time-dependent current was often observed during the first test command evoked in the presence of the drug (Fig. 7). The concentration of TEA in the drug pipette producing about half-maximal depression was 10 mM and a full depression of I_K was seen with 20–25 mM. At the higher concentrations TEA also reduced I_A (see below). The recovery from TEA was relatively fast and was completed

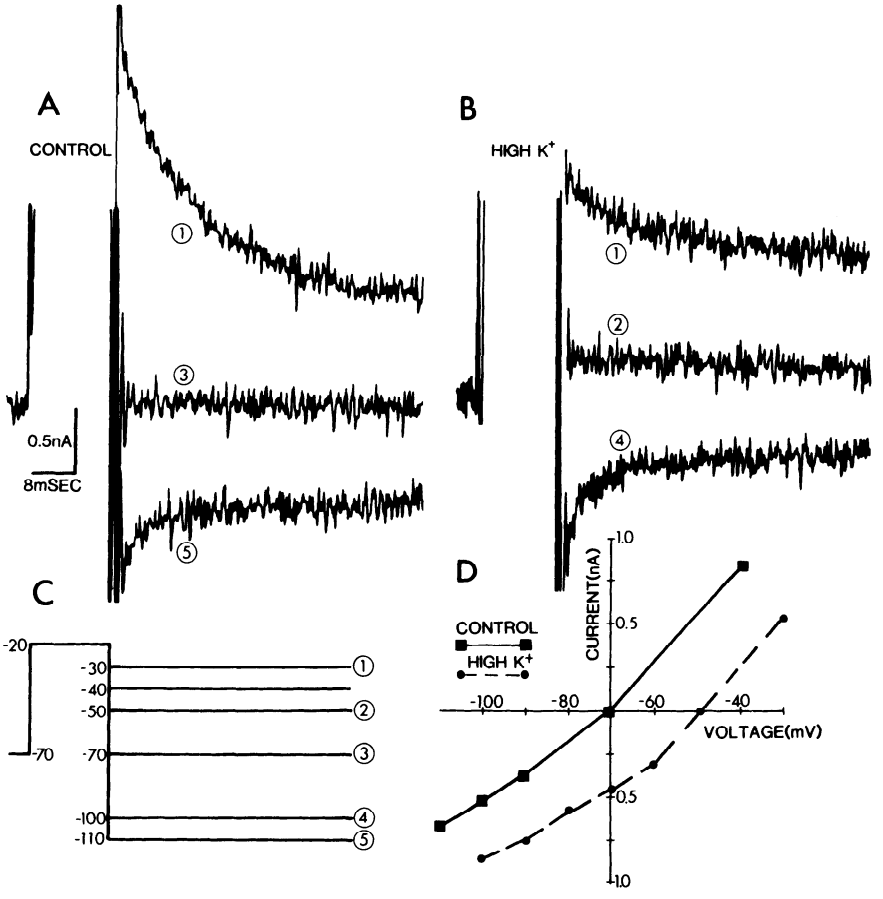

FIG. 9. Reversal potential of I_A. The membrane was held at −70 mV and stepped to −20 mV for 15 ms, and was then stepped back to various potentials depicted schematically in *C*. The tail current detected at each step was plotted in *D* to yield a reversal potential of about −70 mV. *B*: during the application of medium containing 25 mM K^+, the reversal potential shifts to about −50 mV.

within 0.5–1 min after removal of TEA. I_K showed little if any sensitivity to 4-aminopyridine (4-AP) over a range of drug concentration that effectively blocked I_A (Fig. 8). Higher concentrations of 4-AP, (e.g., 10 mM) could reduce I_K. I_K was not affected by extracellular application of 5 mM CS^+ (data not shown).

I_A: *electrophysiological properties*

Like I_K, I_A was detected in the majority of neurons tested (66 of 68). The properties of I_A were routinely studied in media containing sufficient (20–25 mM) TEA to eliminate I_K. In most of the cells analyzed in the present study there was little sign of a concurrent in-

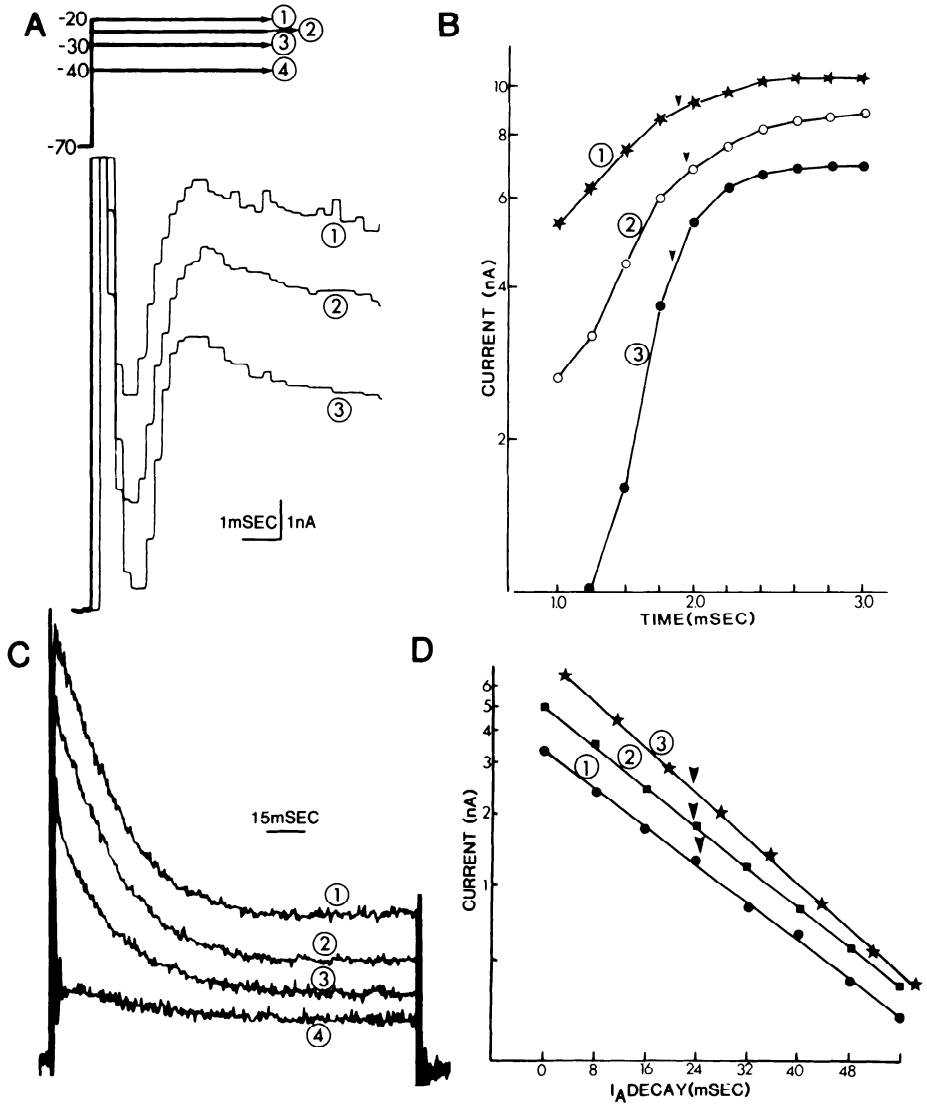

FIG. 10. Activation and decay of I_A. The cell was held at −70 mV and stepped for 150 ms to various potentials depicted schematically in *A*. These current responses were plotted using a Nicolet digital oscilloscope sampling the membrane current at 250 μS/point. Data have been displayed on an expanded time base to elucidate the activation time. I_A peaks within 3 ms from the onset of the voltage command. Note that the capacitative transient (initial large deflection) lasts −0.7 ms. *C*: decay of the three current samples, 1–3, depicted in *A*. Responses decay exponentially, with a time constant of about 24 ms. The medium in this experiment contained TTX and TEA but not Ca^{2+} antagonists, since there was no sign of concurrent inward Ca current. The same is true for the experiment in Fig. 11.

