The Ionic Mechanism of the Slow Outward Current in *Aplysia* Neurons

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SUMMARY AND CONCLUSIONS

1. A slow outward current associated with spike frequency adaptation has been studied in the giant *Aplysia* neurons R2 and LP1. The current was observed during 60-s voltage clamp commands to potentials just below spike threshold.

2. The slow outward current shows a marked voltage dependence at membrane potential less negative than -40 mV.

3. The slow outward current is associated with increased membrane conductance.

4. The K+ sensitivity of the slow outward current was studied by varying the extracellular K+ concentration and also by measuring potassium efflux with a K+-sensitive electrode. Both procedures indicated that the slow outward current was K+ dependent.

5. Tail currents following the activation of the slow outward current were examined. They were shown to have a similar potassium sensitivity as the slow outward current and had a reversal potential near the potassium equilibrium potential for these cells.

6. The sensitivity of the slow outward current to known blockers of K+ currents, tetraethylammonium and 4-aminopyridine, was tested. The sensitivity was much less than that reported for other K+ currents.

7. The sensitivity of the slow outward current to changes of the extracellular concentrations of Na+ and Cl− ions, as well as electrogenic pump inhibitors, was tested. The results indicate that the slow outward current is much less sensitive to these changes than to the manipulations of the extracellular K+ ion concentration.

8. We tested the sensitivity of this current to manipulations of intracellular and extracellular Ca2+ ion concentrations. We found that the current persisted at a slightly reduced level in the absence of extracellular calcium or in the presence of calcium blocking agents, cobalt and lanthanum. Intracellular injection of the calcium chelator EGTA at a concentration sufficient to block the Ca2+-dependent K+ current, seen after a brief (1.4-s) burst of action potentials, had minimal effects on the slow outward current.

9. Procedures thought to increase intracellular Ca2+ were tested. We found that exposure of the cell to solutions containing elevated Ca2+ concentrations for prolonged periods increased the slow outward current. Also, treatment with drugs thought to elevate intracellular Ca2+ increased the slow outward current.

10. In conclusion, the slow outward current results from an increased K+ conductance. There is little evidence for the contribution of other ions or electrogenic pumps. Although this current may have some sensitivity to Ca2+ ions, it is not identical to the classical Ca2+-activated K+ current seen after a brief burst of action potentials.

INTRODUCTION

A slow outward current (SOC) has been described in *Aplysia* neurons, which serves to regulate firing frequency in response to prolonged stimulation (12). SOC appears to be due to a slowly increasing K+ conductance activated by subthreshold depolarizations,
which is at least partially responsible for the phenomenon of spike frequency adaptation (SFA) observed in these cells. Anticonvulsant concentrations of barbiturates cause an enhancement of SOC and a resultant alteration of the adaptation response. Initial studies indicate that this current is sensitive to changes in extracellular K⁺ (12). These data have led us to hypothesize that the SOC represents an increased K⁺ conductance which exerts a hyperpolarizing effect on the membrane and thus leads to SFA in these cells. This hypothesis is consistent with the hypothesis of others that one or more K⁺ conductances underlie SFA in other cell types (27). In this paper we have further examined the ionic dependence of SOC as well as the possibility of electrogenic pump involvement, and have found that SOC exhibits characteristics different from other known potassium currents.

We test the hypothesis that SOC is dependent on an increase in membrane K⁺ conductance. Further, we examine the sensitivity of SOC to changes in the concentration of other ions in the external medium. We find that although a slow increase in K⁺ conductance is sufficient to explain the SOC, the nature of this K⁺ conductance is unique. The SOC does not resemble any of the identified K⁺ conductances in time course or in sensitivity to commonly used K⁺ channel blockers. The manipulation of Ca²⁺ both within and outside the cell indicates at least some dependence of SOC on Ca²⁺, but SOC is not likely to be a Ca²⁺-activated conductance in the usual sense because Ca²⁺ entry is not required.

**Methods**

Experiments were performed on isolated abdominal and pleural ganglia from *Aplysia californica* (Pacific Biomarine) weighing 100–300 g. After removal, the ganglia were pinned to the bottom of a Sylgard-lined (Dow-Corning) chamber and perfused with artificial seawater (ASW). Neurons were impaled with one or two microelectrodes (0.8–2.0 MΩ) filled with 1.5 mM KCl. In most cases the connective tissue was left intact, although a thin slit was occasionally made in the sheath to facilitate impalement.

