CALCIUM-ACTIVATED OUTWARD CURRENT IN VOLTAGE-CLAMPED 
HIPPOCAMPAL NEURONES OF THE GUINEA-PIG

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(Received 15 June 1982)

SUMMARY

1. Slow clamp currents were recorded from CA1 and CA3 pyramidal neurones in 
slices of guinea-pig hippocampus maintained in vitro, using a single micro-electrode 
sample-and-hold technique.

2. Depolarizing voltage commands evoked a time- and voltage-dependent outward 
current which was suppressed by removing external Ca or by adding Cd (0·5 mm) or 
Mn (5 mm). This Ca-dependent current (IC) was not reduced by muscarinic agonists 
(unlike IM) but was greatly reduced by 5–20 mm-tetraethylammonium (TEA).

3. Repolarizing IC tail currents reversed at −73 ± 5 mV in 3 mm-K solution. The 
reversal potential became about 30 mV more positive on raising [K]o to 15 mm. No 
clear change in current amplitude or tail-current reversal potential occurred on 
adding Cs (2 mm), reducing [Cl]o from 128 to 10 mm, or replacing external Na with 
Tris.

4. The underlying conductance GC was activated at membrane potentials positive 
to −45 mV. At −32 mV GC showed an approximately exponential increase with time, 
with a time constant of ~0·6 sec at 26 °C. Repolarizing tail currents declined exponentially with time, the time constant becoming shorter with increasing negative 
post-pulse potentials.

5. When the clamp was switched off at the end of a depolarizing command of 
sufficient amplitude and duration to activate IC, a membrane hyperpolarization to 
−73 mV ensued, of similar amplitude and decay time to that following spontaneous 
action potentials.

6. It is concluded that the clamp current observed in these experiments is probably 
the Ca-activated K current thought to contribute to the post-activation after-
hyperpolarization in hippocampal neurones.

INTRODUCTION

Hippocampal pyramidal cells show prolonged after-hyperpolarizations following 
repetitive action potential discharges or 'bursts' of action potentials (Kandel & 
Spencer, 1961; Schwartzkroin, 1975). This after-hyperpolarization is inhibited by Co,

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Mn or Ba, or by the intracellular injection of EGTA, and has therefore been attributed to the activation of a K conductance by the preceding entry of Ca$^{2+}$ ions (Hotson & Prince, 1980; Alger & Nicoll, 1980; Schwartzkroin & Stafström, 1980; Wong & Prince, 1981; Hablitz, 1981 a, b).

Johnston, Hablitz & Wilson (1980) have previously identified a slow inward current, presumably carried by Ca$^{2+}$ ions, in voltage-clamped pyramidal cells. Using a comparable voltage-clamp technique (see also Adams, Brown & Halliwell, 1981; Adams & Halliwell, 1982; Halliwell & Adams, 1982), we now describe a Ca-dependent component of outward current which may be triggered by this slow inward current and which probably underlies the post-activation after-hyperpolarization. This slow outward current, $I_C$, is distinct from two other slow currents already described in hippocampal pyramidal cells, the M current, $I_M$ (Adams et al. 1981) and an anomalous rectifier current, $I_Q$ (Halliwell & Adams, 1982).

A brief abstract of this observation has been presented (Brown, Griffith & Halliwell, 1982).

**METHODS**

Guinea-pigs (300-400 g) of either sex were decapitated and the hippocampus quickly removed. Transverse slices, 400–500 μm thick, were cut using a vertical guillotine method (Brown & Halliwell, 1981). Six to eight slices were transferred to a holding bath containing oxygenated (95% O$_2$/5% CO$_2$) Krebs solution and maintained at room temperature (22–26°C) for 1–2 hr prior to use. Most experiments were performed at room temperature and the slices remained viable for 10–12 hr; in a few experiments the temperature was raised to 30–36°C during the experiment. The composition of the Krebs solution was (mm): NaCl, 120; KCl, 3; CaCl$_2$, 2.5; MgCl$_2$, 1.2; NaH$_2$PO$_4$, 1.2; NaHCO$_3$, 22.6; glucose, 11.1.

The organ bath used for in vitro electrical recording was similar to that described by Halliwell & Adams (1982). A single slice was placed on a nylon mesh and held completely submerged while the Krebs solution flowed continuously over the slice. The small volume of the organ bath (~ 2 ml), and fast perfusion rate (approximately 4–5 ml/min), facilitated a rapid turnover of solution. All drugs were added to the preparation by this perfusion system.

