Novel Hyperpolarization-activated K⁺ Current Mediates Anomalous Rectification in Crayfish Muscle

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The ionic current underlying anomalous rectification in opener muscle fibers of crayfish was studied under two-electrode voltage clamp. Opener muscle fibers showed a mean resting potential (RP) of -64.8 mV and an input resistance of 0.4 MΩ. Hyperpolarizing voltage command pulses from a holding potential (HP) of -60 mV evoked an instantaneous voltage-independent linear current (I₁) followed by a time- and voltage-dependent inward current (I₅) that reached a steady state within 500 msec. The reversal potential of I₅ (E₅) was estimated from tail current amplitudes. At an extracellular K⁺ concentration ([K⁺]ₑ) of 5.4 mM the mean E₅ was -61.8 mV. E₅ shifted toward positive potentials for a 10-fold increase in [K⁺]ₑ. The conductance underlying I₅ (G₅) increased sigmoidally with hyperpolarization, starting close to the RP, saturating at a G₅,max of about -140 mV, and showing a mean half-activation at -94.4 mV. The activation curve of G₅ shifted 53.6 mV toward positive potentials with a 10-fold increase in [K⁺]ₑ. The activation and deactivation kinetics of I₅ were accurately described by single exponentials with similar time constants (<100 msec). Time constants changed as an exponential function of the membrane potential. I₅, its time course, G₅, and E₅ were not modified in the following conditions: (1) Na⁺- and Ca²⁺-free solutions, (2) intracellular EGTA, (3) extracellular (100 mM) or intracellular tetraethylammonium, (4) extracellular Cs⁺ (up to 50 mM), Rb⁺ (up to 10 mM), Ba²⁺ (13.5 mM), or Mn²⁺ (13.5 mM). However, low extracellular concentrations of Cd²⁺ or Zn²⁺ strongly and reversibly reduced both I₁ and I₅. Therefore, we conclude that anomalous rectification in crayfish muscle is generated by a voltage- and time-dependent K⁺ current I₅. This current displayed many electrophysiological and pharmacological characteristics that distinguished it from all others mediating anomalous rectification described previously.

[Key words: anomalous rectification, inward rectifier, hyperpolarization-activated current, K⁺ current, Cd²⁺ blockade, crayfish muscle]

Katz (1949) reported a higher membrane conductance for inward than for outward current injection in frog skeletal muscle fibers. This nonlinear membrane behavior, termed anomalous or inward rectification, was later found with different characteristics in other systems. Two main varieties of conductances accounting for anomalous rectification have been described. (1) In frog skeletal muscle (Katz, 1949), cardiac muscle (Hall et al., 1963; Sakmann and Trube, 1984), metacerebral giant cells (Kandel and Tauc, 1966), marine eggs (Hagiwara and Takahashi, 1974), tunicate embryos (Miyazaki et al., 1974), and vertebrate neurons (Constanti and Galvan, 1983; Kaneko and Tachibana, 1985; Stanfield et al., 1985; Williams et al., 1988), anomalous rectification is mediated by K⁺, and depends on the extracellular K⁺ concentration ([K⁺]ₑ), and its voltage dependence is a function of the difference between the membrane potential (Vₘ) and the potassium equilibrium potential (Eₖ) (Hagiwara and Takahashi, 1974; Hagiwara and Yoshi, 1979; Leech and Stanfield, 1981). It shows instantaneous voltage-dependent activation followed by a rapid time- and voltage-dependent component, and has been termed inward rectifying current. (2) Another type of inward rectification carried by Na⁺ and K⁺ was found in cardiac muscle (Brown and DiFrancesco, 1980; Yanagihara and Irisawa, 1980), hippocampal pyramidal neurons (Halliwell and Adams, 1982), and lobster slowly adapting stretch receptor neurons (Edman et al., 1987). This current, termed I₅, I₆, or I₇, was later found in several vertebrate neurons (Mayer and Westbrook, 1983; Stanfield et al., 1987; Takahashi, 1990) and was also called I₅ or I₆. If differentiate it from the inward-rectifying current, it has been more generally called hyperpolarization-activated current. It has no instantaneous voltage-dependent component, shows considerably slower activation kinetics, and its activation is independent of [K⁺]ₑ.

