Contribution of Potassium Conductances to a Time-Dependent Transition in Electrical Properties of a Cockroach Motoneuron Soma

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Mills, Janette D. and Robert M. Pitman. Contribution of potassium conductances to a time-dependent transition in electrical properties of a cockroach motoneuron soma. J. Neurophysiol. 81: 2253–2266, 1999. The cell body of the cockroach (Periplaneta americana) fast coxal depressor motoneuron (Df) displays a time-dependent change in excitability. Immediately after dissection, depolarization evokes plateau potentials, but after several hours all-or-none action potentials are evoked. Because K channel blockers have been shown to produce a similar transition in electrical properties, we have used current-clamp, voltage-clamp and action-potential-clamp recording to elucidate the contribution of different classes of K channel to the transition in electrical activity of the neuron. Apamin had no detectable effect on the neuron, but charybdotoxin (ChTX) caused a rapid transition from plateau potentials to spikes in the somatic response of Df to depolarization. In neurons that already produced spikes when depolarized, ChTX increased spike amplitude but did not increase their duration nor decrease the amplitude of their afterhyperpolarization. 4-Aminopyridine (4-AP) (which selectively blocks transient K currents) did not cause a transition from plateau potentials to spikes but did enhance oscillations superimposed on plateau potentials. When applied to neurons that already generated spikes when depolarized, 4-AP could augment spike amplitude, decrease the latency to the first spike, and prolong the afterhyperpolarization. Evidence suggests that the time-dependent transition in electrical properties of this motoneuron soma may result, at least in part, from a fall in calcium-dependent potassium current (I_{K,Ca}), consequent on a gradual reduction in \([Ca^{2+}]_i\). Voltage-clamp experiments demonstrated directly that outward K currents in this neuron do fall with a time course that could be significant in the transition of electrical properties. Voltage-clamp experiments also confirmed the ineffectiveness of apamin and showed that ChTX blocked most of I_{K,CA}. Application of Cd^{2+} (0.5 mM), however, caused a small additional suppression in outward current. Calcium-insensitive outward currents could be divided into transient (4-AP-sensitive) and sustained components. The action-potential-clamp technique revealed that the ChTX-sensitive current underwent sufficient activation during the depolarizing phase of plateau potentials to enable it to shunt inward conductances. Although the ChTX-sensitive conductance apparently makes little contribution to spike repolarization, the ChTX-resistant I_{K,CA} does make a significant contribution to this phase of the action potential. The 4-AP-sensitive current began to develop during the rising phase of both action potentials and plateau potentials but had little effect on the electrical activity of the neuron, probably because of its relatively small amplitude.

INTRODUCTION

It has become clear in recent years that the electrical properties of some neurons can be changed radically by chemical modulators (Harris-Warrick and Marder 1991; Kiehn 1991); for example, some neurons, when isolated from synaptic influences, may be electrically silent, spike regularly, generate spikes in regular bursts, or produce plateau potentials, depending on the presence or absence of specific neuromodulatory compounds. Such transitions in properties can have a profound effect not only on the output of individual neurons but also of the networks in which they participate. It is important, therefore, to establish the ionic mechanisms by which such changes may take place. The soma of the fast coxal depressor motoneuron (Df) of the cockroach, Periplaneta americana, third thoracic ganglion can display two distinct types of electrical activity. In response to long-duration depolarizing pulses or synaptic stimulation, it can generate plateau potentials, each of which may last >1 s and reach a mean membrane potential of \(-37 \text{ mV} \) (Hancox and Pitman 1991, 1993). Neuron, Df, is also capable of generating calcium-dependent action potentials, but these normally are seen only between 1 and 4 h after dissection of the CNS (Hancox and Pitman 1992). Earlier expression of spiking can be brought about pharmacologically; for example, Df can generate spikes after intracellular injection of calcium chelating agents or after extracellular application of the K channel blocker tetraethylammonium (TEA\(^{+}\)) (Pitman 1979). One possible mechanism underlying the transition in membrane response from a plateau potential to an action potential therefore could be a fall in the magnitude of K currents. In this preparation, however, TEA\(^{+}\) appears to suppress both voltage-dependent (I_{K,V}) and Ca-dependent (I_{K,CA}) K conductances (David and Pitman 1995a), so it gives no indication of the roles of each component of the K current in determining the membrane properties of the neuron. Reduction in \([Ca^{2+}]_i\), by intracellular injection of calcium chelators, does suggest, however, that Ca-dependent K channels may be involved in the appearance of spiking activity; this procedure probably acts in two ways: first, reduction in \([Ca^{2+}]_i\) would be expected to increase the magnitude of calcium currents both by increasing the inward gradient of this ion and also by reducing any Ca-mediated Ca inactivation (Mills and Pitman 1997). Second, the fall in \([Ca^{2+}]_i\) also reduces I_{K,CA}, which constitutes a major component of the outward current in this neuron, (David and Pitman 1995a; Thomas 1984). It has been proposed that I_{K,CA} may be large enough to shunt inward calcium currents and thereby prevent the cell spiking (Hancox and Pitman 1992; Pitman 1979; Thomas 1984).

There is a diversity of potassium channels (Jan and Jan 1990) each of which may influence the electrical properties of neurons in different ways. For example, calcium-dependent potassium conductances can contribute toward the resting membrane potential, spike repolarization, afterhyperpolarizations, modulation of repet-
itive firing, spike frequency adaptation, and termination of bursts (Blatz and Magleby 1987; Hermann and Erxleben 1987; Latotire et al. 1989; Rudy 1988; Sah 1996). Calcium-dependent potassium conductances can be classified according to characteristics such as kinetics, calcium sensitivity, and single channel conductance. Different types of \( I_{\text{KCa}} \) also have been distinguished in a number of species by their sensitivity to the neurotoxins apamin and charybdo-toxin (ChTX). Apamin, a bee venom neurotoxin, is known to block a low-conductance calcium-potassium channel (Hugues et al. 1982; Lazdunski 1983; Romey et al. 1984), whereas ChTX, a peptide neurotoxin isolated from the venom of the scorpion, *Leiurus quinquestriatus*, (Gimenez-Gallego et al. 1988; Smith et al. 1986) has been shown to block a variety of calcium-dependent potassium channels of differing conductances (35–250 pS) (Anderson et al. 1988; Garcia et al. 1991; Hermann and Erxleben 1987; Miller et al. 1985; Moczylowski et al. 1988; Schafer et al. 1994). This toxin has been shown to block a Ca-dependent potassium current in dorsal unpaired median cells of the cockroach (Grolleau and Lapied 1995; Wicher et al. 1994).