A single microelectrode voltage clamp circuit (31) provided voltage clamp control of cell membrane potential. In some cases, two-electrode clamping was performed (Dagan 8500). Cells were kept at holding potentials of −45 to −60 mV and stepped to voltages between −30 and −100 mV. Tail currents following depolarization were recorded for up to 4 min to permit analysis of slow kinetic processes (see below). *Aplysia* giant neurons are quite large (up to 1 mm diam) and have relatively low input resistances (0.5 to 1 MΩ), and therefore there can be some difficulty in maintaining clamp control during depolarizations that activate large voltage-dependent conductances. However, the depolarizations in this study were relatively moderate (usually <30 mV), and the clamp currents were accordingly small, <50 nA. The single- and double-electrode voltage clamps used in this study were thus able to maintain adequate voltage control during the step polarizations. Also, in a few cells many of the observations in intact giant cells have been verified in soma that had been isolated by microsurgery. In these cases voltage control is assured due to a spatially compact membrane.

The artificial seawater used in most experiments was a high-Mg²⁺ seawater with the following composition (in mM): CaCl₂ 10, KCl 10, MgCl₂ 70, MgSO₄ 30, NaCl 492, NaHCO₃ 2. The pH of the solution was adjusted to 7.8 with HCl prior to each experiment. This basic formula contains twice the [Mg²⁺] normally used in ASW to suppress the synaptic activity usually seen in the cells under study. In some experiments, 20 mM MgCl₂ was used to obtain a normal Mg²⁺ concentration of 50 mM. Changes in the K⁺ concentration were made by deleting KCl or by adding it hyperosmotically to normal ASW. Ca²⁺-free solutions were made either by simply deleting CaCl₂ from the ASW, or by adding 3 mM EGTA to the Ca²⁺-free ASW. When used, the Ca²⁺ channel blockers Co²⁺ or La³⁺ were either added hyperosmotically to the ASW or substituted for CaCl₂. When La³⁺ was used, NaHCO₃ was replaced by 10 mM Tris. A variety of Na⁺ substitutes were used including glucosamine HCl, Tris HCl, bis-tris-propane HCl, and sucrose. While none of these substitutes was completely satisfactory, neurons remained most viable when sucrose (702 mM) was substituted for NaCl, and only data from those Na⁺ substitution experiments are presented.

**Quantitation of SOC**

The slow currents in *Aplysia* giant neurons have been partially elucidated by cesium injection (11), a maneuver which nonselectively blocks K⁺ conductances. Cesium injection drastically changes the response of the cells to a 60-s depolarization to −30 mV from a holding potential of −50 mV. The slowly increasing outward current obtained upon depolarization and the tail current obtained upon repolarization are blocked to reveal an inward
current that inactivates somewhat during the first 5 s of depolarization and then persists at a steady level. The K\(^+\) currents, obtained by subtraction of the pre- from postcesium injection currents, have a two-phase voltage-dependent onset, and a tail current upon depolarization (11). To quantitate SOC we thus have chosen two means: 1) measurement of the late rise in outward current, which occurs during a period in which the inward current is constant (between 10 and 60 s of the step depolarization; denoted in this report as \(I_{10-60}\)), and 2) analysis of tail currents (e.g., see Fig. 4), since these were abolished by cesium injection (4) and therefore represent the decay of K\(^+\) conductances. Both of these methods provide a means of measuring SOC in the absence of inward current contamination.

Using compartmental analysis ("curve-peeling," Ref. 32), tail currents have been resolved into two exponential decay processes that differ in amplitude and kinetics. When cells were maintained in voltage clamp at a holding potential of -50 mV, tail currents that followed a 60 s step depolarization to between -35 and -30 mV displayed half-lives of 4.82 ± 0.24 s for the first component and 90.8 ± 8.08 s for the second component (n = 23). Amplitudes of the two components measured immediately upon termination of the voltage clamp step (i.e., zero time intercepts, determined by extrapolation) varied depending upon degree of depolarization, but in almost every case the faster, first component was larger than the second or slower component. The amplitude of the first component was 4.35 ± 0.44 nA, and that of the second component was 2.02 ± 0.27 nA (n = 23). No differences were found between R2 and LP1 for these or any other base-line measurements of SOC or tail currents, and in all averaged results that follow data from the two cell types are combined.