A third type of inward rectification carried by Cl⁻ has been reported in Aplysia neurons (Chesnay-Marchais, 1983), amphibian oocytes (Parker and Milelli, 1988), and mammalian hippocampal neurons (e.g., Madison et al., 1986). Fatt and Ginsborg (1958) described a delayed decrease in input resistance evoked by hyperpolarizing current pulses in crayfish muscle, but the underlying conductance had not been characterized under voltage clamp. Therefore, the aim of the present investigation was to characterize the current responsible for the anomalous rectification in crayfish muscle using the two-microelectrode voltage-clamp technique. This voltage- and time-dependent K⁺ current shows no instantaneous component, thus we will call it hyperpolarization-activated current. However, it displayed many electrophysiological and pharmacological characteristics that distinguished it from all others described previously. Since this new hyperpolarization-activated current mediates anomalous rectification and probably acts as a K⁺ balance by reestablishing the transmembrane K⁺ concentration difference after prolonged activation of crayfish muscle, it will be named I₅.
A preliminary report of part of the present results has been published as a short communication (Araque and Bueno, 1991).

**Materials and Methods**

**Preparation.** opener muscles from the propodite of the first walking leg of crayfish (*Procambarus clarkii*) were isolated and transferred to a superfusion chamber (2 mL). Small crayfish (<5 cm) with short muscle fibers (<400 μm) were used for better space-clamp characteristics.

**Microelectrodes and recordings.** Fibers were impaled with two micropipettes usually filled with 1 M KCl (1–5 Ml). When measuring the Cl− equilibrium potential (ECl), micropipettes were filled with 3 M K-acetate (1–5 Ml). In some experiments, the current electrode (filled with 1 M KCl) was substituted, after a control recording, by a new electrode filled either with 700 mM EGTA [ethylene glycol-bis(β-aminoethyl) ether N,N,N′N′-tetraacetic acid], neutralized with KOH (pH 7.2), or with 1 M tetraethylammonium chloride (TEA). EGTA and TEA were ionophoretically injected with 100 nA negative and positive current pulses, respectively, during 13 min. A 1 M KCl–agar Ag–AgCl electrode was used as the indifferent electrode. An Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) was employed for two-electrode current- and voltage-clamp recordings. Probe gains in the voltage-clamp configuration were ×1 and ×10 for voltage and current electrodes, respectively. To decrease the coupling capacitance a grounded shield was installed. A grounded shield was constructed by coating one microelectrode with conductive paint. Voltages were measured with a multimeter (Hewlett-Packard, model 3964a). The time required to reach the command was measured from the holding voltage (VH) and final pulse (VF); voltage-clamp situation, as in all other figures. The currents evoked by VH and VF were measured from the zero current level (upper broken line). For each current, the superscripts 0 and S indicate the initial and steady state levels, respectively. IAMS was the IAMS - IAMdifference.

**Results**

This study is based on data from 127 opener muscle fibers. A representative sample of 31 fibers showed a resting membrane potential (RP) of −64.8 ± 5.8 mV and an input resistance of 0.4 ± 0.2 MΩ. Typical current-clamp responses evoked by depolarizing and hyperpolarizing pulses are shown in Figure 1A. Both small depolarizing and hyperpolarizing current pulses evoked passive responses. Higher-intensity depolarizing pulses elicited active graded responses. With higher-intensity hyperpolarizing currents, the membrane potential (VP) attained an initial peak value at about 50 msec and then gradually decayed, reaching a steady state within about 500 msec. The steady state current versus VP relationship showed a clear nonlinearity in the hyperpolarizing direction, indicating inward or anomalous rectification.

The ionic conductance responsible for the anomalous rectification was investigated under voltage clamp. In order to reduce...
the holding current \( I_{\text{th}} \) and to avoid activation of voltage-gated currents, fibers were clamped at \( H = -60 \text{ mV} \), near the RP. In solutions with raised [K\(^+\)], \( H \) was set close to the attained RP. Figure 2A shows typical currents evoked by 11 msec duration depolarizing pulses from -60 to -40, -15, and 10 mV (the instantaneous linear currents were subtracted) in normal (control), Ca\(^{2+}\)-free (+EGTA), and Ca\(^{2+}\)-free TEA (+TEA) solutions. There is evidence indicating that the depolarization-activated currents shown in Figure 2A are an early inward Ca\(^{2+}\) current \( (I_{\text{Ca1}}) \), an early Ca-dependent K\(^+\) current \( (I_{\text{CaK}}) \), and a late outward K\(^+\) current \( (I_{\text{Ko}}) \) (Mounier and Vassort, 1975a,b; Hencek and Zachar, 1977; Hencek et al., 1978). Both \( I_{\text{Ca1}} \) and \( I_{\text{CaK}} \) were suppressed in Ca\(^{2+}\)-free solution.