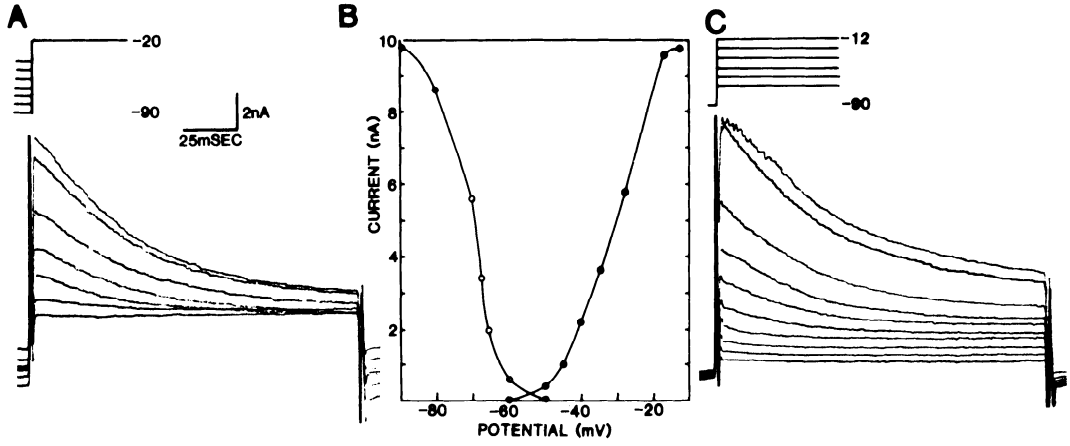

FIG. 11. Activation and inactivation of I_A. *A*: cell was held at various potentials and stepped to −20 mV, as depicted schematically in the top record. The family of current responses are plotted on the left-hand side of *B*. I_A is completely inactivated when the membrane is held at −50 mV and is fully activated by a step from −90 mV. *C*: potential was held at −90 mV and stepped to various depolarized levels, and the resultant family of currents is plotted in the right-hand curve of *B*. Note that the top record is noisy because the voltage clamp becomes unstable and the current-passing capacity of the system begins to saturate. The two curves appear to intersect at about −55 mV.

ward Ca^{2+} current. Thus, no Ca antagonists were used on a routine basis, except when otherwise noted. Also, since the time course of I_A is significantly faster than that of a possible I_C and is associated with much more current than the latter, it was assumed that

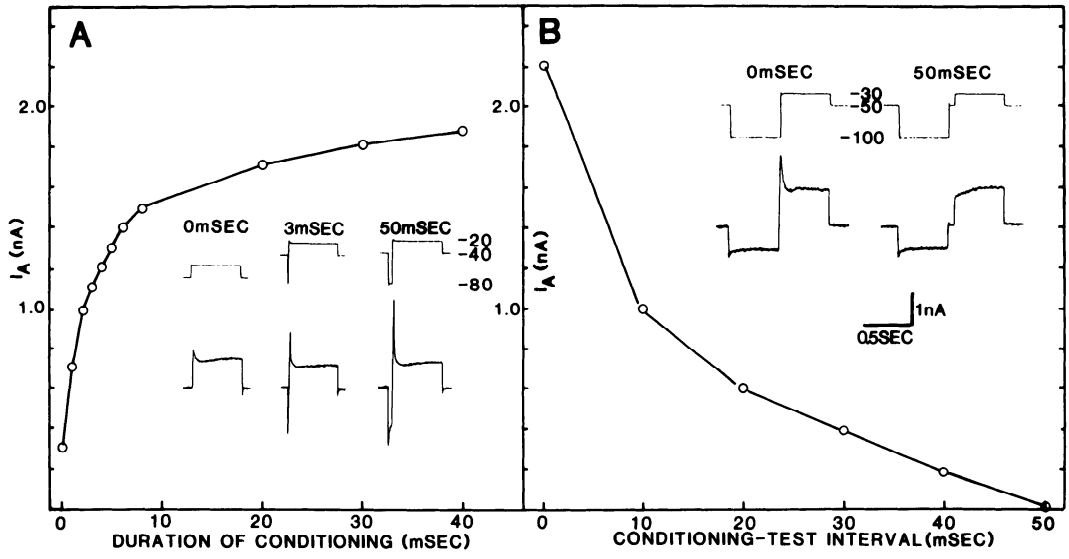

FIG. 12. Effects of conditioning hyperpolarization on the magnitude of I_A. *A*: effects of varying the duration of the hyperpolarizing step to −80 mV. The cell was held at −40 mV to inactivate I_A and then stepped to −20 mV after a variable conditioning period at −80 mV. A conditioning hyperpolarization at −80 mV of about 10 ms is sufficient to activate more than 80% of I_A. Note the presence of a small inward current during the current-response to a step from −40 to −20 mV. This, however, is not affected by a short (less than 200 ms) conditioning hyperpolarization. *B*: effects of conditioning at −50 mV for various periods on the magnitude of I_A following complete removal of inactivation at −100 mV. A delay of 10 ms is sufficient to inactivate more than 50% of the activatable I_A. The medium in this experiment contained TTX and TEA.

I_A parameters reported here are only marginally if at all affected by either I_{Ca} or I_C. The ionic basis of the current response was investigated in 50 cells by examining the effect of changing $[K^+]_o$ on the reversal potential of the tail current detected following a brief (10–20 ms) depolarizing command (Fig. 9). The duration of the depolarizing command was selected to yield a maximal tail current. Commands for durations longer than 20 ms produced progressively smaller tail currents in the presence of TEA. Thus, it is unlikely that I_A tails are contaminated by I_K. Application of K^+ ions from a pipette containing 5 times the normal $[K^+]_o$ consistently shifted the reversal potential in a depolarizing direction from that normally observed, which was about −75 mV. In addition, they caused a substantial reduction in the amplitude of I_A, evoked by the same depolarizing command. Quantitation of the change in $[K^+]_o$ effective at the cell surface was not attempted.

The time required to reach the peak current response was measured from the digitized records following the capacitative current or by measuring the delay to peak in records calculated by subtracting the current response in the presence of a blocking concentration of 4-AP (e.g., Fig. 13*B*). The time to peak ranged between 3 and 6 ms. (Figs. 10 and 13*B*). While this value is markedly dependent on the quality of the clamp and the duration of the capacitative surge, it was clear that in none of the cells examined did a 4-AP-sensitive transient outward current reach a peak more than 10 ms after the onset of the depolarizing command. I_A decayed exponentially with a time constant usually in the range 20–24 ms (Fig. 10*B*), and this decay was independent of the command voltage (Fig. 10*D*). The maximal I_A conductance, G_A, was estimated by extrapolating the exponential decay to zero time (Fig. 10*D*) and then dividing the resultant current by the driving force (see above). The calculated values ranged between 0.2 and 0.3 μs (n = 12). The activation properties of I_A were studied by holding the cell at −90 mV and stepping to different potentials to establish an activation curve. I_A consistently activated at about −50 mV and did not reach saturation before the clamp became unstable (about −10 to −20 mV) (Fig. 11*B, C*).