Data were recorded on a double-width channel of a chart recorder (Gould 2400) and digitized by hand using an Apple graphics tablet. Exponential decay analysis, including estimation of kinetic parameters (half-lives and amplitudes) and curve fitting, were performed on an Apple II computer. Initial results indicate that tail current analysis is a sensitive index of changes in depolarization-induced slow outward current (14) and, where available, the results of such analyses are presented.

RESULTS

Slow outward current in giant neurons

Voltage clamp studies of membrane currents recorded from Aplysia giant neurons R2 and LP1 have revealed that a prolonged depolarizing step to a voltage near spike threshold activates a slow outward current \(I_{10-60}\) that is constant (between 10 and 60 s of the step depolarization; Ref. 4). Figure 1A shows the membrane currents that are activated by 60-s depolarizations of 15-30 mV from a holding potential of -60 mV. Upon depolarization to -45 or -40 mV there is an early increase in outward current during the first 5-10 s of depolarization, after which the current trace remains quite stable. However, upon further depolarization to -35 or -30 mV, there is a slow, late rise in outward current following the initial rise.

The effect of SOC on the current-voltage \((I-V)\) relationship of Aplysia giant cells is shown in Fig. 1B. For this figure, the \(I-V\) curves were derived from an R2 neuron 0.5 and 60 s after initiation of a step depolarization in voltage clamp. The current values taken 0.5 s after initiation of the voltage commands indicate a negative slope conductance region at potentials more positive than -40 mV. In contrast, the \(I-V\) relationship plotted using current values taken 60 s after initiation of the voltage command shows no negative slope conductance region. Rather, one finds an upward deflection of the \(I-V\) curve at potentials more positive than -40 mV.

The contribution of K\(^+\) conductance to SOC

This laboratory has previously reported that SOC is sensitive to changes in \([K^+]_o\). We have examined this finding more closely and have extended our studies to include the sensitivity of SOC to a variety of other ionic substances and pharmacological agents.

Conductance changes (as determined by the slope of the \(I-V\) relationship) during prolonged depolarizations were characterized to further elucidate the ionic mechanism underlying SOC. The conductance of a neuron at the initiation of SOC (10 s into the depolarization) was compared with the conductance after activation of the SOC (60 s into the depolarization). In the experiment shown in Fig. 2, the \(I-V\) relationships determined for a neuron after a 10-s (circles) and 60-s (triangles) command to -30 mV are plotted together. The current values are taken 0.5 s after the step from -30 mV. Depolarization for 60 s causes an increase in the slope conductance of the neuron when compared with the slope conductance measured after only 10 s of depolarization. These \(I-V\) curves intersect at -73 mV, near the K\(^+\) equilibrium potential for these cells (-77 mV, Ref. 28).
Figure 3 shows the SOC that develops during a step depolarization from −70 to −30 mV in a cell exposed to three concentrations of $[K^+]_o$. SOC amplitude, as measured by $I_{10-60}$, is enhanced in low (3-mM) $[K^+]_o$ and depressed in high (30-mM) $[K^+]_o$. The tail currents from a $K^+$ substitution experiment are shown in Fig. 4. Figure 4A is a linear plot of the tail currents obtained with three different values of $[K^+]_o$, while 4B shows the same tail currents on a semilog plot. As can be seen in 4B, the major difference between the curves is one of magnitude, indicating that the difference could be accounted for by a change in the driving force for the current. Assuming that the tail currents are carried by $K^+$, then the tail currents can be converted into tail conductances. This has been done in Fig. 4, C (linear plot) and D (semilog plot). These curves were calculated based on an intracellular $K^+$ concentration of 150 mM (see below). The curves overlap over the full time course of the tail current, providing evidence that this current is carried by $K^+$.