In Figure 2B, the 500-msec-duration hyperpolarizing command pulses from \( H = -60 \text{ mV} \) to different \( V \) between -70 and -160 mV evoked an instantaneous current \( I_{\text{th}} \) followed by a gradually developing inward current that reached a steady state \( I_{\text{th}} \) within about 300 msec. Although both \( I_{\text{th}} \) and \( I_{\text{th}}^{\text{ov}} \) increased with hyperpolarization, the latter increased more than the former. Since at any given voltage pulse \( I_{\text{th}}^{\text{ov}} \) was higher than \( I_{\text{th}} \) (except the smallest pulse that did not activate the current), the time- and voltage-dependent inward current was associated with an increase in the membrane chord conductance (see Spain et al., 1987). Figure 2C shows the hyperpolarization-activated current \( (I_{\text{ha}}) \) in isolation, obtained by subtraction of the instantaneous linear components from the total inward current.

Figure 3 shows the current–voltage \((I-V)\) relationships of the currents shown in Figure 2. Whereas \( I_{\text{Ca}} \) (solid triangles) was suppressed, \( I_{\text{ha}} \) was unaffected in Ca\(^{2+}\)-free solution. The remaining \( I_{\text{Ko}} \) (solid circles above -60 mV) was strongly reduced when TEA was added. Both the steady state total inward current (open circles) and \( I_{\text{AB}} \) (i.e., \( I_{\text{AB}} = I_{\text{Ca1}} - I_{\text{ha}} \)) solid circles below -60 mV) were unchanged in Ca\(^{2+}\)-free and Ca\(^{2+}\)-free TEA solution. Similar results were obtained by intracellular injection of TEA (not shown), indicating that the \( I_{\text{AB}} \) channel has no extracellular or intracellular TEA sensitivity.

**Instantaneous current–voltage relationships**

Figure 4, A and B, exhibits voltage-clamp responses in Ca\(^{2+}\)-free TEA solution evoked by the protocols 1 and 2 used to measure the instantaneous on and off \( I-V \) relationships, respectively. \( I_{\text{th}} \) and \( I_{\text{th}}^{\text{ov}} \) were measured 7 msec after the pulse transients, when the capacitive currents had ended, and the activation or deactivation of \( I_{\text{ha}} \) was negligible. Pulses > -100 mV were not used to measure \( I_{\text{th}}^{\text{ov}} \), because they evoked a significant amount of \( I_{\text{AB}} \) (see below). However, it is conceivable that \( I_{\text{th}}^{\text{ov}} \) remained linear beyond that value, as occurred in other fibers.

The \( I-V \) relation of \( I_{\text{th}}^{\text{ov}} \) obtained with pulses from \( H = -60 \text{ mV} \) to \( V \) between -25 and -100 mV (Fig. 4C, triangles), was linear \((r > 0.99)\). Therefore, the membrane did not exhibit instantaneous inward rectification, the instantaneous linear current obeyed Ohm’s law, and it could be written as

\[
I = \frac{V}{R}
\]
Figure 4. Instantaneous I–V relationships in Ca2+ free TEA solution. A. Total currents evoked by protocol 1 from \( H = -60 \, \text{mV} \) to \( V \) from \(-25\) to \(-120\) mV, in \(-5\) mV increments. B. Total currents in response to protocol 2 from \( H = -60 \, \text{mV} \) to \( V = -130 \, \text{mV} \), followed by \( F \) from \(-30\) to \(-100\) mV, in \(-5\) mV increments. C. I–V relationships of \( I_{\text{c}} \) (triangles) and \( I_{\text{v}} \) (circles) fitted to first-order regressions (\( r > 0.99 \)) (solid lines).