The voltage-dependent potassium channels are another group of potassium channels that are activated by changes in the membrane potential and not by calcium. This group includes the delayed rectifiers that were described first by Hodgkin and Huxley (1952) in the squid giant axon and are responsible for action potential repolarization. The “A” potassium current was first described in molluscan neurons (Connor and Stevens 1971a) and has a lower threshold, faster kinetics and displays steady-state inactivation at more negative membrane potentials than other potassium channels. Currents with similar properties to the A current have been described in other preparations, but there appears to be some variation in the properties of these channels, particularly in the voltage-dependence of inactivation and activation rates (review by Rudy 1988). These currents can regulate the latency to first spike during depolarization, spike frequency and action potential repolarization. 4-Aminopyridine (4-AP) is a potassium channel blocking agent that has its most potent effect on these channels.

In this paper, we have used different techniques to investigate the effects of potassium channel blockers and shown that ChTX blocked a large proportion of outward current in this cell while apamin was without effect. The ChTX-sensitive K current is important in determining the electrical properties of \( \text{D}_f \) somata in the cockroach because it can determine whether the cell responds to depolarization by plateauing or spiking. A smaller ChTX-insensitive \( I_{\text{KCa}} \) and a transient 4-AP-sensitive current also may be involved in the fine tuning of electrical activity.

**METHODS**

All experiments were performed on the metathoracic “fast” coxal depressor motoneuron, \( \text{D}_f \) (Pearson and Iles 1970), of adult male cockroaches (*P. americana*). Animals were decapitated, the mesothoracic and metathoracic ganglia and the first three abdominal ganglia were dissected out, and the metathoracic ganglion desheathed for electrophysiological recording (see Pitman 1975). Experiments were performed in circulating oxygenated saline containing (in mM) 214.0 NaCl, 3.1 KCl, 9.0 CaCl\(_2\), and 10 TES buffer (pH 7.2). Stock solutions (10 \( \mu \)M) of ChTX and apamin (Latoxan, Rosans, France) were made up in saline and stored at \(-4^\circ\text{C}\). 4-AP (Sigma-Aldrich, Poole, U. K.) was made up daily as a 10 mM stock solution. Aliquots (20 or 200 \( \mu \)l) of these agents were added to a side compartment of the chamber (total volume 2 ml), where the oxygenation system mixed and diluted them before they reached the preparation. Concentrations are expressed as final values after mixing in the experimental chamber. Experiments were carried out at room temperature (20–23°C).

\( \text{D}_f \) somata were penetrated with two thin-walled, fiber-filled borosilicate glass microelectrodes (Clark Electromedical, Pangbourne, U. K.). Microelectrodes contained 2 M potassium acetate as electrolyte and, for current-clamp recording, had resistances of 12–20 M\( \Omega \).

**Voltage clamp**

For voltage-clamp recordings, the voltage electrode (for monitoring membrane potential) had a resistance between 8 and 15 M\( \Omega \), whereas the current electrode (used to apply current) had a resistance between 5 and 10 M\( \Omega \). Current was monitored using a laboratory-built “virtual earth” amplifier circuit connected to the reference electrode in the experimental chamber. Data from current-clamp experiments were recorded on tape using a DTR 1204 digital tape recorder (Biological Science Instruments) and displayed on a Gould 1604 oscilloscope. A CED 1401 Plus computer interface (Cambridge Electronic Design) and associated software were used for generating voltage command pulses, recording digitized data, and off-line analysis. Hardcopy data were downloaded from tape or computer using a Gould Colorwriter 6120 plotter or a Hewlett-Packard Laserjet 4P printer. Trains of negative iontophoretic pulses [300 ms, sufficient to hyperpolarize the membrane by 40 mV (2–10 nA) delivered at 0.1 Hz for \( \leq 20 \) min] were used to inject the calcium-chelator, 1,2 bis(2-amino phenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA, Sigma-Aldrich) into the cells. In such experiments, electrodes were filled with solution containing 100 mM BAPTA and 100 mM KCl. All statistical data are presented as means ± SE.

**Action potential clamp**

The procedure in these experiments was to voltage clamp the \( \text{D}_f \) neuron (as described in the preceding section) but using a command signal identical to the electrical activity exhibited by the cell (Doerr et al. 1990). Action potentials or plateau potentials, evoked under current-clamp conditions by applying depolarizing pulses, were collected digitally. The same cell then was voltage clamped at its resting membrane potential, and a typical sample of activity was used as the command signal (using software kindly supplied by John Dempster, University of Strathclyde, Glasgow). A small sustained current was recorded in control conditions which corresponded to the stimulus current that was injected under current-clamp conditions to reach threshold for activity. Because the cell then was clamped by its own action potential or plateau potential, no other current was recorded. When, under these conditions, a current was blocked pharmacologically, the clamp amplifier had to apply a compensation current corresponding to the contribution of the blocked current to the electrical activity.

**RESULTS**

**Time-dependent changes in electrical properties in \( \text{D}_f \)**

Because the mechanically isolated soma of the fast coxal depressor motoneuron (\( \text{D}_f \)) can show a time-dependent change in its electrical response to depolarization (Hancox and Pitman 1992), experiments were designed to determine which currents are responsible for the observed transition in membrane properties. Figure 1A shows the typical time-dependent change in the response to depolarization which is observed in the soma of \( \text{D}_f \). The resting membrane potential was \(-74.6 ± 0.7\ \text{mV} (n = 107\ \text{neurons})\) and did not change significantly during the course of the experiment. Initially after dissection, \( \text{D}_f \) generated plateau potentials of depolarization (Fig. 1Ai). These events had
a threshold between −60 and −40 mV (mean: −50.9 ± 0.74, n = 68 neurons) and reached a mean membrane potential of −37.2 ± 1.1 mV (n = 45 neurons). The duration of plateau potentials varied between preparations and depended on the strength of the applied current pulse. In some preparations, such plateau potentials were sustained throughout a 800-ms depolarization, whereas in other preparations they could be <200 ms in duration. Small-amplitude current injections sometimes produced plateau potentials that inactivated during the pulse, whereas higher amplitude current injections in the same cell produced longer-duration plateau potentials. Over time, an intermediate transitional stage was observed frequently in which oscillations of declining amplitude were superimposed on the plateau (Fig. 1A). One to 4 h after dissection, the Df soma generated action potentials rather than plateau potentials when depolarized (Fig. 1A). The action potentials had a threshold of −50.5 ± 1 mV (n = 14 neurons) and normally did not overshoot zero (mean membrane potential reached: −21 ± 2.5 mV, n = 12 neurons; Fig. 1A).