In two experiments a bipolar $K^+$-sensitive electrode was positioned adjacent to the soma of a giant neuron which had been dissected.
free from the tissue capsule. To obtain these $K^+$ signals it was necessary to actually press against the soma membrane of the neuron with the tip of the $K^+$-sensitive electrode. This procedure likely resulted in the formation of a restricted space in which the potassium efflux could be contained and thus measured. The $K^+$ electrodes had a sensitivity of 30 mV per tenfold change in $K^+$ concentration. In these experiments, the level of extracellular $K^+$ was found to slowly increase (from a base-line level of 10 mM to nearly 20 mM) during the development of SOC and gradually return to base line upon repolarization. At the end of each experiment, the intracellular $K^+$ concentration was determined by impaling the neuron with the $K^+$-sensitive electrode. In the two cases [$K^+$]$_i$ was 145 mM and 157 mM. This compares favorably with the 165.3 ± 3.4 mM obtained by Russell and Brown (28).

Attempts to reverse the tail current met with mixed success. Tail reversal proved to be quite difficult to demonstrate because many cells displayed a slow inward current when hyperpolarized, similar to that seen by Halliwell and Adams, referred to as the "Q-current" (18). Also, the rate constant of tail current decay increased markedly with hyperpolarization thus making reversal of the slow tail currents unlikely. Nevertheless, in a fraction of the cells studied the tail currents following SOC were large enough to overcome the inward (Q-type) current and did reverse. An example is shown in Fig. 5. In 10 mM

![Graph showing conductance changes during development of outward current in R2.](image)

**Fig. 2.** Conductance changes occurring during the development of outward current in R2. Open circles: I-V relationship of the cell after a 10 s depolarization to -30 mV. Open triangle: I-V relationship of the cell after a 60 s depolarization to -30 mV. All current values obtained 0.5 s after step change.

![Graph showing effect of changing external $K^+$ on currents activated by depolarization to -40 or -30 mV.](image)

**Fig. 3.** The effect of changing external $K^+$ on currents activated by depolarization to -40 or -30 mV. Holding potential equals -60 mV.
K⁺ ASW, the tail currents were found to reverse at a voltage very near the expected $E_K (-77 \text{ mV})$.

When $[K^+]_o$ was varied from 3 mM to 30 mM, the reversal potential changed in the appropriate direction with a slope of $35 \pm 4 \text{ mV} (n = 5)$ per tenfold change in $[K^+]_o$.

Although the kinetics of this process are vastly different from other known K⁺ currents, we wished to determine the susceptibility of the SOC to pharmacological blockade compared with other known conductances. Several substances known to block these conductances were applied to the giant neurons and their effects on SOC were observed. The SOC did not resemble any of the currents described by Thompson (30) in its sensitivity to pharmacological blockade. Application of 2 mM 4-aminopyridine (4-AP) only slightly reduced $I_{10-60} (9 \pm 2\%, n = 3)$. Similarly, no reduction in $I_{10-60}$ was observed in 20 mM tetraethylammonium (TEA), while 100 mM TEA reduced $I_{10-60}$ to an average of $54 \pm 7\% (n = 4)$ of control. Figure 6 shows the effects of 2 mM 4-AP and 100 mM TEA on the SOC. Where SOC was reduced by TEA and 4-AP, the tail current amplitude was reduced by a comparable amount. The decrease in amplitude did not result from a selective decrease of either of the components of tail current, i.e., both components were decreased by the same relative amount. Tail current kinetics were not affected. Application of either 10 mM barium or 35 uM muscarine had no obvious effect on $I_{10-60}$ or tail currents, indicating a dissimilarity between SOC and the K⁺-mediated M-current described by Brown and Adams (7).
Involvement of other ionic mechanisms in SOC

The possibility that other ionic mechanisms are involved in generation of the SOC was also investigated. Experiments were performed to determine whether changes in Na\(^+\), Cl\(^-\), or Ca\(^{2+}\) conductance or activation of an electrogenic Na\(^+\) pump might contribute to the development of SOC.

ELECTROGENIC PUMP. No evidence for the participation of an electrogenic Na\(^+\) pump in SOC could be found. In three experiments, ouabain (0.4–1.0 mM) caused an increase in inward current (6–8 nA) at the holding potential of −50 mV and a large increase in base-line synaptic activity with only a slight reduction in SOC or the tails that followed. In addition, cooling to 4°C or addition of the mitochondrial uncoupling agent dinitrophenol either had no effect or actually enhanced SOC. Finally, membrane conductance (as measured by the change in current during a 14-mV hyperpolarizing step), which increased greatly during SOC development, returned to base-line levels with the same time course as the tail currents (Fig. 7). Such conductance changes would not be expected as a result of activation of an electrogenic pump.