\[
P(i) = I_L = G_L (V_m - E_L),
\]

where \( G_L \) and \( E_L \) are the chord conductance and the reversal potential of the voltage-independent linear current \( I_L \), respectively. Similarly, a linear relation was obtained for \( P(v) \) (\( r > 0.99 \)) by stepping \( V_m \) from the \( V \) pulse at \(-130\) mV to \( F \) from \(-30\) to \(-100\) mV (Fig. 4C, solid circles), indicating that the behavior of the total ionic current was also ohmic. Since both \( P(i) \) and \( P(v) \) obeyed Ohm’s law, \( I_{\text{v}} \) did so too, verifying the equation

\[
I_{\text{v}} = G_{\text{v}} (V_m - E_{\text{v}}),
\]

where \( G_{\text{v}} \) and \( E_{\text{v}} \) were the chord conductance and the reversal potential of \( I_{\text{v}} \), respectively. Hence, the total ionic current (\( I_{\text{total}} \)) may be written as

\[
I_{\text{total}} = I_L + I_{\text{v}} = G_L (V_m - E_L) + G_{\text{v}} (V_m - E_{\text{v}}),
\]

and \( I_{\text{v}} \) could be unerringly isolated from \( I_{\text{total}} \) by subtraction of the linear, voltage-independent, current \( I_L \).

The slopes of the linear regressions of the I–V relationships of \( P(i) \) and \( P(v) \) represented the resting chord conductance and the resting chord conductance plus \( G_{\text{v}} \) at \(-130\) mV, respectively. They were 5.5 and 13.5 \( \mu \text{S} \), respectively, confirming that an increase in the membrane chord conductance underlies \( I_{\text{v}} \). The \( P(i) \) and \( P(v) \) I–V relationships intersect at \( E_{\text{v}} \), as can be deduced from Equation 3, verifying the value of \( E_{\text{v}} \) estimated from the tail currents evoked by protocol 2 (see below). Indeed, the intersection point of the I–V curves in Figure 4C (\(-56.5\) mV) agrees well with \( E_{\text{v}} \) estimated by tail currents shown in Figure 6C (\(-53.2\) mV), which were obtained from the same fiber.

Voltage dependence of \( G_{\text{v}} \)
The voltage dependence of \( G_{\text{v}} \) was characterized by a dimensionless activation parameter \( (N) \), similar to the \( n \) and \( m \) parameters defined by Hodgkin and Huxley (1952b), which varied from 0 to 1 as \( G_{\text{v}} \) varied from zero to its maximum value.

Figure 5. Voltage dependence of \( G_{\text{v}} \). A. Total membrane currents evoked by protocol 1 from \( H = -60 \, \text{mV} \) to \( F \) from \(-40\) to \(-160\) mV, in \(-10\) mV increments, and to \( F = -110 \, \text{mV} \). B. Expanded version of same tail currents. C. Activation curve of \( I_{\text{v}} \). \( N_\phi \) values were calculated from Equation 5 and fitted to the Boltzmann formalism (Eq. 6).
(\(G_{AB,\text{max}}\)), and which represents the steady state ensemble probability of \(I_{AB}\) channel activation; \(N_a\) was calculated from currents evoked by protocol 1 (Fig. 5A) with voltage command pulses from \(H\) to different \(V\) (usually from -40 to -160 mV) followed by an \(F\) pulse to a fixed value (usually -110 mV). Since \(F, E_{AB}, E_1,\) and \(G_i\) were constants, Equation 3 could be rewritten as

\[
P_C = G_i (F - E_i) + G_{AB} (F - E_{AB}) = \beta + \alpha G_{AB},
\]

where \(\alpha\) and \(\beta\) were constants. Thus, any change in \(G_{AB}\) will be reflected in \(P_C\). \(P_C\) had a minimum value \((P_C)_{\text{min}}\) when \(G_{AB} = 0\) and it reached a maximum value \((P_C)_{\text{max}}\) when \(G_{AB} = G_{AB,\text{max}}\). Therefore, \(N_a\) was defined as

\[
N_a = (P_C)_{\text{max}} / (P_C)_{\text{max}} - (P_C)_{\text{min}}.
\]

Figure 5C shows the \(N_a\) versus \(V_a\) relation or activation curve of \(I_{AB}\). The data obtained were fitted to the expression

\[
N_a = \left[1 + \exp\left(V_a - V_0\right)/S\right]^{-1},
\]

deduced from the Boltzmann equation, where \(V_0\) is the limiting activation parameter (usually equal to 1), \(V_0\) the voltage at which \(G_{AB}\) is half-activated, and \(S\) a slope parameter.