Neurones in the CA$_1$ and CA$_3$ regions were impaled with a single micro-electrode (1-0 mm o.d., Clark Electromedical Instruments) with a tip resistance of 60–100 MΩ. Special care was taken to use only those electrodes that could pass current without showing strong rectification. Since it was necessary to minimize electrode resistance while maximizing current-carrying ability, 3 M-KCl was used to fill the electrodes. The mean resting potential in ninety-two cells was $-65 \pm 4$ mV (± s.d.). Action potentials, elicited by positive current injection, ranged from 90–110 mV, while cell input resistance ranged between 50–120 MΩ (mean, 69 MΩ).

Cells were voltage clamped using a high-frequency sample-and-hold preamplifier (Dagan 8100), based on the design of Wilson & Goldner (1975). Our technique for using this system has been described previously (Constaniti & Brown, 1981; Halliwell & Adams, 1982). The normal sampling frequency was 3 kHz at 25 or 50% duty cycle, but the sampling frequency was adjusted to a lower value if small changes in capacity-compensation induced changes in recorded steady current levels, since this implied that the recorded voltage was distorted by a residual voltage drop across the electrode impedance at the onset of the next current pulse. Sampled membrane current was filtered at 300 Hz and current and voltage recorded on a Gould 2400 recorder (full-scale deflexion ~ 8 msec) or on tape (Racal 4 DS). For small (< 2 nA) slow currents, recorded (somatic) voltage normally attained > 90% of the command voltage during stepped commands; voltages in Results refer to voltage recorded at the time at which the current was measured, not to command voltage.

Fast Na spikes could not be voltage clamped and were routinely suppressed with tetrodotoxin (TTX, 0.5 μM). Nevertheless, in some cells depolarizing commands from membrane potentials equal to, or greater than, $-50$ mV evoked one or more rather sharp ‘all-or-nothing’ inward currents. These current ‘spikes’ closely resembled those previously observed in voltage-clamped molluscan
neurones by Eckert & Lux (1976) and attributed by them to unclamped axonal spikes; in hippocampal cells they probably represent unclamped dendritic Ca spikes since they could be eliminated by Mn (see Wong & Prince, 1978; Wong, Prince & Basbaum, 1979). These unclamped spikes could frequently be obviated by holding the membrane potential at a value positive to -50 mV before applying the voltage step.

Drugs were obtained from Sigma Chemical Co. Caesium chloride, barium chloride and cadmium chloride were obtained from BDH, Poole, U.K.

RESULTS

Slow outward currents

Fig. 1 A shows currents generated in a voltage-clamped CA3 neurone by a series of stepped hyperpolarizing and depolarizing voltage commands from a holding potential of -50 mV. In this cell, and subsequent Figures, 0·5 μM-TTX was added to the perfusate to block fast inward Na currents. After the initial capacity transient the currents generated by hyperpolarizing commands were essentially ‘square’ and increased linearly with increasing voltage steps down to -75 mV. With larger hyperpolarizations a time-dependent inward rectification was observed due to the advent of the slow inward current $I_h$ (Adams & Halliwell, 1982; see Fig. 2); this could be abolished, and the current–voltage curve linearized to -100 mV, by adding 2 mM-Cs. Following depolarizing commands the current record was more complex, comprising an initial inward current followed by a slow outward relaxation. The current–voltage curve measured after 1 or 2 sec showed strong outward rectification whereas currents measured at 50 msec showed an inward rectification between -50 and -35 mV (Fig. 1 B). On repolarizing the cell to the holding potential there was a clear residual outward current tail ($I_{tail}$ in Fig. 1 B).
M-current contribution to the outward current

One component of slow outward current previously reported in hippocampal neurones is the M current, $I_M$ (Adams et al. 1981; Halliwell & Adams, 1982). This is a non-inactivating K current which is activated above $-60$ mV. It can be readily identified by the fact that it is specifically inhibited by muscarinic acetylcholine-receptor agonists (see Brown & Adams, 1980). Since $I_M$ can be recorded in the same voltage range as the outward currents in Fig. 1, it was necessary to determine to what extent $I_M$ contributed to the total outward current and, if need be, eliminate it.