SODIUM. When exposed to solutions containing 0 Na\(^+\) (equiosmolar sucrose or bis-tris propane) the SOC and the tail currents persisted, although at a slightly reduced am-
The reversal potential of the tail currents was not altered by removal of NaCl from the ASW \( (n = 5) \). It is possible that the depression of SOC and tail currents in Na\(^+\)-free seawater is a nonspecific effect due, for example, to the reduction in ionic strength of the extracellular solution.

**CHLORIDE.** As noted above, replacement of NaCl with sucrose had little effect on the reversal potential of SOC tail currents. In order to further examine the possible role of Cl\(^-\) in SOC, experiments were performed in which sulfate was used to replace 90% of the Cl\(^-\) in the bath. In five such experiments, no significant effect was found on either the SOC itself or the reversal potential of SOC tail currents.

**CALCIUM.** Numerous experiments were performed to examine the sensitivity of SOC to Ca\(^{2+}\). We replaced bath Ca\(^{2+}\) with other divalent cations known to block the Ca\(^{2+}\) channel, such as cobalt or lanthanum, and we found that SOC, while consistently re-

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**Fig. 6.** A: effect of 2 mM 4-AP on currents activated by a 60-s depolarization from -60 to -30 mV. B: effect of 100 mM TEA on currents activated by depolarization from -60 to -30 mV.

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**Fig. 7.** Membrane conductance changes during SOC tail current. The conductance was determined by dividing the change in current during hyperpolarizing voltage steps by the change in the voltage \((-13\) mV\). The changes in current levels were measured 100 ms from the beginning of the voltage steps using an expanded record of the current trace. The conductance during the tail current was compared with base line, as measured by the conductance prior to SOC activation. *Inset* is a current trace demonstrating the method of this experiment. Holding potential equals \(-45\) mV; depolarized potential is \(34\) mV.
duced, was never eliminated. Substitution of 10 mM Co^{2+} for Ca^{2+} reduced I_{10-50} by 33% and 53% in two experiments. Three mM La^{3+} reduced I_{10-50} by 48.2% ± 7.2% (n = 5). An example of each of these experiments is shown in Fig. 8. Another Ca^{2+} channel blocker, Cd (1-2 mM), also reduced SOC only incompletely. It has been previously shown that SOC is sensitive to pharmacological enhancement by barbiturates (12). In every case where the substitution of another cation for Ca^{2+} resulted in a reduction of the SOC, the addition of 100 μM pentobarbital resulted in an increase in the SOC, usually to greater than control levels.

To further test the Ca^{2+} dependency, we reduced [Ca^{2+}]_o to a concentration at which Ca^{2+} entry is not likely to occur under any circumstances by adding 3 mM EGTA to Ca^{2+}-free ASW. Under these conditions, [Ca^{2+}]_o should be <10^{-8} M (29), less than the expected [Ca^{2+}]. The results from such experiments were similar to those in which Ca^{2+} channel blockers were used; SOC was diminished in most cells but not eliminated. In both the Ca^{2+} channel blocker experiments and the EGTA experiments, the effect on SOC required prolonged exposure to the Ca^{2+} deficient medium. This is in marked contrast to the effect of low Ca^{2+} ASW on the postspike hyperpolarization that has been shown to be dependent on Ca^{2+} entry (22). While the latter phenomenon was virtually eliminated within 10 min of [Ca^{2+}]_o removal, maximal reduction of the SOC could take 1 h or more.

One unexpected result was that in a few cells, SOC amplitude actually increased upon the application of EGTA ASW. This might be explained by the effect of Ca^{2+}-deficient medium on the voltage sensitivity, or stability, of the membrane (15). It has long been recognized that spike threshold is strongly influenced by the external Ca^{2+} concentration,

![Fig. 8](image_url)

**Fig. 8.** A: depression of the SOC in R2 by 10 mM Co^{2+} solution containing normal Ca^{2+} and by a solution containing 10 mM Co^{2+} and 0 Ca^{2+}. Current records are from depolarizations from −60 to −30 mV. B: effect of La^{3+} addition on the membrane currents of R2 activated by depolarization from −60 to −30 mV.
and this effect is seen in these cells as well. Since SOC amplitude is a function of membrane depolarization, it is possible that in at least some cells the effect of Ca$^{2+}$ depletion \textit{per se} was more than compensated for by the effect of that depletion on the voltage sensing mechanism of the membrane. In any case, it is clear from the Ca$^{2+}$ channel blocker and EGTA experiments that SOC is not immediately dependent on Ca$^{2+}$ entry across the membrane for its origin.