Results obtained from 17 muscle fibers indicate that the activation of \(G_{AB}\) increased sigmoidally with hyperpolarization, started close to the RP, and could be fitted \((r > 0.99)\) with the Boltzmann equation. The voltage dependence showed a mean half-activation at -94.4 ± 7.1 mV, a slope factor of 12.4 ± 2.7, and a \(G_{AB,\text{max}} = 7.8 ± 3.6 \mu S\). \(N_a\) approached unity at -130 mV and usually saturated at \(V_a > -140\) mV. Hyperpolarization beyond about -140 mV sometimes evoked a large, slow, long-lasting inward current, probably due to membrane breakdown. Thus, strong hyperpolarizations were employed only when this current was not present.

**Kinetic behavior of \(I_{AB}\)**

The time course of both \(I_{AB}\) activation and deactivation (i.e., during the \(V\) and \(F\) pulses, Fig. 6, A and B, respectively) could be fitted \((r > 0.95)\) by single-exponential functions of the form

\[
I_{AB(t)} = a + b \exp(-t/\tau_a),
\]

\[
I_{AB(t)} = c + d \exp(-t/\tau_d),
\]

where \(a, b, c,\) and \(d\) were constants and \(\tau_a\) and \(\tau_d\) were the activation and deactivation time constants, respectively. While \(\tau_a\) decreased with increasing hyperpolarization (Fig. 6D, circles), declining e-fold for 23.6 mV, \(\tau_d\) decreased e-fold every 31.7 mV with depolarization (Fig. 6D, triangles), both being exponential functions of \(V_{m}\). The similarity of \(\tau_a\) and \(\tau_d\) magnitudes indicates that both the \(I_{AB}\) activation and deactivation kinetics may be described by a single time constant \((\tau_{AB})\). For convenience, \(\tau_{AB}\) corresponded to \(\tau_a\) for relatively large hyperpolarizations, whereas it corresponded to \(\tau_d\) for the remaining voltages.

According to what is deduced from Hodgkin and Huxley (1952a), the time constant of voltage-gated currents with an activation curve like that of \(I_{AB}\) should be bell-shaped functions of \(V_{m}\) with a peak at the half-activation voltage \(V_0\) (see also Mayer and Westbrook, 1983). Figure 6D shows that \(\tau_{AB}\) was a bell-shaped function of \(V_{m}\) with a peak around \(V_0\) (compare with Fig. 5, same fiber).

**Ionic nature of \(I_{AB}\)**

The reversal potential of \(I_{AB}\) was estimated from the reversal potential of tail currents evoked by protocol 2. Figure 6B shows the expanded tail currents in Figure 4B. Since the fully activated \(I_{AB}\) did not exhibit rectification and its deactivation was time dependent, then

\[
I_{\text{tail}} = I_{(F)} - I_{(V)} = \Delta G_{(F)} (F - E_{AB}),
\]

where \(\Delta G_{(F)}\) is the difference between the conductance at the beginning and at the end of the \(F\) pulse. Figure 6C shows the \(I-V\) relation of \(I_{\text{tail}}\) from Figure 6B and the corresponding first-order regression fit \((r > 0.99)\). The reversal potential of \(I_{AB}\) estimated by linear interpolation was -53.2 mV. The mean value of \(E_{AB}\) was -61.8 ± 6.3 mV \((n = 28)\), which did not differ substantially from the average RP.

**Effects of \(Ca^{2+}\).** The effects of \(Ca^{2+}\)-free solutions and of in-
concentrations block both the inward-rectifying (Hagiwara et al., 1976; Halliwell and Adams, 1978) and the hyperpolarization-activated currents (DiFrancesco and Ojeda, 1980; Halliwell and Adams, 1980; Hagiwara et al., 1978). Therefore, we can conclude that the actions of raised \([K^+]_o\), not due to redistribution of \(Cl^-\), and that \(I_{AB}\) was selectively mediated by \(K^+\). The surprisingly low \(E_C\) values are not unexpected according to similar results obtained from other crustacean muscles (see, e.g., Mounier and Vassort, 1975a; Henesey et al., 1978).

Although it has been firmly established that alterations of \([K^+]_o\) result in a rapid redistribution of \(Cl^-\) across the cell membrane in many systems, Boistel and Fatt (1958) showed no important \(E_C\) changes in raised \([K^+]_o\), and \(Cl^-\)-free solution in crayfish opener muscle fibers. Their observations were based on inhibitory postsynaptic potential amplitude measurements made within the initial 15 min after the solution change and therefore do not preclude slower changes. Figure 8 shows the effects of raised \([K^+]_o\), on \(E_{AB}\), the \(I_c\) reversal potential, and \(E_C\). While the reversal potential of \(I_c\) and \(E_{AB}\) shifted from \(-60.1\) and \(-63.5\) to \(-46.5\) and \(-49.4\) mV, respectively, \(E_C\) did not change much (from \(-53.3\) to \(-55.8\) mV), in 5.4 and 10.8 mM \([K^+]_o\), respectively. \(E_{AB}\) was the difference between \(E_{AB}\) with and without GABA (see Materials and Methods), and \(E_{CI}\) was the reversal potential of \(I_{CI}\).