![Image of current-voltage curves](image)

**Fig. 2.** Current–voltage curves measured from current excursions at the end of 1 sec voltage commands ($I_{\text{exc}}$) from a holding potential of $-50$ mV in a CA3 neurone a before (●) and b during (▼) superfusion of the slice with 50 μM-carbachol. Two clamp currents following the same voltage command are shown to illustrate the outward currents before (a) and during (b) carbachol.

In our experiments, $I_M$ was normally prominent in CA1 neurones but, though present, was rather smaller and variable in CA3 neurones. Thus, addition of muscarine (10–20 μM, $n = 7$) or carbachol (50–100 μM, $n = 10$) frequently reduced the steady outward current generated between $-60$ and $-30$ mV and eliminated a fast component to the repolarizing tail current (see, for example, Fig. 2). However, the major part of the time-dependent outward current and steady-state outward rectification recorded at potentials positive to $-30$ mV remained and therefore could not be attributed to $I_M$.

**Ionic dependence of outward current**

**Calcium.** When the perfusion fluid was changed to a Ca-free solution (containing 10 mM-Mg), the initial inward inflexion and time-dependent outward currents at command potentials above $-30$ mV were progressively lost (Fig. 3 A). A similar, and reversible, depression of initial inward and delayed outward currents occurred on
adding 5 mM-Mn \((n = 3)\) or 0.2-0.5 mM-Cd \((n = 12)\) (see Kostyuk & Krishtal, 1977) to the perfusion fluid (Fig. 3B). The decline of the outward current was accompanied by a reduction in the amplitude of the repolarizing tail currents, as shown in the lower right corner of Fig. 3B. These experiments strongly suggest that the slow time-dependent outward current was largely dependent upon a prior, or simultaneous,

\[
\text{Control} \quad 0 \text{Ca}^2+ \quad 3 \quad 6 \quad 9 \quad 15 \text{(min)} \quad 0.5 \text{nA} \\
\text{Cd}^2+ 0.5 \text{mm} \quad \text{Rinsing} \quad \text{Control} \quad 0.5 \text{nA}
\]

Fig. 3. Effects of (A) 0 Ca (10 mM-Mg) and (B) addition of 0.5 mM-Cd to normal Krebs solution on the outward currents generated by 20 mV depolarizing commands from \(-50\) mV. In A, (CA1 neurone), 1 sec commands were applied (reading from the left): 3 min before, and 3, 6, 9 and 15 min after, switching to the Ca-free solution (at arrow). In B, (CA3 neurone), left and right columns show currents generated by 20 mV, 2 sec depolarizing and hyperpolarizing commands from \(-50\) mV 1 min before and 8 min after adding Cd\(^{2+}\), and 20 min after returning to normal Krebs solution. The lower right-hand records show superimposed tracings of the repolarizing tail currents at the end of the depolarizing command, recorded at a higher gain.

inward Ca current. This provides a further distinction from \(I_M\), since the latter is insensitive to Ca or Ca-channel blockers (Brown & Adams, 1980; Adams & Halliwell, 1982).

**Potassium.** As shown in Fig. 1, the outward current generated by a depolarizing command is followed by a clear tail current \((I_{\text{tail}}\text{ in Fig. 1B})\) when the cell is repolarized to the holding potential. The reversal potential for this tail current was determined by applying post-pulses to different potentials. In normal Krebs solution (containing 3 mM-K) the initial amplitude of this tail current declined linearly as the post-pulse potential was made increasingly negative (Fig. 4) and became flat (or occasionally reversed, see Fig. 9B1) at a potential of about \(-73\) mV ± s.d. 5 mV
On raising \([K]_0\) both outward currents and tail currents were depressed and the tail-current reversal potential \(V_{\text{rev}}\) shifted toward a more depolarized level (Fig. 4). Thus, raising \([K]_0\) to 9 and 15 mm shifted the reversal potential by 18 and 29 mV respectively, corresponding to a 41 mV shift per ten-fold elevation of \([K]_0\). Pooled data from fifteen cells also showed a 41 mV shift in \(V_{\text{rev}}\) per ten-fold increase in \([K]_0\).