Despite this conclusion, one cannot escape the fact that SOC is somewhat sensitive to changes in [Ca$^{2+}$]$_i$. Since the relevant effect of Ca$^{2+}$ withdrawal might be a change in [Ca$^{2+}$]$_i$, a series of experiments was done to test the effect of manipulating [Ca$^{2+}$]$_i$ on SOC.

In four experiments neuron R2 was exposed to 100 mM Ca$^{2+}$ for 1 h. The intention of these “calcium soak” experiments was to raise the level of intracellular Ca$^{2+}$. Figure 9 depicts the results from one of these experiments. In this experiment the cell exhibited a depression of the slow currents while in the presence of high Ca$^{2+}$, but a significant enhancement of the SOC shortly after returning to normal saline. Other cells showed an enhancement of SOC both in the presence of elevated Ca$^{2+}$ and following the return of [Ca$^{2+}$]$_o$ to normal. In most cases, the effects of exposure to high Ca$^{2+}$ lasted more than 2 h.

A number of substances have been shown to interfere with intracellular Ca$^{2+}$ uptake mechanisms. These include the mitochondrial uncoupling agent 2,4-dinitrophenol (DNP) at a concentration of 0.1 mM (4, 10, 21) and mersalyl acid, a potent inhibitor of both mitochondrial and nonmitochondrial uptake systems at a concentration of 50 μM (4). Both of these agents enhanced SOC amplitude.

The mitochondrial uncoupling agent DNP increased $I_{10-60}$ by 59.5% ± 10.3% ($n = 4$) at a concentration of 1 mM. The enhancement of SOC by DNP occurred quite rapidly, usually within 10–15 min. Mersalyl acid proved to be a much more potent agent than DNP. Mersalyl acid, 100 μM, increased $I_{10-60}$ by 245 ± 89% ($n = 4$). At mersalyl acid concentrations of 25 and 50 μM, $I_{10-60}$

![Image of Figure 9](image-url)
FIG. 10. Effect of EGTA injection on SOC and postspike current. SOC stimulus was a 60-s depolarization to -30 mV from a holding potential of -60 mV. Postspike current was elicited by switching to current clamp for 1.4 s to elicit 14 action potentials in control, and for 1.5 s to elicit 15 action potentials after EGTA injection. EGTA was pressure injected through a separate micropipette filled with 250 mM EGTA and 500 mM KCl. The pipette also contained 10 mM arsenazo III to visually verify the intracellular injection. The chart recorder speed was increased during the train of action potentials in order to count the number of spikes; during this period the time calibration is 1 s.

was increased by lesser amounts. In addition, mersalyl acid depolarized the cells and produced a large increase in membrane conductance. When mersalyl acid was washed from the bath, these latter effects reversed, but enhancement of SOC contained for hours after return to drug-free medium. This distinguishes mersalyl acid from DNP, whose effects were quite reversible.

Lewis and Wilson (22) have shown that intracellular injection of the Ca$^{2+}$ chelator EGTA blocks postspike current (PSC) in Aplysia neurons. The PSC is a K$^+$ current dependent on the entry of Ca$^{2+}$ that occurs during a train of action potentials. However, intracellular injections of EGTA that block the PSC have minimal effects on SOC (Fig. 10).

DISCUSSION

The SOC was first described in neurons that exhibit a depression of firing rate following the application of barbiturates (12). It was discovered that phenobarbital and pentobarbital enhance the development of the SOC, with the result that the repetitive firing of action potentials is inhibited. Subsequently, similar slowly activating membrane currents have been observed in other cell types. Merickel and Gray (24) have identified a very slow outward current in the neurons of the cyberchron network in Helisoma. Partridge (26) has described a slow outward current in Helix and Lymnaea with similar properties that is insensitive to extracellular Ca$^{2+}$. Other investigators have demonstrated a slow outward current in different neurons of Aplysia similar to that observed in R2 and LP1 (8). A slow outward current mediated by an increase in K$^+$ conductance has also been identified in mouse neuroblastoma cells (25).