Inactivation was detected at potentials depolarized to −90 mV with 50% inactivation occurring at about −70 mV. Usually, inactivation was complete when the cell was clamped at potentials positive to −50 mV. There was little, if any apparent overlap between the activation and inactivation curves, indicating that I_A does not contribute to the steady-state conductance of the neurons. The process of inactivation was analyzed in two types of experiments. In one, the duration of conditioning hyperpolarization needed to remove inactivation of I_A was measured. It was

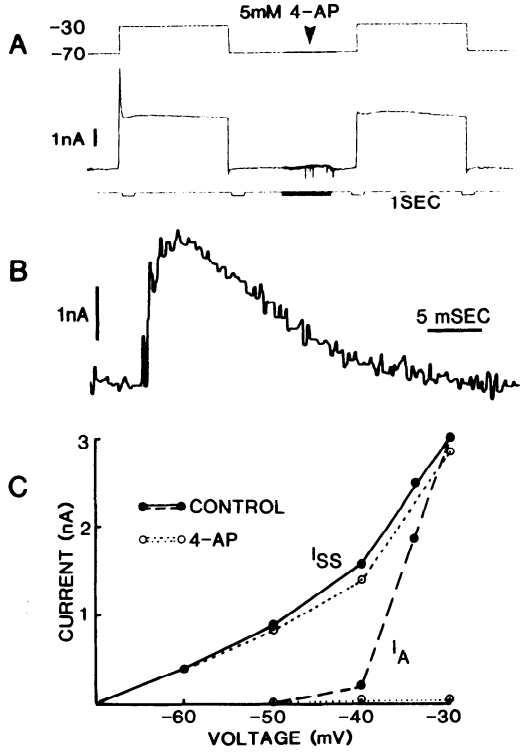

FIG. 13. Effects of 4-AP on I_A. *A*: a continuous record showing the outward current response to a 1-s step to −30 from −70 mV. 5 mM Co^{2+} has been included in the recording medium in order to block a possible effect of 4-AP on I_{Ca}. Following the response, the chart recorder speed was slowed down (note 1-s marks below current trace) and 5 mM 4-AP applied at level of the cell body. The next voltage command to −30 mV evoked little if any transient outward current. The current response in 4-AP was subtracted from the control using the Nicolet digital oscilloscope to yield the amplitude and time course of I_A, depicted in *B*. This illustrates that I_A reaches a peak within 3 ms and decays with a time constant of about 25 ms. *C*: current-voltage plot to illustrate that 4-AP has little effect on the steady-state current (I_{ss}) while eliminating I_A.

found that a brief hyperpolarization was sufficient to remove inactivation of I_A (Fig. 12*A*), with removal of inactivation reaching 95% of its maximal value with a 50-ms pulse. In another type of paradigm, the cell was first conditioned at a hyperpolarized level to eliminate inactivation of I_A, then stepped for a variable period to −50 mV before triggering I_A with a more depolarizing command. The conditioning step at −50 mV inactivated I_A with a time constant of about 20 ms (Fig. 12*B*).

I_A: *pharmacology*

The sensitivity of I_A to various substances known to interact with K^+ conductances in other membranes was measured in 29 cells. I_A was relatively insensitive to TEA at concentrations that eliminated I_K (Fig. 8) but was reduced at higher concentrations in some cells. I_A was effectively blocked in a dose-dependent, reversible manner by 4-aminopyridine (4-AP) in all 29 cells tested (Fig. 13). A depressant effect was detectable at 0.5 mM and was nearly

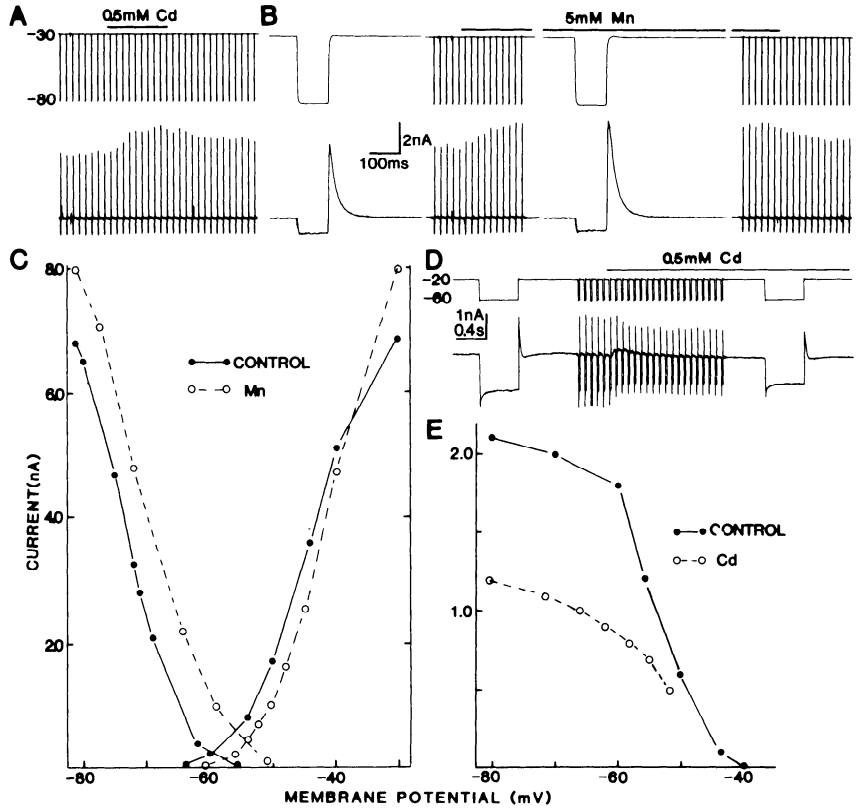

FIG. 14. Complex effects of Cd^{2+} and Mn^{2+} ions on transient outward current responses in cultured hippocampal neurons. *A–C* are results from the same cell. *A*: in this experiment the cell was held at −30 mV and stepped to −80 mV for 100 ms to remove inactivation of I_A and then returned to −30 mV to activate I_A. Topical application of Cd^{2+} ions and Mn^{2+} ions rapidly and reversibly increases the amplitude of I_A while just slightly decreasing leak conductance. The two traces in *B* shown at faster speed illustrate that Mn^{2+}, like Cd^{2+}, has little if any detectable effect on the time course of I_A decay. *C*: activation and inactivation curves summarizing current responses obtained during depolarizing commands from a holding potential of −80 mV (curves on right-hand side) or from −30 mV with interposed conditioning steps to hyperpolarized potentials (curves on left-hand side). Inactivation is shifted in a depolarizing direction parallel to the control curve, while activation is slightly shifted in a depolarizing manner over the range −60 to −40 mV and then crosses the control curve at −30 mV. Thus, I_A would be depressed by Mn^{2+} over the −60- to −40-mV range but enhanced at −30 mV. *D* and *E*: Cd^{2+} ions applied to a cell repeatedly stepped from −20 to −60 mV reduce I_A and the leakage conductance and markedly shift the inactivation curve for I_A so that nearly 50% less current is evoked at −20 mV following a conditioning step into the −60- to −80-mV range.

maximal with 5 mM 4-AP. Both the time course of the depression and the subsequent recovery were characteristically slow, with full recovery sometimes requiring over 10 min (data not shown). The depressant effects of 4-AP did not appear to depend on prior activation of I_A, since the current response could be virtually eliminated during the first activation of I_A in the presence of 4-AP (Fig. 13*A*). Since 4-AP can enhance Ca^{2+} conductances in these cells (14, 20), some of the experiments were performed in solutions containing sufficient Co^{2+} (four cells) or Cd^{2+} (two cells) to block Ca^{2+} conductances. Under these conditions, 4-AP depressed I_A with only little effect on leakage conductance. This was measured either at the end of the 0.5-s depolarizing command (Fig. 13*C*) or during a conditioning pulse to −90 mV, which was used in some cells prior to activation of I_A (not shown). The complete depression of I_A by 4-AP allowed us to subtract the remaining current from the total current recorded under control conditions to reveal the time course and extent of I_A (Fig. 13*B*).