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**Fig. 4.** The effect of raising external potassium concentration \([K]_0\) on tail-current reversal in a CA3 neurone. A post-pulse protocol was used so that, following a constant depolarizing command, the membrane potential could be stepped to different levels to reverse the amplitude of the tail current. The records in A show outward currents generated by 1 sec depolarization to \(-29\) mV from \(-50\) mV, followed by the repolarizing tail currents (arrowed) recorded at a post-pulse potential of \(-61\) mV. Records were obtained first in normal Krebs solution containing 3 mm-K, then 15 min after raising \([K]_0\) to 9 mm and finally 15 min after raising \([K]_0\) to 15 mm. Note that the tail current at \(-61\) mV is outward in 3 mm-K, flat in 9 mm-K and inward in 15 mm-K. In B the peak amplitudes of the tail currents \(I_{\text{tail}}\) measured at a constant time after the capacity transient had subsided (i.e. at 40 msec after the start of the post-pulse command step), are plotted against the post-pulse potential (outward currents positive).

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over this range of \([K]\). Comparable tail currents (see Fig. 5) and reversal potentials were observed in muscarine solution \((n = 5)\), showing that they were not seriously distorted by M currents.

**Caestium.** Neither outward currents nor repolarizing tail currents were materially altered by adding Cs (2 mm) to the perfusion fluid. As previously shown by Adams & Halliwell (1981) and Halliwell & Adams (1982) the principal effect of Cs was to block the time-dependent inward current, \(I_Q\), activated by hyperpolarizing to
potentials more negative than $-70 \text{ mV}$, and thereby to abolish 'anomalous rectification' at hyperpolarized levels.

**Sodium and chloride.** The low slope of the relationship between $V_{\text{rev}}$ and $[\text{K}]_o$ suggests that the outward current may not be a pure K current but may be contaminated by other ions or currents. To test this, external Na was replaced by Tris (Tris(hydroxymethyl)aminomethane) while the total osmolarity and pH (7.4) were maintained at the control level. Fifteen minutes were allowed for adequate exchange of solution and removal of Na$^+$ ions. In two experiments $V_{\text{rev}}$ for the tail currents (determined using the post-pulse protocol described above) did not change after adding the Na-free solution. Likewise, Cl substitution experiments were performed using sodium isethionate (2-hydroxyethanesulphonate, 120 mM) to replace NaCl. In two experiments $V_{\text{rev}}$ was not altered after adding the low-Cl solution. Therefore, it seems unlikely that either Na or Cl contribute significantly to the Ca-dependent current.

**Outward current kinetics**

**Tail currents.** Following modest depolarizing commands, yielding outward currents of $\lesssim 2 \text{ nA}$, the repolarizing tail currents showed an exponential decay. The time constants for this decay at temperatures between 24 and 26 $^\circ\text{C}$ were (mean $\pm$ S.E. of the mean), $171 \pm 33$ msec ($n = 5$) at a post-pulse potential of $-50 \text{ mV}$ and $234 \pm 29$ msec ($n = 9$) at $-40 \text{ mV}$. At more positive post-pulse potentials, or after larger or more prolonged depolarizing commands, the tail currents were often very prolonged, lasting several seconds, and did not conform to a single exponential.

The tail-current decay rate accelerated as the post-pulse potential was made more positive. Thus, in the experiment illustrated in Fig. 5A, time constants were about 290 msec at $-37 \text{ mV}$, 185 msec at $-41 \text{ mV}$, 140 msec at $-46 \text{ mV}$ and 95 msec at $-55 \text{ mV}$. (In the test at $-37 \text{ mV}$, there was a small residual component of tail current after the second step, on returning to the holding potential, suggesting that not all of the K channels had closed after 2 sec at $-37 \text{ mV}$). In order to track the tails at more hyperpolarized levels, the K concentration was raised to yield inverted tails: the time constant then continued to shorten down to $-75 \text{ mV}$, approximately e-fold for 18 mV hyperpolarization (Fig. 5B). Time constants were unaffected by muscarine, showing that they were not contaminated by M-current tails.

In some CA$_1$ cells the outward current generated by depolarizing steps, in the voltage range to $-30$, was greatly reduced by muscarine or carbachol. However, larger depolarizing voltage commands generated outward currents and repolarizing tail currents (as shown in Fig. 2) that showed similar kinetic properties to those in cells with no $I_M$.