Our results are in close agreement with Boistel and Fatt (1958) since they indicate either little \(Cl^-\) redistribution or an extremely slow effect following \([K^+]_o\), changes.

Therefore, we can conclude that the actions of raised \([K^+]_o\), were not due to redistribution of \(Cl^-\), and that \(I_{AB}\) was selectively carried by \(K^+\).

**Effects of monovalent cations Cs\(^+\) and Rb\(^+\)**

It is strongly established that low extracellular \(Cs^+\) concentrations block both the inward-rectifying (Hagiwara et al., 1976; Gay and Stanfield, 1977) and the hyperpolarization-activated currents (DiFrancesco and Ojeda, 1980; Halliwell and Adams, 1982; Mayer and Westbrook, 1983; Spain et al., 1987; Takahashi, 1990). However, \(I_{AB}\) was unaffected by different \(Cs^+\) con-
centrations (up to 50 mM). On the other hand, Rb+ interacts with K+-permeable channels in several tissues including both types of cationic inward rectifiers (Hagiwara and Takahashi, 1974; Standen and Stanfield, 1980; DiFrancesco, 1982), which are blocked by low concentrations of Rb+ (under 10 mM). Nevertheless, the extracellular addition of 10 mM Rb+ did not change $I_{ab}$ (not shown).

Effects of divalent cations

Ba$^{2+}$, Mg$^{2+}$, and Mn$^{2+}$. Low concentrations of Ba$^{2+}$ are known to block the inward-rectifying currents (10–100 μM; Hagiwara et al., 1978) but not the hyperpolarization-activated currents (Yanagihara and Irisawa, 1980; Halliwell and Adams, 1982; Mayer and Westbrook, 1983; Takahashi, 1990). When extracellular CaCl$_2$ was equimolarly replaced by BaCl$_2$, $I_{ab}$ was not modified. $I_{ab}$ was also unchanged when CaCl$_2$ was replaced either by MgCl$_2$ or MnCl$_2$, while the depolarization-activated inward Ca$^{2+}$ current was totally suppressed in those conditions.

Action of Cd$^{2+}$ and Zn$^{2+}$. The effects of 5 mM extracellular Cd$^{2+}$ are shown in Figure 9A. Low extracellular Cd$^{2+}$ concentrations (<5 mM) strongly and reversibly reduced both $I_{ab}$ and the instantaneous linear current (Fig. 9, B and C, respectively), while the holding current $I_{ho}$ was not significantly affected. Similar results were obtained with millimolar extracellular Zn$^{2+}$ concentrations (Fig. 10). The effects of Cd$^{2+}$ and Zn$^{2+}$ on $I_{ab}$ were dose dependent, both with an IC$_{50}$ of about 300 μM (A. Araque, D. Cattaert, and W. Buño, unpublished observations). It is noteworthy that the anomalous rectification observed with hyperpolarizing pulses in current-clamp conditions was also suppressed by Cd$^{2+}$ and Zn$^{2+}$ (not shown).

Discussion

The above-described experiments indicate that a hyperpolarization-activated time-dependent inward K$^+$ current $I_{ab}$ operates in opener muscle fibers of crayfish. This current underlies the inward (i.e., anomalous) rectification existing in current-clamp conditions (Fig. 1A). Although the effects of $I_{ab}$ activation were similar to those of other inward-rectifying and hyperpolarization-activated currents, $I_{ab}$ displayed many electrophysiological and pharmacological characteristics that distinguished it from all others previously described.