I_A was not affected by extracellular Cs^+ and was also present when Na^+ was totally replaced in the medium by either $Tris^+$ or $choline^+$ (three cells, data not shown). The possible involvement of Ca^{2+} in the regulation of I_A was examined in cells recorded in a medium containing (in mM): 4 Ca^{2+}, 4 Mg^{2+}, and 20 TEA before and during topical application of 0.2 Cd^{2+} (13 cells) or Mn^{2+} (3 cells). The effects of Cd^{2+} were complex and depended on the initial clamp potential as well as the magnitude of the depolarizing command. Two opposite effects of Cd^{2+} were seen when a fully activated I_A was generated by stepping from −90 mV to potentials positive to −30 mV. Cd^{2+} (three cells) and Mn^{2+} (three cells) both enhanced I_A (Fig. 14*A, B*), sometimes by up to 50%. This effect appeared to be due to shifts in both activation and inactivation properties of I_A (Fig. 14*C*). In 10 cells, Cd^{2+} suppressed I_A by up to 50% (Fig. 14*D*). This effect was especially apparent in the inactivation paradigm (Fig. 14*E*). Even in cells where Cd^{2+} or Mn^{2+} enhanced a fully activated I_A (Fig. 14*A–C*), it suppressed a partially activated I_A (e.g., when stepping to potentials negative to −40 mV (Fig. 14*C*)). The enhancing effect of Cd^{2+} on I_A probably does not reflect suppression of a concomitant inward Ca^{2+} current. Such suppression should lead to prolongation of I_A decay, since I_{Ca} decays much more slowly than I_A (unpublished observations). At any rate, there was only a minimally detectable I_{Ca} in the cells analyzed for I_A, as noted above. This might be due to a collapse of the Ca^{2+} ion gradient. Indeed, when cells were purposely loaded with Ca^{2+}, Cd^{2+} could still enhance I_A

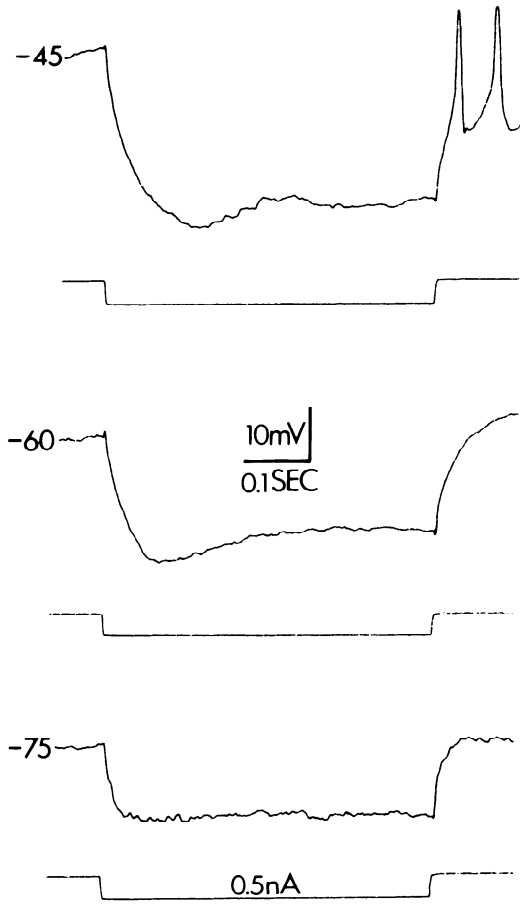

FIG. 15. Inward rectification of a rat hippocampal neuron. The cell is current clamped at different potentials and a constant hyperpolarizing current pulse (0.5 nA) is applied through the bridge circuit in the recording electrode. Top, the cell is depolarized to −45 mV and the current pulse yields a hyperpolarizing response that peaks at 100 ms and then relaxes 3–4 mV in a depolarizing direction. Anodal break potentials are evident on return to −45 mV. Middle, the cell is held at −60 mV, and a 4- to 5-mV depolarization is seen after the hyperpolarizing voltage response peaks. Hyperpolarization to −75 mV reveals the presence of a noninactivating steady-state increase in membrane conductance and absence of a depolarizing sag.

(unpublished observations). Furthermore, enhancement of I_A by Cd^{2+} or Mn^{2+} was not associated with a change in the rate of I_A decay (compare records shown in Fig. 14*B*). When stepped to potentials negative to −30 mV, Cd^{2+} suppressed I_A in the other 10 cells studied. These results suggest that Ca^{2+} ions may be involved in a complex manner in regulating I_A in cultured hippocampal cells. A further examination of the modes of interactions of Ca^{2+} with I_A is planned.

I_{AR}: electrophysiological properties

The membrane properties of some neurons were first examined with a single recording electrode over a wide range of membrane potentials. A time- and voltage-dependent sag in the voltage response to a constant-current pulse was recorded in some of these cells. At depolarized potentials, the initial voltage deflection was reduced after 100–150 ms. This "sag" was not seen if the cell was hyperpolarized. Instead, the same current pulse produced a smaller voltage deflection in the hyperpolarized cell (Fig. 15). This time- and voltage-dependent rectification corresponds to the anomalous rectification described previously in a variety of excitable membranes. Twenty-seven of 41 cells studied over the range

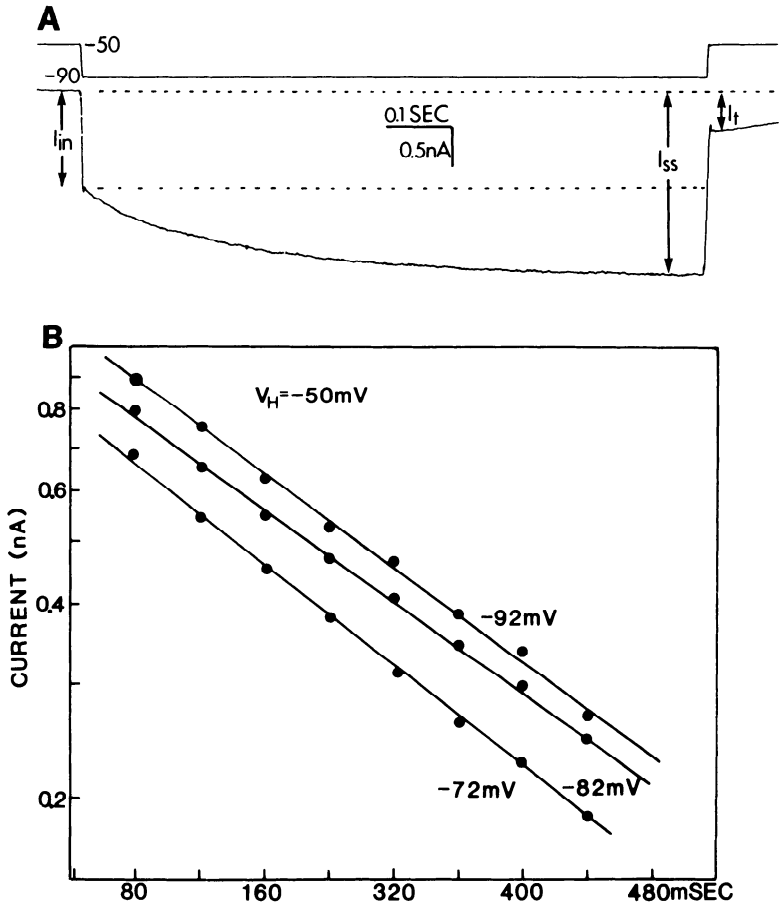

FIG. 16. Time course of the anomalous rectifier, I_{AR}. The cell was voltage clamped at −50 mV and stepped to −90 mV. An instantaneous current deflection (I_{in}), representing the leakage conductance, is followed by a slowly developing inward current response whose time constant of activation in this cell was about −200 ms. Termination of the voltage step results in a larger instantaneous deflection, indicating an increase in conductance, and a slowly relaxing tail current (I_t). *B*: semilogarithmic plots of the increase in current as a function of time for steps to −72, −82, and −92 mV from a holding potential of −50 mV.

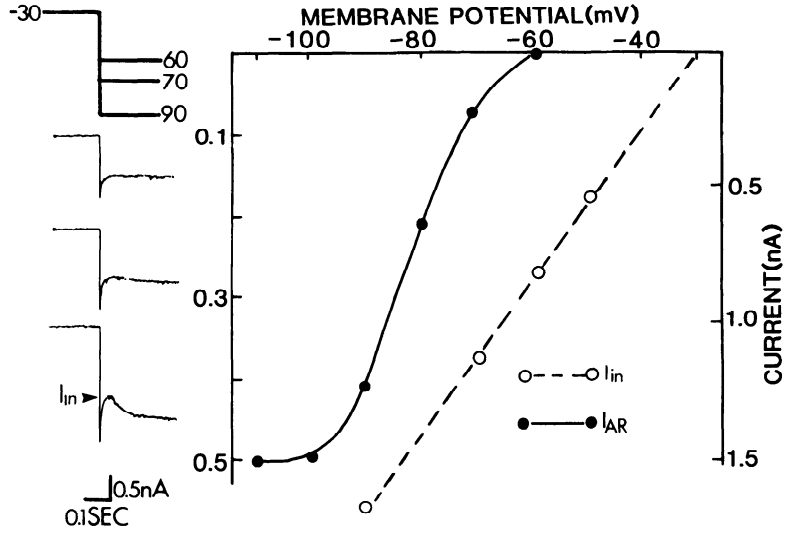

FIG. 17. Activation properties of I_{AR}. The cell was held at −30 mV and stepped to hyperpolarized levels (depicted schematically on the left). I_{AR} becomes detectable at −70 mV and is fully activated at −100 mV. Current responses at −60, −70, and −90 mV are shown at left. The current-voltage relations are plotted at the right for both the instantaneous current response (I_{in} is indicated by arrowhead on lowermost current trace) and for the net amplitude of I_{AR}. Note differences in ordinates for amplitudes of I_{in} (right ordinate) and I_{AR} (left ordinate).