**Tail currents at raised temperature.** In four experiments, the perfusion temperature was raised to 30 $^\circ\text{C}$. Tail currents at $-40 \text{ mV}$ shortened to $188 \pm 20$ msec at 30 $^\circ\text{C}$ (cf. $234 \pm 29$ msec at 24–26 $^\circ\text{C}$). This effect was reversible on re-cooling (Fig. 5C). The $Q_{10}$ between $-26$ and $-36 \text{ C}$ was about 1.6 (Fig. 5D). Tail currents retained their voltage sensitivity at the higher temperature.

**On currents.** The onset of the outward currents was clearly contaminated by inward currents. To circumvent this, the underlying conductance change was determined from the amplitude of the repolarizing tail currents at various times after the initiation of a depolarizing command (Fig. 6). The reversal potential for the tail current did
Fig. 5. Voltage-sensitivity and temperature-sensitivity of tail currents. 

A, semi-logarithmic plots of the decay time course of the repolarizing tail currents recorded at post-pulse potentials of −37, −41, −46 and −55 mV following a 0.7 sec depolarizing command from −50 to −28 mV (see inset). Temperature, 24 °C. 

B, plot of the reciprocal time constant of the tail-current decay ($\tau^{-1}$) against post-pulse potential obtained from another experiment (temperature 24 °C) using a 1 sec prepulse to −32 mV in normal Krebs solution (▽), Krebs solution with 20 μM-muscarine added (■) and Krebs solution containing 9 mM-K (●). The latter was added to invert the tail, in order to measure the decay at the more hyperpolarized levels (which would otherwise be near to the reversal potential, see Fig. 4). The curve is drawn assuming an e-fold shortening of $\tau$ per 18 mV hyperpolarization from −40 mV. 

C, effect of warming the superfusion fluid from 26 °C (●) to 32.5 °C (△), and recoling to 26 °C (▲), on the time course of sample tail currents recorded at −40 mV after a 1 sec prepulse to −22 mV. Time constants were 250 msec at 26 °C and 170 msec at 32.5 °C. 

D, average tail currents recorded in three different experiments at between −35 and −40 mV on warming from 26 to 32.5 °C (●), 26 to 30 °C (▽) and 30 to 36 °C (▲). Each point is the mean of several determinations in the same cell at the temperature indicated.
not differ appreciably when measured between 700 msec and 3 sec after the start of the depolarizing command, indicating that the tail-current amplitudes were not seriously affected by extracellular K shifts. At a command potential of \(-32\) mV the apparent conductance showed an approximately exponential increase to an asymptote at 2 sec, with a time constant of about 0.6 sec. At more positive potentials the outward current increased more rapidly (see Fig. 1) and accurate measurements from the tail currents were rather impracticable.

![Graph](image)

Fig. 6. Onset of outward current. The record in A shows the outward current generated by a 3 sec command to \(-32\) mV from a holding potential \((V_H)\) of \(-60\) mV. A series of equal commands of variable shorter duration were then applied, and the repolarizing tail currents recorded as shown in the lower record. The graph in B was constructed by dividing the initial amplitude of the tail current by the driving force \(V_H - V_{rev}\) where \(V_{rev}\) is the reversal potential of the tail current \((-72.5\) mV; see Fig. 4). The curve is drawn for an exponential increase in conductance to an asymptotic value of 19 nS with a time constant of 0.6 sec.

Repolarizing tail currents also increased in amplitude after increasingly positive command potentials of constant duration, in the manner illustrated in Fig. 1. The conductance increase measured from such tail currents accorded approximately with that deduced from the outward current at the end of the command pulse after leak subtraction (Fig. 7). This suggests that the inward current at the end of the command pulse was relatively small compared to the outward current. The conductance increased steeply with depolarization above a threshold of between \(-45\) and \(-35\) mV. Outward currents could only be followed over a relatively limited range (up to \(-20\) mV or so), which probably encompasses only the foot of the activation curve; this did not appear to follow a simple exponential dependence on voltage.

**Effects of tetraethylammonium (TEA)**

TEA (5–20 mM; \(n = 8\)) produced a small net inward current at the holding potential (normally \(-50\) mV) and rapidly reduced the amplitude of the time-dependent outward current (Fig. 8A). In consequence, outward rectification of the current–voltage curve positive to \(-50\) mV was reduced, leaving a variable degree of inward rectification (Fig. 8B).