There are two main varieties of currents accounting for inward rectification in neurons, muscle fibers, and oocytes (see introductory remarks): first, a K$^+$ current that has a characteristic

Figure 8. Effects of increased [K$^+$], on $E_{rev}$ the $I_{ab}$ reversal potential, and $E_{cl}$ in Ca$^{2+}$-free solution. A and B. Tail currents of $I_{ab}$ (left) and $I_k$ (right) evoked in 5.4 and 10.8 mM [K$^+$], respectively. C and D. Tail amplitudes of $I_{ab}$ and $I_k$ (measured at arrows in A and B), respectively, versus $V_h$. I-V relationships of the difference between $P_{rev}$ with and without GABA. Circles and triangles, 5.4 and 10.8 mM [K$^+$], respectively, fitted to first order regressions ($r > 0.99$) (solid lines).

Figure 9. Effects of extracellular Cd$^{2+}$. A. Total inward currents evoked by protocol 1, from $H = -60$ to $V = -130$ mV, in -10 mV increments, in normal solution (control), 5 mM Cd$^{2+}$, and recovery after washout. B, I-V relationships of $P_{rev}$ (open symbols) and $I_{ab}$ (solid symbols) in normal (circles) and 5 mM Cd$^{2+}$ (triangles) solutions. C, I-V relations of $P_{ch}$ in normal (circles) and 5 mM Cd$^{2+}$ (triangles) solutions, fitted by first-order regressions ($r > 0.99$).
Figure 10. Effects of extracellular Zn$^{2+}$. A–C are as in Figure 9, but with 3 mM Zn$^{2+}$. B, I–V relationships of $I_{\text{in}}$ (open symbols) and $I_{\text{leak}}$ (solid symbols) in normal (circles) and 3 mM Zn$^{2+}$ (triangles) solutions. C, I–V relations of $P_{\text{in}}$ in normal (circles) and 3 mM Zn$^{2+}$ (triangles) solutions, fitted by first-order regressions ($r > 0.99$).

instantaneous voltage-dependent component followed by a rapid voltage- and time-dependent element—its conductance is a function of $[K^+]_o$, and has an activation curve that is contingent on the $V_m - E_k$ difference (e.g., Hagiwara and Takahashi, 1974; Hagiwara and Yoshii, 1979; Leech and Stanfield, 1981); and second, a slower time-dependent hyperpolarization-activated current termed $I_{\text{H}}$, $I_{\text{A}}$, or $I_{\text{AB}}$, carried by Na$^+$ and K$^+$. It lacks the instantaneous voltage-dependent component, and its activation is independent of $[K^+]_o$ (Brown and DiFrancesco, 1980; Yanagihara and Irisawa, 1980; Halliwell and Adams, 1982; Edman et al., 1987).

Two different mechanisms have been proposed to explain both current types. The hyperpolarization-activated currents are pictured as due to the intrinsic voltage-sensitive gating mechanisms of channels that when open are ohmic. On the other hand, the instantaneous voltage-dependent component of the inward-rectifying current is represented as generated by the rectifying quality of the open channels themselves (Sakmann and Trube, 1984), without the participation of intrinsic gating mechanisms. Evidence exists indicating that intracellular Mg$^{2+}$, at physiological concentrations, acts as the voltage-dependent blocker that grants instantaneous rectification to the otherwise ohmic open channels (Matsuda et al., 1987; Matsuda, 1991). However, the time-dependent component of the inward rectifier can also be explained by a gating mechanism (Hagiwara et al., 1976; Hagiwara and Yoshii, 1979; Leech and Stanfield, 1981). If such a gating mechanism also accounts for the instantaneous current component is still questionable (Ishihara et al., 1989; Silver and DeCourcey, 1990; Matsuda, 1991; Mitra and Morad, 1991).

In crayfish opener muscle fibers, the instantaneous current was voltage-independent and displayed a linear $I$–$V$ relationship; hence it could be the leak current $I_L$. Therefore, opener muscle fibers lack the instantaneous voltage-dependent component that is characteristic of inward-rectifying currents (see the references above). The activation of $I_{\text{leak}}$ was time dependent, suggesting that rectification was due to the channel's kinetic characteristics. At a fixed degree of activation $I_{\text{leak}}$ itself obeyed Ohm's law; therefore, rectification was not due to the attributes of the ohmic open channel. The time dependence of $I_{\text{leak}}$ also implies that a gating mechanism is responsible for its activation.