Action potentials recorded from Df somata mechanically isolated from their processes similarly fail to overshoot zero (Hancox and Pitman 1992). Plateau potentials and time-dependent spikes are calcium dependent because both can be blocked by 1 mM Cd²⁺ but not by tetrodotoxin (≤1 μM) (Hancox and Pitman 1991, 1992). Two components of the calcium current have been identified: one that activates at membrane potentials positive to −60 mV and is blocked by nifedipine and a second component that activates at membrane potentials positive to −40 mV and is blocked by micromolar cadmium ions (Mills and Pitman 1997). The nifedipine-sensitive component appears to underlie the plateau potential, and both the nifedipine- and Cd²⁺-sensitive components contribute to spikes.

Although action potentials normally cannot be evoked from the neuron shortly after dissection, external application of the potassium channel blocker, TEA⁺ (10 mM, n = 9) or intracellular injection of calcium-chelating agents, such as BAPTA (n = 19), could enable Df to spike within minutes (Fig. 1, B and C) (cf. Pitman 1979). To eliminate the possibility that the effects observed resulted from the hyperpolarizing pulses used to inject the BAPTA rather than from the reagent itself, control trains of hyperpolarizing pulses were applied to microelectrodes filled with 2 M K acetate. These had no effect on membrane properties. The spikes produced in the presence of bath-applied TEA⁺ or after injection of BAPTA reached a mean membrane potential of −4.8 ± 2.2 (n = 9 neurons) and −15.7 ± 2.3 (n = 18 neurons), respectively. The effects of these agents on electrical activity indicate that K currents may have an important role in dictating the type of electrical activity generated by this neuron; BAPTA may operate both by enhancing I_{Ca} and by depressing I_{K,Ca}, whereas TEA⁺ causes a nonselective suppression of most, if not all, components of K current (personal observations). It is likely, therefore, that one or more components of K current may suppress spiking by shunting the depolarizing effects of I_{Ca}; a progressive reduction in K current thus could contribute to the time-dependent transition of the somatic response of Df to depolarizing stimuli. To establish the role of different components of K current in determining the type of electrical activity generated by Df at any time, we have investigated the effects of different K channel blockers under current-clamp, voltage-clamp, and action-potential-clamp conditions.

**Effect of potassium channel blockers on electrical activity recorded under current-clamp conditions**

Because I_{K,Ca} forms a large proportion of depolarization-induced outward current in this neuron (David and Pitman 1995a; Thomas 1984) and experimental reduction of [Ca²⁺] can enable the soma of Df to generate action potentials, it is very likely that this current is normally at least partially responsible for suppression of spiking. To establish whether suppression of I_{K,Ca} alone could mimic the time-dependent transition in the electrical characteristics normally observed in Df, we have used the bee venom neurotoxin, apamin, and the scorpion neurotoxin, chaobotoxin (ChTX), each of which blocks a different class of calcium-dependent potassium channel. Depolarization of the cell shown in Fig. 2A produced plateau potentials of similar amplitude, duration and threshold in control saline and 10 min after application of 1 μM apamin. Similar results were obtained in a total of six preparations.

**FIG. 1.** A: time-dependent excitability change in the soma of fast coxal depressor motoneuron (Df). i: in a freshly dissected, recently impaled neuron, a plateau potential is evoked by depolarization. ii: in the same neuron 30 min after dissection, a series of oscillations of successively decreasing amplitude is superimposed on the plateau. iii: train of action potentials is evoked by depolarization applied 80 min after dissection. B: neuron that under normal circumstances generated plateau potentials on depolarization (seen as a rapid depolarization part way through the current pulse, *) produces action potentials when potassium currents are suppressed by external application of TEA⁺ (10 mM) for 5 min. C: effect of intracellular injection of the calcium chelating agent 1,2 bis(2-aminophenoxy)ethane-N,N',N,N'-tetraacetic acid (BAPTA) on the electrical response of motoneuron Df to depolarizing current pulses. i: under control conditions, depolarization evokes a plateau potential. ii: after BAPTA injection, depolarization drives a train of spikes. Timing of depolarizing current pulses is indicated by the horizontal bars. BAPTA was injected by applying 300-ms pulses of an amplitude sufficient to hyperpolarize the membrane by 40 mV (2–10 nA) at 0.1 Hz for ±20 min.
ChTX, on the other hand, rapidly caused neurons to change their response to depolarization from plateau potentials to spikes (Fig. 2B, n = 8). The neuron shown in Fig. 2B initially generated plateau potentials in control saline. Within 30 s of applying ChTX (100 nM), the soma responded to depolarization by generating shorter-duration, higher-amplitude plateau potentials on which were superimposed oscillations (n = 4, c.f. Fig. 1A). After 1 min in ChTX, nonovershooting spikes reaching an absolute membrane potential of −20 mV were generated. After 2 min, the spikes began to overshoot 0 mV. While the action potentials illustrated in Fig. 2B attained a positive potential, this was not true of all neurons; the mean membrane potential reached by spikes after 5 min in ChTX was −7.9 ± 5 mV (n = 8). Although the absolute level of the action potential peak potentially also could be influenced by changes in the resting membrane potential, this did not have to be considered here because 100 nM ChTX produced no detectable change in the resting membrane potential (n = 8 neurons). In effect, ChTX, caused cells to rapidly undergo a transformation in excitable properties similar to that seen over a period of hours in control neurons (Hancox and Pitman 1992). This suggests that the time-dependent change in activity seen in cells in normal saline at least in part could be due to a progressive decrease in the ChTX-sensitive component of the calcium-dependent potassium conductance.

Because motoneuron Df is known to have complex dendritic arborizations, experiments were performed on cell bodies that had been separated mechanically from their processes to eliminate the possibility that the effects of ChTX resulted from differential effects of this agent on the soma and dendrites of the neuron or from poor space-clamping. Under these conditions, ChTX still could transform the response of the soma to depolarization from a plateau potential to a series of action potentials (n = 4).