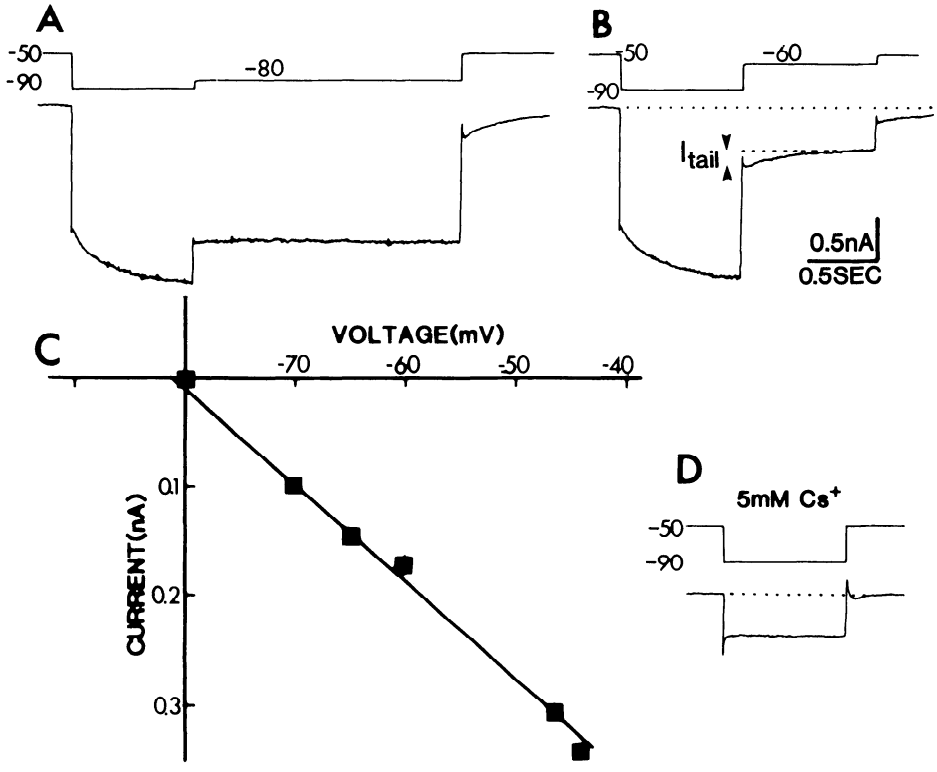

FIG. 18. Reversal potential of I_{AR}. *A* and *B*: an initial step is made to −90 mV to activate I_{AR}, followed by steps back to various potentials. The resulting current relaxations are plotted in *C*. *D*: these relaxation currents are dependent on activation of I_{AR}, since they are absent when I_{AR} is blocked by Cs^+.

of potential from −70 to −110 mV showed evidence of this type of rectification.

The currents underlying this membrane property were studied in 27 cells exhibiting anomalous rectification. Hyperpolarizing commands from holding potentials in the range −40 to −70 mV evoked time- and voltage-dependent increases in membrane current, I_{AR} (Fig. 16*A*). Membrane current began to increase with an exponential time course, almost immediately following a voltage step, the time constant of activation being 150–250 ms (Fig. 16*B*). In some cells the onset of the time-dependent increase in inward current was preceded by a rapid, transient inward current that decayed within 50 ms. The ionic nature and biophysical properties of this transient inward current are yet to be analyzed. I_{AR} appeared to show little if any decrement following its activation. A return to a more depolarized level was associated with a slowly relaxing outward current response.

Activation curves for I_{AR} were constructed by holding the cell at −30 mV and stepping to various hyperpolarized levels. The cell was held at a depolarized potential so as to allow detection of a second, time-dependent K^+ conductance, I_M. The first deviation from a linear current-voltage relation was detected when the potential was stepped to −70 mV. A full activation was seen when the membrane was stepped to −100 mV with half-maximal activation being at about −85 mV (Fig. 17).

Activation of I_{AR} superficially resembled the time-dependent changes in membrane current associated with the decay of another non-inactivating K^+ conductance (I_M) reported to exist in several types of neurons, including hippocampal cells (20). I_M was observed in too few cultured hippocampal cells to allow careful study of its properties. When detectable, the inactivation of I_M occurred over a potential range decidedly depolarized to that associated with activation of I_{AR}.

The ionic mechanisms of I_{AR} were analyzed in two types of experiments. The reversal potential of the tail current was measured in double-pulse experiments (Fig. 18), in which the membrane was first stepped to −90 mV and then back to various potentials. Care was taken to avoid activation of other conductances (e.g., I_A) by not stepping to depolarized potentials where these would activate (i.e., depolarized to −40 mV). Tail currents disappeared between −75 and −80 mV, similar to the reversal potentials of I_K and of I_A. It is difficult to show a true reversal of I_{AR} tail since the expected reversed tail is in the activation range of this current. Evidence that the tail current did indeed reflect I_{AR} can be seen in Fig. 18*D*, where extracellular application of Cs^+ ions greatly reduced both I_{AR} and its associated tail current. The dependence of I_{AR} on $[K^+]_o$ was measured by increasing $[K^+]_o$ from 5 mM to a value close to 25 mM, the concentration in the pressure pipette. The amplitude of I_{AR} was increased in the depolarizing direction, probably because of a larger driving force underlying this current due to a shift in E_K in the depolarizing direction (Fig. 19).

The maximal conductance associated with I_{AR}, G_{AR}, was estimated by subtracting I_{ss} from

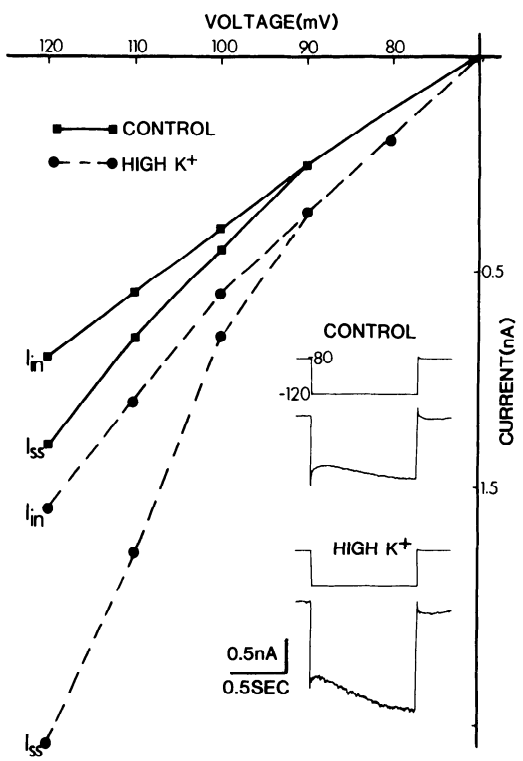

FIG. 19. Effect of elevated $[K^+]_o$ on the magnitude of I_{AR}. A current-voltage plot depicting instantaneous (I_{in}) and steady-state (I_{ss}) current responses to voltage steps from a holding potential of −70 mV. The instantaneous current is measured after the decay of the transient inward current. Topical application of medium containing elevated (25 mM) K^+ results in an increase in I_{in} and a larger increase in I_{ss}.