Since the activation and the deactivation kinetics of $I_{\text{AB}}$ were the same, the simplest representation to explain the above described phenomena—if an ohmic open channel with an intrinsic gating mechanism is assumed—is a two-state model like the one proposed by Chesnoy-Marchais (1983) to explain inward rectification carried by Cl$^-$ in *Aplysia* neurons. Interestingly, this seems to be the only electrophysiological similarity between $I_{\text{AB}}$ and the hyperpolarization-activated Cl$^-$ current described by Chesnoy-Marchais (1983). Indeed, this current is carried by Cl$^-$, its activation curve depends on [Cl$^-$], and its time course is relatively slow (with time constant in the second range). Contrastingly, $I_{\text{AB}}$ is carried by K$^+$, its activation curve depends on $[K^+]_o$, and does not depend on [Cl$^-$], (not shown), and its time course is faster (with a time constant at least two orders of magnitude lower).

Approximating the inward-rectifying K$^+$ currents (Hagiwara and Takahashi, 1974; Hagiwara and Yoshii, 1979; Leech and Stanfield, 1981), $I_{\text{AB}}$ was specifically mediated by K$^+$, and the voltage dependence of its activation curve was a function of the $V_m - E_k$ difference. On the other hand, the conductance of the inward rectifier of marine oocytes is proportional to the square root of $[K^+]_o$ (Hagiwara and Yoshii, 1979). However, the conductance underlying $I_{\text{AB}}$ was independent of $[K^+]_o$. Contrastingly, the leak conductance increased 1.4 ± 0.1 times in doubled $[K^+]_o$. Although the conductance of other hyperpolarization-activated currents (e.g., Mayer and Westbrook, 1983; Spain et al., 1987) increased in raised [K$^+$], the hyperpolarization-activated current in rat spinal motoneurons did not display a similar behavior (Takahashi, 1990).

Although the electrophysiological characteristics of $I_{\text{leak}}$ partially overlap with those of the other types of cationic inward rectification (see Discussion above), the prominent dissimilarity was the pharmacological sensitivity to different extracellular ions. Indeed, while the inward-rectifying currents are blocked by low concentrations of Ba$^{2+}$ (0.01–5 mM, Standen and Standfield, 1978; 10–100 $\mu$M, Hagiwara et al., 1978; 500 $\mu$M, Constanti and Galvan, 1983), $I_{\text{leak}}$ was not altered in the presence of 13.5 mM Ba$^{2+}$. On the other hand, low concentrations of extracellular Cs$^+$ are known to block both the inward-rectifying (0.5–1 mM, Hagiwara et al., 1976; 2.5 mM, Gay and Stanfield, 1977) and the hyperpolarization-activated currents (20 mM, DiFrancesco...
and Ojeda, 1980, 0.5-3 mm. Halliwell and Adams, 1982; 1-10 mm. Mayer and Westbrook, 1983; 2 mm, Takahashi, 1990; however, concentrations of Cs⁺ up to 50 mm did not modify \( I_{AB} \). Furthermore, \( I_{AB} \) was not modified by extracellular addition of 10 mm Rb⁺, although this ion blocks the other cationic inward rectifications at concentrations under 10 mm (Hagiwara and Takahashi, 1974; Standen and Stanfield, 1980; DiFrancesco, 1982). Contrarily, \( I_{AB} \) was blocked by the application of low concentrations of extracellular Cd²⁺ and Zn²⁺.

In conclusion, our results provide evidence of the existence of a new type of voltage- and time-dependent inward rectification, selectively mediated by K⁺, whose activation curve depends on \( V_m - E_K \) (assuming a fixed \( [K^+]_o \)) but whose conductance is unaffected by \( [K^+]_o \). It is blocked by low concentrations of Cd²⁺ and Zn²⁺ but is insensitive to other ions that are potent blockers of other inward rectifications; that is, Cs⁺, Ba²⁺, and Rb⁺.

Although we can only speculate about the functional meaning of \( I_{AB} \), the following possibilities are likely besides the K⁺ balance function described above. \( E_{K_p} \) is close to the RP; therefore, \( I_{AB} \) could stabilize the membrane potential preventing large hyperpolarizations. This function may be important in those systems where hyperpolarizations due to postsynaptic inhibition are present (Takeuchi and Takeuchi, 1965, 1967). It is conceivable that owing to its voltage sensitivity and kinetics, \( I_{AB} \) could also modulate synaptic efficacy. The synaptic transmitters or modulators operative in this system could control its excitatory behavior and the synaptic integration at the postsynaptic membrane through the activation or suppression of \( I_{AB} \). Interestingly, we have found that \( I_{AB} \) was markedly increased by the crustacean neuromodulator octopamine (A. Araque and W. Buño, unpublished observations).

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