In most cells, the currents evoked by depolarizing commands from \(-50\) mV
remained net outwards in TEA solution, as in Fig. 8. However, these could frequently be converted to a slow inward current by re-setting the holding potential to a more positive level, as shown in Fig. 8C at $V_H = -30$ and $-20$ mV. These slow inward currents resembled in form those previously described by Johnston et al. (1980) and are described in greater detail in the subsequent paper (Brown & Griffith, 1983).

![Graph](image)

Fig. 7. Conductance increase at the end of 1 sec depolarizing commands from a holding potential of $-45$ mV. The records in A show (at the top) the currents during and after a depolarizing command to $-32$ mV and (below) the change in tail-current amplitude recorded at $-45$ mV as the command potential was varied between $-38$ and $-30$ mV. The graph in B shows the increase in conductance (calculated as described in Fig. 6) measured from the amplitude of the repolarizing tail current ($O$, $G_{tail}$) and from the final amplitude of the outward current corrected for the ‘leak’ measured from equivalent hyperpolarizing commands ($\bullet$, $G_{se}$).

**Membrane hyperpolarizations**

To test whether the outward current observed in the present experiments might be an appropriate current for the spike after-hyperpolarization in hippocampal neurones (see Introduction), both the hyperpolarization following spontaneous action potentials and the outward current were studied in the same cell (Fig. 9). In this cell, the post-spike hyperpolarization attained a maximum potential of about $-74$ mV and decayed over 1–2 sec. This potential is close to the reversal potential of the tail currents following outward clamp currents recorded in this cell (Fig. 9B). Furthermore, when the clamp was switched off during an outward clamp current, a membrane hyperpolarization ensued (Fig. 9C), of very similar amplitude and time course to that observed after the spontaneous spikes (cf. Fig. 9A).
Fig. 8. Effects of tetraethylammonium (TEA) on the outward currents. The records in A show currents recorded during a 1 sec, 16 mV depolarizing command before, and 1, 2, 3, 4 and 8 min after, adding 10 mM-TEA to the perfusion fluid (CA1 neurone, \( V_H = -50 \)). B, the complete current–voltage curve (measured at 1 sec) before (●) and during (▼) TEA perfusion. The records in C are from a CA3 neurone, also in the presence 10 mM-TEA. The currents produced are the result of a 1 sec command pulse to -12 mV (left column) from holding potentials of -50, -40, -30 and -20 mV. The right-hand column shows ‘leak’ currents induced by corresponding hyperpolarizing pulses. Note that in C the depolarizing command step from a holding potential of -50 mV generates a clamp current very similar to that produced in A after 8 min TEA perfusion, but that, when the holding potential is changed to a more depolarized level, the clamp current becomes net inward.

DISCUSSION

Previous experiments on voltage-clamped hippocampal neurones have revealed a slow outward current, activated at membrane potentials from -70 mV upwards, which was relatively insensitive to \([\text{Ca}]_0\) but was inhibited by muscarinic agonists and by Ba (Adams et al. 1981; Halliwell & Adams, 1982). This current appeared homologous with that previously observed in sympathetic ganglia, and designated the ‘M current’ (see Brown & Adams, 1980).

In the present experiments we have detected a second slow outward current in both CA1 and CA3 neurones, activated from about -45 mV upwards, which persisted in the presence of sufficient cholinergic agonist to block the M current. Unlike the M current, this current was suppressed in a Ca-free solution or on adding a Ca antagonist to the bathing fluid. We therefore conclude that it is a species of Ca-activated outward current, examples of which have been demonstrated in a variety of cells (see Meech,
Fig. 9. Membrane hyperpolarizations and outward currents generated in the same CA1 neurone. A, from a resting potential of $-68$ mV this neurone fired spontaneous action potentials (TTX not present; peaks attenuated by pen recorder) with prolonged after-hyperpolarizations attaining a membrane potential of $-74$ mV. B, under voltage clamp, outward currents could be generated (B1); using the post-pulse protocol (see Fig. 4), $V_{rev}$ of the tail current in this cell was $-73$ mV (B2). C, from a holding potential of $-60$ mV a large outward current was generated. During the outward current the voltage clamp was switched back to current clamp (at arrow), resulting in a membrane hyperpolarization to $-73$ mV, the same value as $V_{rev}$. D, plots of the decay of the membrane hyperpolarizations (in current clamp) versus time for the spontaneous hyperpolarization (▲, in A) and the hyperpolarization generated after the outward clamp current (●, in C).