I_{in} in response to a command potential in the range −100 to −110 mV and then dividing this current by the driving force. The latter was calculated using the potential difference between the K^+ equilibrium potential (−75 mV), which was chosen as a mean of several cases where I_K, I_A, and I_{AR} reversal was measured, and −110 mV. Altogether, G_{AR} attained lower values than those associated with the other K^+ conductances, with an average of 15.6 nS being measured in 27 cells.

I_{AR}: pharmacology

I_{AR} was markedly reduced by extracellular application of 5 mM Cs^+ in five cells tested (Fig. 20). The application of Cs^+ ions was also associated with a gradual increase in outward current and a sizable decrease in the steady-state conductance measured instantaneously with a 50-mV command. The possible involvement of Na^+ in I_{AR} was examined in five cells by extracellular application of medium in which 105 mM Na^+ had been replaced in an equimolar manner by choline$^+$. Application of choline-containing medium, like Cs^+, caused a marked increase in input resistance of the cells. Concurrently with the reduction in leak conductance there was a complete disappearance of I_{AR} and its associated tail current (Fig. 21). In the absence of Na^+ ions, I_A could still be recorded (Fig. 21*C*, lowest pair of traces). Finally, I_{AR} was not altered by top-

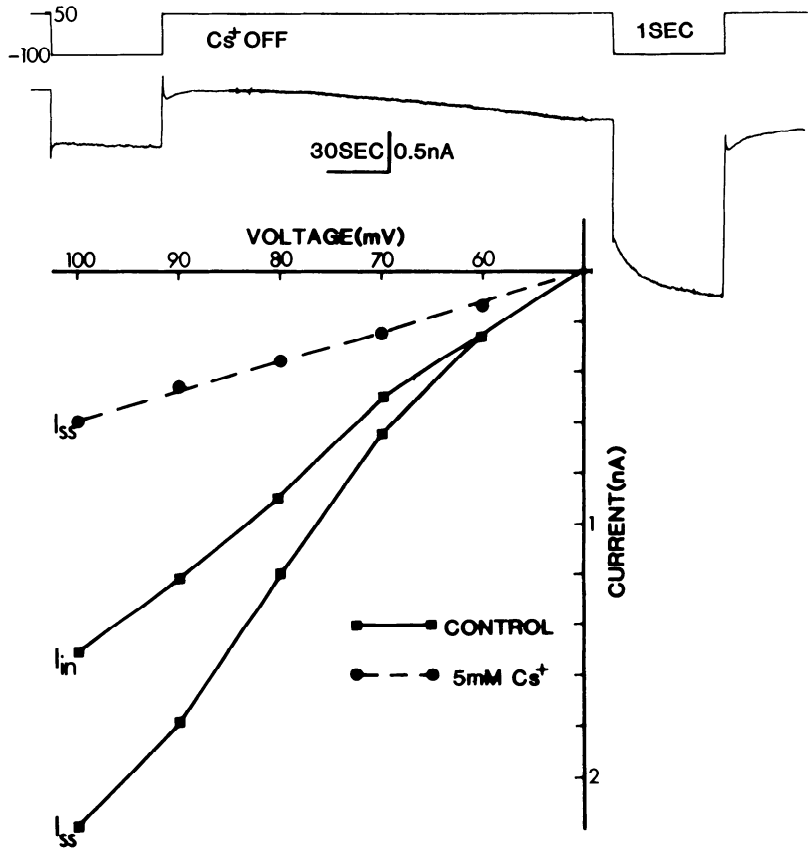

FIG. 20. Effects of 5 mM Cs^+ on I_{AR}. Specimen record is a continuous chart recorder illustrating the response to a 0.5-s voltage command in the presence of Cs^+ and shortly after Cs^+ was removed. The response after recovery was similar to that recorded before Cs^+ application (not shown). Note the change in chart speed between the successive voltage commands. Bottom, current-voltage curves summarizing the current responses to the voltage commands in the absence and presence of Cs^+. The instantaneous current, measured after the decay of the transient and the steady-state current have the same relationship with membrane voltage in the presence of Cs^+.

ical applications of either TEA or 4-AP or Ba^{2+}, which blocks Ca^{2+}-dependent K^+ conductances in these cells (unpublished observations).

Drug effects on voltage responses in current-clamped cells

The electrical activity of six hippocampal cells was recorded under current clamp in medium containing (in mM): 4 Mg^{2+}, 4 Ca^{2+}, and 1.0 TTX. Depolarizing current stimuli of sufficient intensity evoked characteristic transient and delayed forms of rectification in all six cells (Fig. 22). The delayed rectification was selectively blocked by topical application of TEA (Fig. 22*A*), while the transient rectification was selectively blocked by 4-AP (Fig. 22*B*). Cd^{2+} ions had little if any effects on

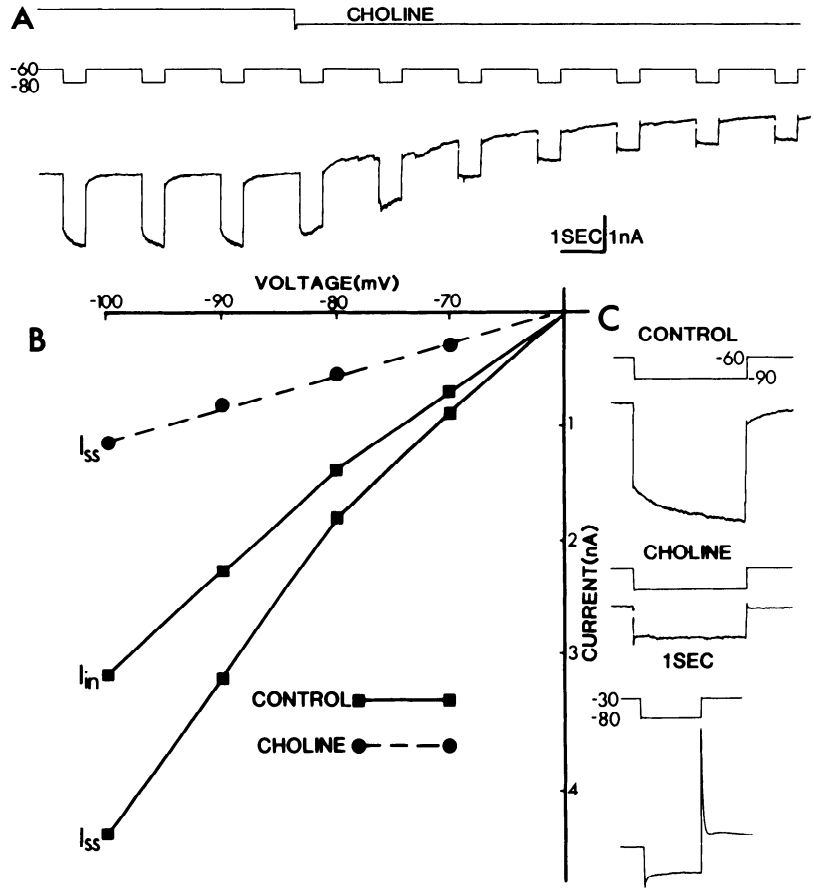

FIG. 21. I_{AR} is eliminated by topical application of a low-Na^+ medium. *A*: cell was clamped at −60 mV and then repeatedly stepped to −80 mV for 500 ms once every 2 s to monitor the amplitude of I_{AR} before and during the topical application of solution in which Na^+ ions have been replaced in equimolar manner by choline$^+$ ions. The start of the application is indicated in the topmost trace. The application of choline rapidly eliminates I_{AR} and induces a slowly developing outward current as well as marked reduction in the instantaneously measured slope conductance. *B*: plots of *I–V* relations before and during topical application of choline$^+$ were derived from a series of current responses evoked by 1.0 s hyperpolarizing commands of increasing amplitude from a holding potential of −60 mV. Both the instantaneously measured current, I_{in}, and the steady-state current, I_{ss}, have been plotted under control conditions since they were detectably different, reflecting the presence of time-dependent I_{AR}. Specimen records are shown in *C*. In the presence of choline$^+$, I_{AR} has been eliminated and I_{in} and I_{ss} become identical. The lowest pair of traces in *C* show the same cell in the presence of choline$^+$ held at −30 mV and then stepped to −80 mV for 1 s. I_{AR} is not detectable but on returning to −30 mV, a sizable transient outward current response, I_A, is still clearly evident.