In preparations that had been allowed a sufficient interval from the time of dissection to enable them to generate spikes when depolarized (2 h), application of ChTX caused an increase in spike amplitude and duration. B: charybdotoxin (ChTX) converts the response to depolarization of Df from plateauing to action potential peak. Shortly after application of 100 nM ChTX, Df produces higher-amplitude, shorter-duration plateau potentials on which are superimposed oscillations (n = 4, c.f. Fig. 1A). After 1 min in ChTX, nonovershooting spikes reaching an absolute membrane potential of −15 mV. After application of 100 nM ChTX for 1 min, the spike amplitude increased to overshoot 0 mV (Fig. 2C). The spike duration of 7 ms (measured at half spike amplitude) and the absolute membrane potential reached by the afterhyperpolarization (−55 mV) were not changed in the presence of ChTX. Although these observations may suggest that this conductance does not play a significant role in spike repolarization and afterhyperpolarization, the increase in spike amplitude could cause greater activation of other voltage-dependent outward conductances, which may mask any change in the afterhyperpolarization produced by suppression of Ik,Ca.

Many different types of neuron possess a low-threshold, transient current (Rogawski 1985; Rudy 1988) that was described first in molluscan neurons (Connor and Stevens 1971a) and termed the 'A current.' This current influences the excitable properties of a number of different types of neuron. For example, it regulates the frequency with which neurons produce repetitive spiking (Connor and Stevens 1971b), delays the onset of spiking during depolarization (Byrne 1980; Getting 1983), produces spike frequency adaptation (Partridge and Stevens 1976), and contributes to spike repolarization (Beluzzi et al. 1985; Storm 1987). Because this current can have such a significant effect on the excitable properties of neurons, we applied the selective A current blocking agent 4-AP to motoneuron Df to determine whether it influences its excitable properties. Under current-clamp conditions, application of 1 mM 4-AP leads to an increase in synaptic activity (n = 7) and slight depolarization of the resting membrane potential (2.8 ± 0.5 mV, n = 6). In neurons that displayed a plateau potential on depolarization, 4-AP increased the amplitude of the superimposed oscillations (n = 3, Fig. 3A). In 4-AP, the oscillations reached a more positive absolute membrane potential (by ≤5 mV) and were followed by a more pronounced phase of repolarization. Application of 4-AP to neurons that already spiked when depolarized, increased action potential amplitude 1–3
mV ($n = 5$) and latency to first spike was decreased (Fig. 3B, i and ii). In some preparations, spike afterhyperpolarizations developed more slowly in the presence of 4-AP (Fig. 3B, i and ii, $n = 3$). In other preparations, this was not so noticeable ($n = 2$). In some preparations, 4-AP increased the frequency of spikes evoked by depolarizing pulses of a given amplitude ($n = 3$ of 5).

In some experiments in which ChTX and 4-AP had been coapplied to block outward currents, (Fig. 3C) subsequent administration of TEA$^+$ (50 mM) caused a marked increase in both the amplitude and duration of action potentials (Fig. 3Cii). The magnitude of the spike afterhyperpolarization also was increased significantly.

**Time dependence of outward currents measured under voltage-clamp conditions**

In physiological saline, depolarizing command pulses elicited an outward current, mainly attributable to flux of potassium ions, which was sufficiently rapid and large to obscure a smaller inward calcium current. Altering external chloride concentration had no observable effect on the current-voltage relationship, suggesting that there was no significant chloride conductance in these cells.

To determine whether there is a fall in outward current that corresponds with the transition in the electrical events recorded from the neuron, the amplitude of this current was monitored over time. Within half an hour of dissection, the neuron in Fig. 4 was displaying plateau potentials on depolarization. Electrical activity and the amplitude of outward current then were measured every 15 min. With increasing time from dissection, a decrease in outward current was observed ($n = 3$; Fig. 4). During this time, activity induced in this neuron by depolarization under current-clamp conditions changed from plateauing into spiking (not shown). The peak amplitude of inward calcium currents in this neuron is normally <200 nA (Mills and Pitman 1997) in recordings made up to several hours after dissection. Inward sodium currents are too small to be detected. Any increase in these currents therefore would be too small to produce sufficient direct contribution to membrane currents to account for the comparatively large (>1-μA) reduction when measured at a command potential of −10 mV time-dependent decrease in net outward current observed. The explanation for this reduction in net outward current therefore is almost certainly a fall in the potassium current. It is possible, however, that this fall in outward current could result indirectly from a small change in inward current amplitude; a small change in Ca influx could have a profound effect on $I_{K_{Ca}}$ because this neuron appears to be very sensitive to changes in $[Ca^{2+}]_i$ (Thomas 1984).

**Effects of apamin and ChTX on outward currents**

Because apamin and ChTX differed in their ability to alter the type of activity produced by $D_i$ under current-clamp conditions, the effect of these toxins on outward currents was determined. Figure 5 shows currents and current-voltage ($I-V$) relationships obtained from $D_i$ before and after application of apamin. The holding potential was −70 mV, which is close to the normal resting potential of the neuron; 50-ms-duration command pulses, applied every 15 s, stepped the membrane potential to values between −60 and +100 mV in 10-mV increments. The control $I-V$ relationship of the $D_i$ soma has a characteristic form; outward currents increase steeply between about −40 and +80 mV. Beyond approximately +80 mV, the net outward current shows a sharp decline before undergoing a further increase at still more positive potentials (not shown). This N-shaped $I-V$ relationship is due to the presence of a large $I_{K_{Ca}}$ in this neuron (cf. David and Pitman 1995a; Thomas 1984). The $I-V$ relationship illustrated in Fig. 5 covers this range to demonstrate the form that is diagnostic of $I_{K_{Ca}}$, even though membrane potentials between −70 and 0 mV are most physiologically relevant in this neuron.

Apamin (1 μM) did not depress outward currents measured under these conditions nor did it alter their time course (Fig. 5). This is consistent with lack of effect of this toxin observed under current-clamp conditions. Application of the Ca-channel blocker Cd$^{2+}$ in the presence of apamin reduced a large proportion of the outward current and abolished the decline in outward current seen as the command potential steps are increased from +80 and +100 mV (responsible for conferring the N shape to the $I-V$ relationship of the neuron). This con-
firms the presence of a large Ca-dependent outward current component in this neuron; the lack of effect of apamin, therefore, cannot be attributed to absence of $I_{K_{Ca}}$.