The current tails reversed at about $-73$ mV in the standard bathing medium (containing $3$ mM-K); and, over a limited range from $3$ to $15$ mM-K, this reversal potential shifted in the depolarizing direction by some $40$ mV per ten-fold increase in $[K]_o$. This suggests that the outward current is substantially a K current, but may not be exclusively so; for this reason we have designated it $I_C$, by analogy with that in molluscan neurones (Thompson, 1977).

The intracellular K concentration in hippocampal neurones in vitro is unknown. Olfactory cortex slices maintained under similar incubation conditions to those employed in our experiments, but with $5-9$ mM extracellular K, yielded an over-all intracellular K concentration of near $130$ mM (Harvey, Scholfield & Brown, 1974), giving a K-equilibrium potential, ($E_K$), of about $80$ mV. This accords with the reversal potential ($-80$ mV) for the glutamate-induced after-hyperpolarization in hippocampal pyramidal cells in $5-4$ mM-K, which is thought to be generated by a Ca-dependent 'K current' (Nicoll & Alger, 1981). The reversal potential for another K current, $I_M$, appears to
be about $-78$ mV in hippocampal slices maintained under the same conditions as those we have used (i.e. at ambient temperature in 3 mm-K; Halliwell & Adams, 1982). A reversal potential of $-73$ mV for $I_C$ in 3 mm-K may therefore be a few mV less than that expected for a 'pure' K currents.

Ca-activated K currents in mammalian central neurones were first inferred from the hyperpolarizing effects of dinitrophenol and Ca injections in spinal motoneurones (Godfraind, Kawamura, Krnjević & Pumain, 1971; Krnjević & Lisiewicz, 1972), and an appropriate Ca-dependent slow outward current has been recorded from motoneurones under voltage clamp (Barrett, Barrett & Crill, 1980). This current was activated some 20 mV positive to rest potential; both activation and decay were exponential with a maximal time constant (near to threshold) of 40–50 msec. The hippocampal current recorded in our experiments is clearly much slower than this at a comparable potential (see Fig. 5); this difference cannot be attributed solely to the lower temperature used in our in vitro experiments, since, even at 36 °C, a time constant of the order of 150 msec was recorded. Accordingly, the after-hyperpolarization resulting from residual $I_C$ when the clamp is switched off was more prolonged in the hippocampal cells, lasting several hundred msec (Fig. 13; cf. Fig. 10 in Barrett et al. 1980). In this regard, the hippocampal $I_C$ more closely resembles that in mammalian sympathetic ganglia (see Brown, Adams & Constanti, 1982).

The rates of both activation and deactivation of $I_C$ varied with voltage. Activation of $I_C$ speeded up with increasing depolarization (Fig. 1), whereas the repolarizing tail currents clearly accelerated as the membrane hyperpolarized. Since $I_C$ is dependent on a prior inward current, the onset and duration of $I_C$ might merely reflect that of the priming inward Ca current. This does not appear to be true for the offset of $I_C$ since, at hyperpolarized potentials, the $I_C$ tail outlasted the inward current tail by an order of magnitude (see Brown & Griffith, 1983). $I_C$ decay rates may therefore be determined either by the rate of decline of intracellular Ca, or by a separate, and additional, voltage-dependence of the K-channel closing rate (see Gorman & Thomas, 1980; Adams, Constanti, Brown & Clark, 1982; Hermann & Hartung, 1982). The present experiments do not readily permit the relationship between $I_C$, $I_{Ca}$ and [Ca]$_{in}$ to be determined and hence the present description of $I_C$ can only be regarded as phenomenological.

Nevertheless, our findings are helpful in confirming the presence under voltage clamp of a Ca-activated current previously inferred from observations on spike after-hyperpolarization (see Introduction). It is clear from Fig. 9 that this current is capable of generating a membrane hyperpolarization of similar amplitude and time course to the post-spike hyperpolarizations in pyramidal cells. The sensitivity of this current to TEA parallels that of the corresponding current in sympathetic neurones (Adams et al. 1982); the ability of TEA to induce seizure discharges in hippocampal neurones (Schwartzkroin & Pedley, 1980) suggests that $I_C$ may play an important role in controlling hippocampal cell excitability.

Supported by a grant from the Medical Research Council. We thank Dr James Halliwell for his help in the initiation of these experiments.
REFERENCES


K CURRENTS IN HIPPOCAMPAL NEURONES