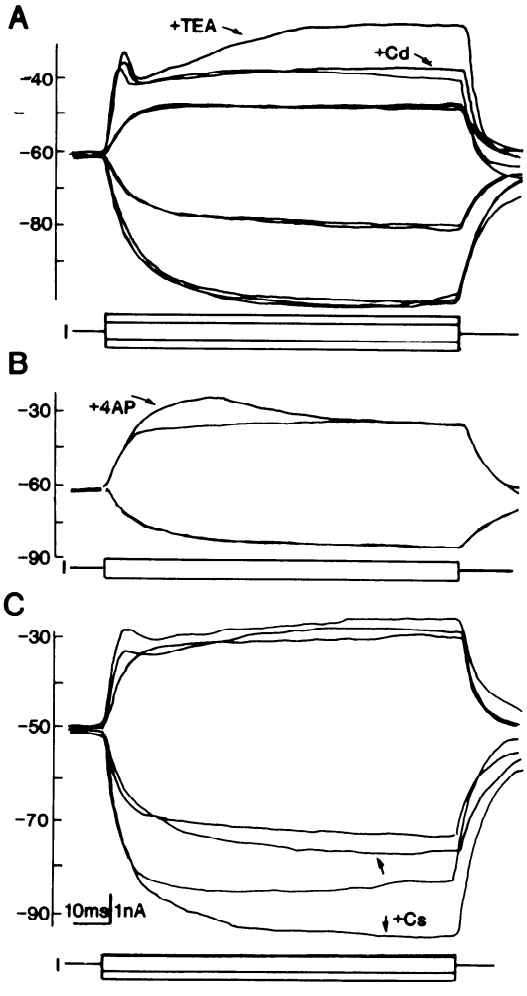

FIG. 22. Voltage responses to depolarizing and hyperpolarizing current pulses in cultured hippocampal neurons. In each section, pulses of 0.4 and/or 0.8 nA have been applied in cells current clamped at the resting membrane potential in the presence of 1 μM TTX. *A*: voltage responses to ±0.4-nA pulses are relatively symmetrical, exponential in time course, and unaffected by either 20 mM TEA or 0.5 mM Cd^{2+}. Voltage responses to ±0.8-nA pulses are clearly asymmetrical, with rectification developing after about 10 ms during the depolarizing response at −40 mV. The late component of membrane rectification is attenuated by TEA with little effect on the transient rectification occurring at 10 ms. Cd^{2+} has little if any effects on either the transient or delayed rectifying components and neither TEA or Cd^{2+} alter the hyperpolarizing responses. The unlabeled trace evoked by +0.8 nA is the voltage response obtained under control conditions. *B*: 4-AP blocks the early phase of rectification in another cell without affecting the delayed component or the hyperpolarizing response. The unlabeled one of the pair of traces evoked by depolarizing current is the response obtained under control conditions. *C*: topical application of 5 mM Cs^+ markedly attenuates the anomalous rectification recorded at hyperpolarizing potentials with little if any effects on the transient or delayed rectifications evoked in the depolarizing direction. The top traces in each pair evoked in the hyperpolarizing direction, which are unmarked, are the control responses.

either transient or delayed components (Fig. 22*A*). In four of the six cells an anomalous form of rectification was detected when the cells were hyperpolarized by current pulses of sufficient intensity. This rectification was blocked by 5 mM Cs^+ (Fig. 22*C*) (but not affected by either 4-AP, TEA, or Cd^{2+}). Finally, Cs^+ had little effect on the rectifications evoked at depolarized potentials.

DISCUSSION

In the present experiments we have characterized, in a semiquantitative manner, some properties of some of the K^+ conductances that are present in the majority of cultured hippocampal neurons. Another K^+ conductance, activated by depolarization and dependent on Ca^{2+}, will be considered elsewhere. Two of the K^+ conductances described in this paper, I_K and I_A, have been observed in virtually every cultured neuron and were activated over a range of membrane potential depolarized relative to the K^+ equilibrium potential. The current-carrying capacity of the system and difficulties encountered in clamping whole cells at positive membrane potentials prevented us from examining I_K and I_A in more detail over a wider range of potential. Although we were not able to account quantitatively for the membrane-potential behavior generated by these conductances, we were able to compare their properties over a sufficient range of potential to reveal that they play important yet different roles in shaping the electrical activity of hippocampal cells. These functional roles appear to be similar to those described for I_K and I_A in other membranes.

A major problem of interpretation of our results is in the inability to assess accurately the adequacy of the space clamp of these neurons. When measured at 35°C and without drugs in the medium, these cells have a relatively short electrotonic length ($L \cong 0.9$). While this is considered "short" by most (12, 25), it still does not allow an accurate evaluation of the contribution of remote processes to the measured currents. This limitation is

of particular concern in view of the suggestion that dendrites of hippocampal neurons can generate regenerative potentials and may possess some of the voltage-dependent K^+ channels discussed here (56). An inadequate space clamp would primarily distort our estimates of the time constants of activation and decay of I_A and I_K. These problems obviated a more quantitative level of analysis of the currents, as has been done elsewhere (15). Most of the experiments were done under conditions designed to isolate one of the K^+ conductances for study. Thus, experiments focused on I_A were done in the presence of TEA and some also in the presence of Co^{2+} or Cd^{2+}. Studies on I_K were usually done in the presence of Co^{2+}, while those on I_{AR} were carried out in medium containing TEA. Furthermore, all of the experiments were done at room temperature so as to slow down the kinetics of the conductances and achieve better control over membrane voltage. While this poses problems when attempting to relate our values to those obtained using current clamp in situ at 37°C, we felt justified in studying excitability at a temperature where we could impose better control over membrane potential. Finally, cultured hippocampal neurons have thinner, shorter (and possibly more electrically compact) apical dendrites than those of hippocampal neurons studied in situ (unpublished observations). This difference may be related to the extensive number of afferents terminating on hippocampal neurons in situ.

I_K and I_A

Both conductances involve primarily K^+ ions, as judged by the sensitivities of the reversal potentials of their tail currents to changes in $[K^+]_o$. The potential at which I_A first becomes detectable is typically 5–10 mV hyperpolarized relative to the potential at which I_K becomes evident. I_A activates rapidly, reaching a peak within 3–5 ms, and inactivates quickly ($\leq$30 ms). I_K activates and inactivates at about a 10- to 20-fold slower rate than I_A. The activation time course of I_K might be considerably quicker when the membrane is stepped to positive potentials during an action potential. We could not approach this potential given the limitation of our recording system. Nevertheless, even though the slow time course of I_K might be misleading, it is still far slower than I_A.

I_K observed in our experiments is not likely to be contaminated to a significant degree by Ca^{2+}-dependent K^+ current, I_C. It has been recently reported that I_C is sensitive to TEA (12). Thus while it might contribute to the TEA-sensitive I_K, we would argue that its contribution is minimal. Some of our experiments on I_K were performed in the presence of sufficient Co^{2+} to block I_{Ca} and I_C (e.g., Fig. 2). The time constant of I_C decay reported in other membranes is much slower than that of I_K, but the latter is fitted quite well by a single exponential in these cells (Fig. 5). Application of Cd^{2+} to current-clamped hippocampal cells did not ameliorate delayed rectification observed in these cells at depolarized potentials. The relationship between I_A and Ca^{2+} ions, however, is more complex. Transient outward currents with Ca^{2+}-dependent and Ca^{2+}-independent components have been reported (4, 31, 39, 44), and it has also been proposed that intracellular Ca^{2+} ions may act to reduce I_A (6). The effects of Ca^{2+} antagonists on I_A in hippocampal neurons are complex, with enhancement and/or suppression being recorded in different cells. In fact, a particular effect could only be observed over restricted ranges of membrane potential. The effects of the Ca^{2+} antagonists on I_A are further compounded by blocking actions on Ca^{2+}-dependent inward currents in some cells (unpublished observations). Presumably this action of Cd^{2+} on Ca^{2+} currents would be revealed as a slowing down of I_A decay as well as an increase in its amplitude. However, we recorded effects of Ca^{2+} antagonists on I_A amplitude without any detectable effect on decay kinetics. Clearly, the role of Ca^{2+} ions in the expression of transient outward currents will be better understood following experiments using direct intracellular injection of Ca^{2+}, as has been done in sympathetic neurons (4).