The effect of ChTX on currents evoked at different membrane potentials is shown in Fig. 6. At potentials between $-40$ and $+70$ mV, ChTX (100 nM) reduced the net outward current by $66.1 \pm 1.2\%$ (7 neurons) (measured at the peak outward current). At membrane potentials more positive than approximately $+80–100$ mV, however, there was a fall in the proportion of the total outward current blocked by ChTX (Fig. 6, A and C). This is presumably because the contribution of $I_{K_{Ca}}$ to the total outward current declines as the membrane potential approaches the calcium equilibrium potential. In the presence of a dose of ChTX (100 nM) sufficient to produce its maximal effect on $I_{K_{Ca}}$, addition of 0.5 mM Cd$^{2+}$ still could cause a further reduction in the outward current measured at some membrane potentials. Combined application of ChTX and Cd$^{2+}$, for example, blocked $82 \pm 7\%$ ($n = 8$) of the peak outward current at $+70$ mV; the current that has been blocked represents $I_{K_{Ca}}$. The additional block produced by Cd$^{2+}$ suggests that there may be a ChTX-insensitive component of $I_{K_{Ca}}$ in Df. When Cd$^{2+}$ was applied before ChTX ($n = 3$, not shown), no further depression of outward current was produced by ChTX showing that ChTX was not affecting any cadmium-insensitive outward conductances.

To investigate different components of outward current, those recorded in the presence of a drug were subtracted from those obtained in control conditions. The ChTX-sensitive current obtained in this way is shown in Fig. 6Bi. The $I_{K_{Ca}}$ that was insensitive to ChTX but sensitive to cadmium ions (ChTX-insensitive $I_{K_{Ca}}$) was obtained by subtracting the currents in the presence of both ChTX and Cd$^{2+}$ from those in the presence of ChTX alone (Fig. 6Bii; note that these currents do not give an indication of the whole current that is sensitive to Cd$^{2+}$, which also would include the ChTX-sensitive component). Differences currents (Fig. 6Bi) show that ChTX blocked the sustained outward current as well as the peak outward current. This also is reflected in the I-V relationships measured at 45 ms (Fig. 6Ci) and at 8 ms (Fig. 6Cii) after the beginning of a 50-ms pulse. The kink in the I-V curve seen as command pulses are increased from $+80$ to $+120$ mV (which normally gives the characteristic N-shaped I-V relationship of the neuron) is reduced by ChTX and abolished by Cd$^{2+}$ (currents sampled at 8 and 45 ms; Fig. 6C). The ChTX- and Cd$^{2+}$-resistant outward current, which activated at potentials more positive than $-40$ mV, also showed transient and sustained components; the transient component became more prominent at very positive membrane potentials (Fig. 6A, *), where it peaked at 3 ms after the beginning of the depolarizing pulse. Because ChTX and Cd$^{2+}$ have little effect on currents recorded 3 ms after the onset of command pulses (Fig. 6, A and Cii), it appears that the initial rapid rise outward current in Df is primarily calcium insensitive.

**Effect of 4-AP on outward currents**

The fact that electrical activity recorded under current clamp is influenced by 4-AP provides indirect evidence for a transient
potassium current in D_f. In fact, the current that remained in the presence of Cd\(^{2+}\) was blocked by 1 mM 4-AP (Fig. 7A). It was essential to use relatively low concentrations of 4-AP to observe this effect; at concentrations \(\geq 5\) mM, 4-AP caused an unexpected increase in an outward conductance that obscured the block and resulted in complex effects on the I-V relationship. In some molluscan neurons, 4-AP has been shown to display a similar concentration-dependent effect; at low concentrations, it suppresses outward current, but at high concentrations, it increases \(I_{K,Ca}\) (Hermann and Gorman 1981). 4-AP (1 mM) decreased the slope of the I-V relationship (recorded in the presence of Cd\(^{2+}\)) measured at 3 and 8 ms but...
had little effect on current measured at 45 ms after the beginning of the depolarizing pulse (Fig. 7B), reflecting the rapid activation and inactivation kinetics of the 4-AP-sensitive currents. The time to the peak of the current was between 3 (at command potentials >0 mV) and 4 ms (at more negative command potentials), and the current decay could normally be fitted using a single exponential that was faster at more positive command potentials (Table 1). The fast kinetics and sensitivity to 4-AP suggest that this current may be similar to the fast transient A current originally described in molluscan neurons by Connor and Stevens (1971a) and subsequently observed in a number of different preparations (review by Rudy 1988).

Steady-state inactivation of the outward current

Typical A currents display almost complete steady-state inactivation at −40 mV, whereas their threshold for activation is normally lower than those for other K currents (Rudy 1988). The inactivation properties of outward currents in D. therefore were investigated. Current subtractions were used to study Cd2+- and 4-AP-sensitive currents. The Cd2+-sensitive current consisted of rapidly and slowly decaying transient components and a sustained component (Fig. 8A). The 4-AP-sensitive current was comparatively small and appeared to consist of a single component that almost completely decayed during 50-ms command pulses (Fig. 8B).

Steady-state inactivation was determined by applying 800-ms conditioning prepulses to step the membrane potential to values between −100 and 0 mV (10-mV increments) from a holding potential of −70 mV. This duration was sufficient to allow inactivation to reach a steady level. Each prepulse was used (1), a large current was elicited by the test pulse. However, as the conditioning pulse was made more positive, the amplitude of the total outward current evoked by a test pulse decreased. To represent this graphically, the peak current recorded after a given prepulse (I) was normalized to the peak current observed after a prepulse to −100 mV (Imax), then plotted against prepulse potential (Fig. 8C). Steady-state inactivation curves were determined for total outward current, Cd2+-sensitive current, 4-AP-sensitive current, and the current that was insensitive to both 4-AP and Cd2+ ions, by subtracting the currents remaining in the presence of channel blockers from currents recorded in normal saline solution (Fig. 8C). The mean steady-state inactivation curves were fitted by a Boltzmann distribution. The total outward current displayed steady-state inactivation that reached a maximum of between 50 and 60% at prepulse potentials more positive than −15 mV and had an E0.5 (which is the potential at which inactivation is half completed) of −33 mV. The Cd-sensitive current also displayed partial steady-state inactivation, which reached a maximum of ~70% at potentials more positive than −10 mV and had an E0.5 of −26 mV. The 4-AP-sensitive current displayed slight inactivation at the resting membrane potential of −70 mV in some preparations (in 4 of 8 preparations), but a conditioning pulse to −55 mV normally was required before inactivation occurred in the remaining preparations; maximum steady-state inactivation was observed at potentials more positive than −15 mV, where it approached 100%; the E0.5 was −36 mV.