The results described here indicate that the SOC results primarily, if not exclusively, from an increased conductance to K$^+$. The SOC was enhanced when the driving potential for K$^+$ was increased by lowering [K$^+$_o], and was depressed when the [K$^+$_o] was raised, lowering the driving potential. The reversal potential of the SOC was dependent on the extracellular K$^+$ concentration. Also, the extracellular level of K$^+$ immediately adjacent to the soma membrane increases with a similar time course to that of SOC activation.

Although the relationship between extracellular K$^+$ and reversal potential was not as strong as predicted (35 mV/decade obtained vs. 58 mV predicted), this could be explained by the difficulty in obtaining reversal potentials for the tail currents due to interference with other slow currents (Q current) and changes in the kinetics of tail current decay with hyperpolarization. The persistence of the current in solutions in which Na$^+$, Ca$^{2+}$, and Cl$^-$ were greatly reduced or eliminated indicates that the SOC is not comprised of a large flux of these ions across the cell membrane.

An electrogenic pump has been shown to contribute significantly to the resting-membrane potential of R2 (9). Ouabain, cooling, and 0 K$^+$, all of which have been shown to depress the pump (9), were not effective in blocking generation of the SOC. Indeed, the SOC was consistently enhanced when [K$^+$_o] was lowered. Similarly, mitochondrial uncoupling agents enhanced the SOC, a result unexpected if the SOC was dependent on an energy-consuming pump (5, 10, 21).

The fact that injection of Cs$^+$ reduces or
eliminates the SOC (11) supports the hypothesis that the SOC is primarily a K⁺ current. Cs⁺ appears to be a nonspecific K⁺ channel blocker capable of reducing a variety of K⁺ conductances in different neuron types (2, 11, 13). However, the SOC was not affected by other, more specific K⁺ channel blockers in a manner consistent with any of the currently characterized K⁺ channels (30). SOC was resistant to blockade by the K⁺ current blockers TEA and 4-AP. While 100 mM TEA did reduce the SOC by 50%, this is much less than the reduction seen in K current described by Thompson (30). Two mM 4-AP caused a 9% reduction in SOC, much less than the reduction in A-current. Finally, the SOC was not blocked by barium, indicating a dissimilarity with the K⁺-mediated M-current described by Adams et al. (1, 7).

The SOC does not resemble the C-current (9) or other previously described Ca²⁺-activated K⁺ currents (3, 16, 17, 19, 23, 30) in that the SOC persists in no added Ca²⁺/EGTA solutions, in the presence of Ca²⁺ channel blockers, and with intracellular EGTA. However, the SOC does show a gradual reduction in size in a Ca²⁺-deficient medium or in the presence of Ca²⁺ channel blockers. It is not clear whether this gradual depression of the SOC indicates a true dependence of the SOC on Ca²⁺, or whether these are nonspecific or toxic effects due to Ca²⁺ deficiency or the presence of the channel blockers. What is clear from these experiments is that the development of SOC is not dependent on Ca²⁺ entry.

However, the results of other experiments do suggest that [Ca²⁺]ᵢ has at least some influence on the generation of SOC. The mitochondrial uncoupling agent DNP and mersalyl acid, a potent inhibitor of intracellular Ca²⁺ sequestration, both enhanced the SOC. These substances have in common the ability to increase intracellular Ca²⁺ (6, 10, 20). It is possible that the development of SOC is sensitive to the level of [Ca²⁺]ᵢ, although SOC was maintained under conditions in which large increases in [Ca²⁺]ᵢ are blocked (Fig. 10). The means by which [Ca²⁺]ᵢ might change in the absence of Ca²⁺ influx, and the magnitude of any increase of [Ca²⁺]ᵢ during SOC development are not clear and remain the focus of future research.

In conclusion, the SOC appears to be a K⁺-mediated current, although it is not readily identifiable as any of the currently recognized K⁺ currents. Further, the SOC is sensitive to Ca²⁺, although the exact nature of this sensitivity is not clear.

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