We calculated the values of the conductances activated at the peak of I_A and I_K at potentials where there was good voltage control and no current saturation. For direct comparison, I_K and I_A were activated by the same step from the same holding potential in the same cell. The K^+ conductance associated with I_K is always considerably greater than that associated with I_A. The decay of the two K^+ conductances, as reflected in the decays of their tail currents, is different, with I_A decaying far more rapidly than I_K. It should be

noted, though, that the decay rates of tail currents of the two conductances are dependent on voltage, becoming faster at hyperpolarized levels. Some of the properties associated with I_K and I_A are summarized along with those of I_{AR} in Table 1.

The relative specificity of TEA and 4-AP for I_K and I_A, respectively, allowed us to examine how the two conductances contribute to the membrane-potential behavior of hippocampal cells under current-clamp conditions (37). Application of brief (100 ms) depolarizing current pulses to TTX-treated cells current clamped to potentials at or below their resting level produces transient and delayed rectifications, both of which intensify as the cell is depolarized. The transient and delayed rectifications can be selectively blocked by 4-AP and TEA, respectively. In non-TTX-treated cells, TEA increases the duration of action potentials and decreases the amplitude of the hyperpolarizing phase, with little if any effects on other membrane properties studied (40). These results are consistent with the well-studied and long-established role of I_K in the repolarizing phase of a Na^+-dependent action potential. In contrast, 4-AP usually has only a small effect on the duration of a Na^+-dependent action potential or the amplitude of its afterhyperpolarization or other membrane properties in most cells studied (42). In the presence of sufficient Cd^{2+} to block I_{Ca}, 4-AP alters the threshold and rheobase for triggering single and repetitive action-potential activity. If we assume that 4-AP is selectively depressing I_A, then these excitability-enhancing actions of 4-AP suggest that I_A functions primarily to regulate the threshold and discharge rate of action potentials rather than the properties of an individual action potential. The relatively rapid time course of I_A, which overlaps with the kinetics of I_{Na}, supports such a notion. Given that these experiments were carried out at room temperature, an even faster rise time would be expected to occur at physiological temperatures. The properties of I_A reported here are qualitatively similar to those reported in other preparations; however, unlike some ganglion cells in invertebrates (1, 5, 15–17, 33, 35) or vertebrates (4, 31), I_A activates substantially when evoked from the level of the resting potential, which ranges between −60 and −80 mV in these cells (21, 40). Activation from the resting level has also been reported in a preliminary study of cells in the hippocampal slice, using the single-electrode voltage-clamp technique (21). These observations indicate that I_A may not necessarily be linked to afterhyperpolarization (16), but rather may be activated during the rapid depolarizing phase of an excitatory synaptic potential (19). It is tempting to speculate that the "dendritic spike" often seen in hippocampal cells (40) represents concomitant activation of a depolarizing conductance and I_A, thus resulting in an abbreviated spike.

Another characteristic of I_A resident in hippocampal (and spinal cord) cells is its relative sensitivity to 4-AP, which contrasts with its apparent insensitivity when recorded in sympathetic ganglion cells (11, 31). From our experiments it appears that in cultured CNS neurons, I_A need not be activated to be blocked by 4-AP, as reported for 4-AP's interaction

TABLE 1. *Comparison of physiological and pharmacological properties of K^+ conductances in cultured hippocampal neurons*

Conductance Species	Incidence, %	Activation Threshold, mV	Inactivation Threshold, mV	Rise Time, ms	Decay Time, s	Conductance, nS	Blocker
I_K	100	−40	−70	100	3.00	350.0	TEA
I_A	90	−50	−80	3–5	0.03	200.0	4-AP
I_{AR}	60	−70	None	100	None	15.6	Cs^+

Incidence, percentage of cells studied expressing the current response. Activation, membrane potential at which current response first becomes detectable. Inactivation, membrane potential at which current response first begins to inactivate. Rise time, time required to reach peak current response. Decay time, time constant for decay of current response from peak value. Conductance, see text. Blocker, substances that block the K^+ conductances during extracellular applications.

with transient outward currents in other cells (47, 48). Furthermore, 4-AP is relatively specific for I_A and has little effect on I_K in the concentration range studied, unlike its reported effects on I_K in other neuronal membranes (24, 51). This may indicate that I_A and I_K are more distinct in mammalian neurons than elsewhere. The presence of I_A in virtually every cultured hippocampal cell and in the majority of cultured spinal neurons (42) indicates that this conductance probably plays an important role in regulating the electrical activity of a variety of CNS neurons. It is still not a ubiquitous conductance in mammalian neurons, being noticeably absent from dorsal root ganglion (or spinal sensory) neurons (29, 30, 32), neuroblastoma cells (34), and some cultured hypothalamic cells (unpublished observations). Future experiments should allow us to characterize the biological differences between cells that exhibit I_A and those that do not.

I_M and I_{AR}

Two other K^+ conductances have been detected in hippocampal neurons. Both of these conductances activate slowly and show little, if any inactivation. One, I_M, is activated at potentials more depolarized than −60 mV, while the other, I_{AR}, is activated at potentials more hyperpolarized than −70 mV. The macroscopic conductances associated with I_M and I_{AR} are small compared to I_K and I_A (see Table 1). I_M is noticeably absent from most of the hippocampal cells studied and has not been detected in cells cultured in this laboratory from the spinal cord (unpublished observations). I_M may only be expressed in certain types of CNS neurons and these may not survive in our cultures. Alternately, the cells may have lost these K^+ channels in culture. It is worthwhile noting that cultured hippocampal cells do not depolarize or show an apparent increase in input resistance in response to acetylcholine as their in situ counterparts do (41). I_{AR} is present in the majority of cells studied but its magnitude varies considerably among cells and does not approach values observed in dorsal root ganglion (DRG) cells (32). The apparent difference in magnitude of this current might underlie the difference in the inversion potentials of I_{AR} in the two preparations. The conductance in hippocampal cells appears to involve primarily K^+ ions, as judged by the reversal potential of its tail current and the effects of changing $[K^+]_o$ on the amplitude of I_{AR}. I_{AR} is blocked quite effectively by Cs^+ ions in concentrations equivalent to $[K^+]_o$. Furthermore, the conductance is activated at potentials equivalent to or more hyperpolarized than the K^+ equilibrium potential. While it is likely that K^+ is the main ion carried, the possible involvement of Na^+ ions cannot be ruled out. The replacement of Na^+ by choline$^+$ ions does eliminate the slow inward current but does not shift the inversion potential in the hyperpolarizing direction, as seen elsewhere (23). High concentrations of choline$^+$ may reduce the conductance mechanism pharmacologically by interacting with it directly rather than ionically by substituting nonpermeant cations for permeant cations. Other, somewhat different types of anomalous rectifications have been reported. For example, Hotson et al. (27) have suggested that both Ca^{2+} and Na^+ ions may be involved. Their results differ from ours and those of Halliwell and Adams (23) in that they could record a rectifying response in the presence of TTX. Still another, transient form of rectification at hyperpolarized potentials has been reported in the olfactory cortex (18). Thus, there may be several types of rectification that function at hyperpolarized potentials.

In summary, although the three K^+ conductances observed here act to restore the potential of the cell to near the K^+ equilibrium potential (about −75 mV), each has distinctly different properties. The selective antagonism of the three conductances further suggests that if activation of a common conductance mechanism occurs, certain steps in the activation process are not shared. Functionally, these conductance repolarize the cell at different rates from different membrane potentials, and they serve to stabilize the membrane potential relative to the threshold for activation of TTX-sensitive Na^+ channels. In so doing they influence the rate at which Na^+ channels are activated, and this presumably is critical for neuronal function.

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