**Action potential clamp**

This method is a powerful tool for observing the contribution made by specific ion conductances toward different components of electrical events recorded from excitable cells.
Using this technique, representative plateau potentials or action potentials were recorded from Df under current clamp, then used as the command signal applied to the same cell under voltage-clamp conditions. When the neuron is clamped by its own activity, no net current will be measured once activity has synchronized. However, if a specific current is blocked pharmacologically, the clamp amplifier has to compensate for the contribution that this current would normally make to the electrical activity. The compensation current therefore should be identical to the original contribution of the blocked ion conductance (Doerr et al. 1989). An example of the effects of ChTX on a Df neuron that was clamped by one of its typical plateau potentials is shown in Fig. 9. On depolarization under current-clamp conditions, this neuron produced plateau potentials similar to that shown in Fig. 9ii. This typical plateau potential was used as the command signal under voltage-clamp conditions. Because motoneuron Df is not normally spontaneously active, it must be depolarized by injection of a small amount of current. The onset of this current pulse is visible on the records (see arrows). Under control conditions, application of the prerecorded plateau potential produced only a very small compensation current (at arrow) that represents the current injected under current-clamp conditions to depolarize the cell to threshold. After application of 100 nM ChTX to the preparation [which under current-clamp conditions converted the response of the neuron to depolarization from a plateau potential to an action potential (not shown; c.f. Fig. 2)], a downward compensation current was observed because the clamping amplifier had to inject negative current to compensate for the absence of the current blocked by ChTX (Fig. 9, ii–iv). The ChTX-compensation current activated during the depolarizing phase of the plateau potential, but peaked during the repolarizing phase of the oscillations (Fig. 9iiv). Under current-clamp conditions, the ChTX-sensitive current would shunt the inward current underlying these events and thereby limit their amplitude. Activation of this current also could account for the inability of the Df soma to spike at short intervals after dissection (see preceding text). The compensation current declined in amplitude to almost zero by the end of the plateau potential, suggesting that this ChTX-sensitive conductance is unlikely to be responsible for plateau potential termination.

The effects of ChTX on cells that were already spiking indicated that this conductance cannot be primarily responsible for spike repolarization and afterhyperpolarization because spikes evoked in the presence of ChTX still repolarized rapidly and had distinct afterhyperpolarizations (see Fig. 2). The action-potential-clamp method was used to test whether the ChTX-insensitive, calcium-dependent potassium conductance, on the other hand, does contribute toward these events. The current-clamp recording shown in Fig. 10A was made from Df in the presence of ChTX, which caused this neuron to spike on depolarization. After application of micromolar Cd$^{2+}$, the amplitude of the spike and afterhyperpolarization were decreased and the rate of depolarization and repolarization were slowed (Fig. 10A, spikes in Cd$^{2+}$ marked by *). These effects may not necessarily be due to a decrease in a calcium-dependent potassium conductance but rather from a reduction in voltage-dependent potassium conductances resulting from the decrease in spike amplitude. This problem is circumvented by using the action-potential-clamp technique; the membrane potential excursion produced by a control action potential as the command signal will be the same before and after administration of Cd$^{2+}$. Interpretation of resultant current therefore will not be complicated by variations in the extent to which voltage-dependent conductances are activated. A series of typical action potentials was used as the command signal when the neuron was held under voltage-clamp conditions (Fig. 10Biii). The control response consisted of a small sustained upward compensation current (corresponding to the injected current that was required under current-clamp conditions to reach the spike threshold). After application of 20 μM Cd$^{2+}$, biphasic transient compensation currents were observed. The relatively small upward current, which coincided with spike depolarization and the peak of the action potentials resulted from block of the Cd$^{2+}$-sensitive calcium current (Mills and Pitman 1997). The larger transient downward compensation currents, coinciding with the spike repolarizations, were due to block of a calcium-dependent potassium current. Because the cell already was bathed in ChTX, the potassium current responsible for these compensation currents must be the ChTX-insensitive, Cd$^{2+}$-sensitive $I_{K,Ca}$.

We already have indicated that 4-AP increases the amplitude of oscillations superimposed on plateau potentials but does not cause a transition to spiking (see Fig. 3). To establish the contribution of the 4-AP-sensitive current to plateau potentials, we used the action-potential-clamp method. Figure 11 shows the results of applying this technique to the neuron used in Fig. 3. A typical control plateau potential was used as the command signal under action-potential-clamp conditions (Fig. 11Aiii).
An upward compensation current was recorded, which reflects the current applied to evoke the template plateau potential; the plateau potential signal itself evoked no compensation current (Fig. 11Ai). In the presence of 1 mM 4-AP, transient downward compensation currents were observed that activated during the membrane potential oscillations and peaked during the repolarizing phase of the oscillation (Fig. 11A, ii–iv). The effect of the 4-AP-sensitive current, like that of the ChTX-sensitive current, therefore would be to shunt the inward currents underlying the oscillations superimposed on plateau potentials. The amplitude of the 4-AP-compensation current is considerably smaller than the ChTX-compensation current. This probably accounts for the inability of 4-AP to cause D_{1} to undergo the transition from plateau potential activity to spikes. During the sustained phase of the plateau potential, no significant compensation current was observed, suggesting that this conductance does not contribute to plateau termination.

Application of 1 mM 4-AP to a spiking neuron under current-clamp conditions can decrease the latency to first spike, increase spike amplitude, and slow the development of the spike afterhyperpolarization (see Fig. 3B). This same preparation was clamped by a series of typical action potentials (Fig. 11Bii). In control responses, each template action potential was associated with an extremely small compensation current (Fig. 11Bi). The compensation currents observed in the presence of 4-AP are shown in Fig. 11Bii; these developed during the depolarizing phase of each action potential and peaked during spike repolarization (Fig. 11Bii). The 4-AP-sensitive current therefore would tend to reduce spike amplitude and contribute toward the afterhyperpolarization.

These experiments show that outward conductances in the soma of D_{1} can influence the electrical activity displayed by this neuron. A ChTX-sensitive i_{K,Ca} is the major outward conductance in this cell and is important in determining whether the cell spikes or displays plateau potentials on depolarization. Other smaller conductances such as the ChTX-insensitive i_{K,Ca} and the 4-AP transient currents contribute...
toward spike repolarization and appear to play a role in the fine-tuning of electrical activity.

**DISCUSSION**

In this paper, current-clamp, voltage-clamp, and action-potential-clamp techniques have been used to explore the roles of potassium conductances in determining the type of electrical activity displayed by the cockroach $D_f$ motoneuron soma. Depolarizing pulses evoke plateau potentials at short intervals after dissection; after $\sim 1 \text{ h}$, however, depolarization elicits action potentials (Hancox and Pitman 1992). Similar changes are seen if the soma is divided mechanically from its processes (Hancox and Pitman 1992). This indicates that the observed transition in electrical properties occurs in the soma itself and is not an indirect result of a change in the length constant or other characteristics of the neuron that enable the dendrites to exert a greater effect on somatic recordings. It was suggested that, shortly after dissection, $[\text{Ca}^{2+}]_i$ is relatively high and that this results in elevation of calcium-dependent potassium current ($I_{K,Ca}$), which, in turn, shunts inward currents, so preventing the soma from spiking (Hancox and Pitman 1992). In line with this proposal, we have found that there is a decrease in net outward current with increasing time from dissection, which correlates well with the change in electrical properties of the soma. We propose that plateau potentials can occur shortly after dissection, despite enhancement of $I_{K,Ca}$, because this current is not strongly activated at the membrane potentials attained during plateau potentials. The inward current underlying the plateau potential is probably the low-threshold, nifedipine-sensitive calcium current we have described recently (Mills and Pitman, 1997). We have shown that the $D_f$ soma possesses $I_{K,Ca}$, a transient 4-AP-sensitive and a delayed rectifier current, each of which may have a different role in regulating the electrical properties of the neuron.

**Calcium-dependent potassium current**

$I_{K,Ca}$ is the largest current recorded from the soma of this neuron. Because apamin had no effect on membrane currents in $D_f$, it appears that, like *Aplysia* neurons (Hermann and Hartung 1983) and larval muscle fibers of *Drosophila* (Elkins et al. 1986; Gho and Mallart 1986), this neuron lacks an $I_{K,Ca}$ similar to the apamin-sensitive low-conductance subtype found in a range of vertebrate and invertebrate preparations (Lazdunski 1983). ChTX, on the other hand, almost completely blocked $I_{K,Ca}$ recorded from the soma of $D_f$. Experiments with isolated somata indicate that the ChTX current is present on the soma itself. Application of different concentrations of ChTX indicates that there is a small ChTX-insensitive component of $I_{K,Ca}$ that can be blocked by Cd$^{2+}$. A slight possibility remains, however, that ChTX produces incomplete block of a single class of current. When ChTX was applied after Cd$^{2+}$, there was no additional inhibition of $I_{K,Ca}$, indicating that ChTX does not affect any calcium-independent (Cd$^{2+}$-insensitive) conductances.

Although the identity of the small ChTX-resistant component of $I_{K,Ca}$ has not been established, it may be similar to one of the different subtypes of $I_{K,Ca}$ that have been identified in insect neurons. For example, in *Drosophila* muscle and “giant” neurons, two subtypes of $I_{K,Ca}$ have been identified; a fast transient and a slow non-inactivating component (Elkins et al. 1986; Gho and Mallart 1986; Saito and Wu 1991; Salkoff 1983; Singh and Wu 1989). A *Drosophila* mutant, slowpoke (slo), lacks a component of $I_{K,Ca}$ in muscle and giant neurons (Elkins et al. 1986; Saito and Wu 1991; Singh and Wu 1989); these cells are more excitable and display broader action potentials than the wild-type, indicating that, in these preparations, $I_{K,Ca}$ is important in spike repolarization (Elkins et al. 1986; Singh and Wu 1990). Irregular regenerative responses, including oscillations of variable amplitude superimposed on slow waves of depolarization have been recorded from some subsets of neurons in slo mutants (Saito and Wu 1991). In Kenyon cells from the honeybee (Schäfer et al. 1994), and in cockroach DUM neurons (Grolleau and Lapied 1995), a ChTX-sensitive $I_{K,Ca}$ with fast transient and sustained components has been identified. Although the ChTX-sensitive $I_{K,Ca}$ identified in $D_f$ displays some inactivation during a sustained depolarization, a fast ChTX-sensitive transient component was not observed. It is possible, however, that such a component may have been present but extremely small compared with the sustained component.

Application of ChTX to $D_f$ rapidly causes the neuron to generate spikes instead of plateau potentials when depolarized. This transition in response is similar to the time-dependent change that normally develops in this preparation during a period of hours, indicating that this conductance may be important in determining electrical properties of the neuron. The gradual fall in $K$ currents with time from the onset of recording (perhaps due to falling levels of intracellular calcium), which we report here, may be responsible for the appearance of spikes, and their subsequent gradual increase in amplitude as a function of time from dissection.

The activation characteristics of $I_{K,Ca}$ determined in voltage-clamp experiments, could explain why $D_f$ is initially capable of generating plateau potentials but not spikes. The potential reached by a plateau potential rarely exceeds $\sim 30 \text{ mV}$, whereas the membrane potential reached during a spike can be close to 0 mV. At $\sim 30 \text{ mV}, I_{K,Ca}$ may not be large or fast enough to shunt the inward current underlying the plateau potential. At more positive membrane potentials, however, $I_{K,Ca}$ activation is faster and larger and is more likely to be capable of shunting the currents responsible for spiking. When $I_{K,Ca}$ is reduced, either by direct pharmacological block or by lowering intracellular calcium levels, the inward currents underlying spiking would be shunted to a smaller extent, thereby allowing the cell to spike. The action-potential-clamp technique, makes it possible to establish the contribution of individual conductances to electrical events with a precision not possible with conventional voltage-clamp method. For example, this method has enabled us to show that the ChTX-sensitive conductance activates sufficiently fast at the onset of a plateau potential to shunt inward currents, so, at the same time, limiting plateau potential amplitude and preventing action potential generation. During plateau potentials, this current decays to such an extent that it could not be responsible for termination of these events.

In *Aplysia*, ChTX produces different effects on action potentials depending on the cell type; in bursting cells, ChTX prolongs the action potential duration but it has no effect on beating or silent cells after direct stimulation (Hermann and Erxleben 1987). Hermann and Erxleben (1987) found that
ChTX also could depolarize the resting membrane by blocking a resting potassium conductance, thus causing an increase in firing frequency. In Df, however, ChTX had no effect on the resting membrane potential. ChTX did not appear to affect spike duration or reduce the amplitude of the spike afterhyperpolarization in Df even though the activation characteristics of the ChTX-sensitive current are such that this conductance should contribute toward speeding spike repolarization. In fact, application of ChTX to neurons that were already able to generate action potentials increased the afterhyperpolarization amplitude, probably because ChTX produced an increase in action potential amplitude, so causing stronger activation of the voltage-dependent K currents (cf. effects of 4-AP). A similar phenomenon is probably responsible for the initial shortening of plateau potential duration by ChTX, since it causes an increase in the amplitude of plateau potentials amplitude.

4-AP-sensitive current

The small amplitude of the 4-AP compensation current compared with that for ChTX may explain why 4-AP did not convert activity in Df from plateauing to spiking but merely increased the amplitude of the oscillations that normally are superimposed on plateau potentials. The small depolarization observed after application of 4-AP most probably resulted from synaptic input impinging on Df because this drug increased the level spontaneous synaptic activity. This is supported by the observation that 4-AP did not appear to influence voltage-dependent currents activated at the resting membrane potential of Df.

Although the 4-AP-sensitive current did not induce spiking in the neuron soma, it did contribute toward spike repolarization and afterhyperpolarization as demonstrated in current-clamp and action-potential-clamp experiments. Under current clamp, the effects of 4-AP on spike afterhyperpolarization varied from preparation to preparation; in some, it was prolonged, whereas in others it was unaffected. The explanation for this is probably the same as that for the effect of ChTX on spike repolarization and afterhyperpolarization; 4-AP produces a small increase in spike amplitude in some preparations by reducing shunting of inward conductances. In those preparations where this occurred, activation of other voltage-dependent outward conductances, and hence afterhyperpolarization, may be increased. Under action-potential-clamp conditions, the 4-AP-sensitive compensation current activated during the depolarizing phase of the action potential and peaked during spike afterhyperpolarization, confirming that this conductance does contribute toward the afterhyperpolarization. In addition to this current, a small, ChTX-insensitive component of \( I_{K,Na} \) also contributes to repolarization.

The threshold of activation and inactivation of the 4-AP-sensitive current in Df occur at more positive membrane potentials than those of a typical A-type current and are more similar to the ‘‘high-threshold A-type’’ currents that have been observed in some preparations (review by Rudy 1988) such as the current observed in Drosophila muscle [A1 transient current that is removed by the Shaker mutation: (Salkoff and Wyman 1981; Solc et al. 1987; Zagotta 1988)]. The transient conductance in Df appears to influence the latency to first spike, spike repolarization, and firing frequency as does the A current in many preparations. These observations contrast with those made on another cockroach neuron, a basalar/coxal depressor motoneuron known as cell 3, which is a partial synergiist of Df (Nightingale and Pitman 1989). Although cell 3 and Df have many similarities, when studied either under current or voltage clamp, little or no transient outward current was observed in cell 3 when command steps were applied from a holding potential less negative than -70 mV. When the membrane potential was held at more negative values, however, command steps to -45 mV generated transient outward currents, the amplitude of which increased as the holding potential was made more negative. Inactivation of the transient outward current in cell 3, therefore appears to occur at more negative potentials than similar currents in other neurons. Such currents in Df, on the other hand, inactive at less negative potentials than is usual.

Delayed rectifier current

The current remaining after application of Cd\(^{2+}\) and 4-AP displayed activation and inactivation properties of a typical delayed rectifier current. We did not further explore the influence of this current on electrical activity in Df mainly because we found no means to manipulate this current pharmacologically. As a result, this current could only be investigated by first eliminating all other outward currents; it was impossible, therefore, to directly determine the contribution made by the delayed rectifier current to the electrical properties of the neuron. For this reason, it was not studied in depth.

To summarize, the ChTX-sensitive current is the largest component and is important in determining whether the neuron responds to depolarization by spiking or by producing plateau potentials; when this conductance is high, the cell cannot spike. The other smaller conductances appear to be important in the fine tuning of electrical activity. Obviously any change in inward conductances would be important in determining electrical activity not only directly but also via their indirect effect on outward conductances. For example, the calcium conductance in this cell shows calcium-dependent inactivation (Mills and Pitman 1997). Therefore high intracellular calcium levels would not only decrease the amplitude of \( I_{Ca} \) but also would enhance \( I_{K,Ca} \) and thus may disable spiking in the soma of Df shortly after dissection via these two synergistic mechanisms.

Although it is likely that the transition from plateauing to spiking may result from a gradual fall in [Ca\(^{2+}\)], it is not clear whether the neuronal properties observed immediately after dissection represent a normal state or whether they are consequences of trauma associated with dissection (e.g., temporary hypoxia or excessive neuronal activity produced by stress associated with handling and dissection which could cause release of abnormally large amounts of neurotransmitters and neuromodulators). If this were the case, the postoperational change in neuronal properties could reflect gradual recovery. Any neuromodulator that induces a change in intracellular calcium levels may be expected to exert an important influence on output from this motoneuron. For example, muscarinic agonists have been shown to elevate intracellular calcium levels (David and Pitman 1996a) and decrease \( I_{Ca} \) (David and Pitman 1995b). These agents convert activity in Df from spiking to plateauing (unpublished data). Increases in intracellular cyclic AMP inhibit \( I_{K,Ca} \) in Df (David and Pitman 1996b) so
that neuromodulators that increase cyclic AMP also would be expected to alter electrical activity. The possibility cannot be excluded, however, that, under physiological conditions, the soma of Df does not normally spike and that development of somatic action potentials reflects an early response to axotomy. If this were the case, the transition in properties could result either from redistribution of ion channel proteins or from de novo synthesis of new channel proteins (cf. Pitman et al. 1972). Whatever the case, a similar excitation has not be found in motoneuron Ds, a synergist of Df or in inhibitory motoneurons, indicating that the transition we have observed in Df must be selective for certain neurons.

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