Serotonin increases an anomalously rectifying K⁺ current in the Aplysia neuron R15

(ABSTRACT/Communicated/Proc. Natl. Acad. Sci. USA) Aplysia neuron

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Communicated by S. Hagiwara, February 28, 1983

ABSTRACT Previous work has shown that serotonin causes an increase in K⁺ conductance in the identified Aplysia neuron R15. This response is mediated by cAMP-dependent protein phosphorylation. The results presented here show that the K⁺ channel modulated by serotonin is an anomalous or inward rectifier (designated Iₐ) that is present in R15 together with the three other distinct K⁺ channels previously described for this cell. Several lines of evidence indicate that this inward rectifier is partially activated in the resting cell and is further activated by serotonin. Voltage clamp analysis of resting and serotonin-evoked membrane currents at various external K⁺ concentrations shows that both currents have reversal potentials close to the potassium equilibrium potential, exhibit similar dependences in magnitude on external K⁺ concentration, and display marked anomalous rectification. The effects of particular monovalent and divalent cations are also similar on the resting and serotonin-evoked currents. Ba⁺⁺, Cs⁺, and Nal⁺ block both currents while Th⁺⁺ can substitute for K⁺ as a charge carrier and channel activator in both. These properties are characteristic of anomalous rectifiers in other systems. Furthermore, measurement of the voltage dependence of inactivation for the fast transient K⁺ current shows that this current cannot account for the anomalously rectifying K⁺ conductance in R15. The inward rectifier is, therefore, a separate current mediated by its own channels, the activity of which can be modulated by serotonin.

In 1949, Katz described a markedly nonlinear current–voltage relationship for the membranes of frog muscle fibers bathed in K⁺ medium (1). This ability to pass large inward but only small outward K⁺ currents has been called anomalous or inward rectification (2). Since then, anomalously rectifying K⁺ currents have been reported for a variety of muscle fibers (2–7) and for the egg cell membranes of tunicates (8) and starfish (9). However, in neuronal systems, anomalous rectification has been less precisely defined as an increase in membrane resistance with depolarization (10–13) and, in many cases, this resistance change is known to be due to the activation of an inward current carried by Ca⁺⁺, Na⁺, or both (14, 15). It has also been hypothesized that activation of the fast transient K⁺ current (Iₐ) found in molluscan (16–18) and crustacean neurons may contribute to anomalous rectification (19–22). In this paper we describe the modulation by a neurotransmitter of an anomalously rectifying K⁺ current (Iₐ) in a nerve cell. We show that, in Aplysia neuron R15, the Iₐ is increased by serotonin and that it is conducted by channels distinct from those mediating Iₐ.

MATERIALS AND METHODS

Aplysia californica were obtained and maintained as described (23). Standard Aplysia saline contained 460 mM NaCl, 55 mM MgCl₂, 11 mM CaCl₂, 10 mM KCl, 10 mM Hepes, pH 7.4. Neuron R15 is located in the abdominal ganglion of Aplysia and can be identified easily from one animal to another. The cell was voltage clamped conventionally with a one- or two-electrode system and held at −80 mV for the duration of the experiment. Membrane current at other membrane potentials was monitored by applying command pulses ranging in duration from 0.5 to 10 sec, shifting the membrane potential to values between −50 mV and −160 mV. There was no significant time-dependent current change in this membrane potential range. The serotonin response reaches its maximum after 15–25 min of perfusion, and there appears to be no attenuation of the responses during up to 120 min of perfusion with serotonin.

RESULTS

We have shown previously that serotonin causes a cAMP-dependent increase in the K⁺ current in neuron R15 (23). To investigate the properties of the K⁺ channel mediating this response, the neuron was voltage clamped and the membrane current was recorded over a membrane potential (Eₑ) range from −50 mV to −160 mV. Fig. 1 illustrates the effects of changing the external potassium concentration, Kₑ, on the "steady-state" resting and serotonin-evoked currents measured at the end of 0.75-sec pulses to various Eₑ values from a holding potential of −80 mV. The pairs of current vs. voltage curves intersect at the reversal potential (Eₒ) of the serotonin-evoked current. The Eₒ in each case occurs at the potassium equilibrium potential (E_K) calculated for each Kₑ from the Nernst equation. This close correspondence shows that the serotonin-evoked current is highly selective for K⁺, even when Kₑ is low. The evoked current depends in direction and magnitude on Eₑ − E_K rather than on the Eₑ alone. This current is thus an anomalous rectifier since the inward current is large and the outward current is small. At Eₑ values progressively more positive than the E_K, the evoked current decreases to zero, while in the negative direction conductance, measured by the slope of the current vs. voltage curve, increases and reaches a constant level about 10 mV negative to the E_K. Furthermore, as with other anomalous rectifiers (9, 24), this constant conductance level depends on the Kₒ. Conductance decreases with decreasing Kₒ and, at Kₒ = 1 mM, no serotonin-evoked current can be measured. The application of a broad range of Kₒ values makes clear the distinction between an anomalously rectifying K⁺ current and anomalous rectification arising from activation of an inward current, a distinction that has not been made clear in earlier descriptions of membrane conductance at hyperpolarized potentials in molluscan neurons (25–27).

As shown in Fig. 1, the resting conductance also decreases as the Kₒ decreases, suggesting that it is composed in part of...
a current that has properties similar to those of the evoked current. This is confirmed by the application of ions known to block anomalous rectifiers in other systems. Ba\(^{2+}\) blocks several types of K\(^+\) channel, including the anomalous rectifier (28, 29), and Rb\(^+\) and Cs\(^+\), although they can pass through less-selective K\(^+\) channels (30), enter and block anomalously rectifying K\(^+\) channels (27, 31, 32). The effects of 10 mM Ba\(^{2+}\), 10 mM Cs\(^+\), and 5 mM Rb\(^+\) on the combined evoked and resting currents are shown in Fig. 2 A–C. Both the serotonin-evoked and the resting currents are substantially reduced. If the blocking ions are applied before serotonin, the resting current is greatly reduced and there is no serotonin response. Both the blocked component of the resting current and the evoked current reverse at the E\(_K\). The component of the resting current blocked by Cs\(^+\), Rb\(^+\), and Ba\(^{2+}\) is also an anomalous rectifier, since a relatively small outward current has been suppressed but the ions have blocked a large inward current. The blocked inward resting current is proportional to the K\(_r\) in a manner similar to the evoked current.

Unlike Cs\(^+\) or Rb\(^+\), Tl\(^+\) can pass through even highly selective K\(^+\) channels (33, 34). K\(^+\) is thought to play a part in the activation of anomalous rectifier channels in addition to its role as charge carrier (6, 34), and Tl\(^+\) can substitute for K\(^+\) to activate the gating mechanism of the anomalously rectifying K\(^+\) channels in frog sartorius muscle (35) and starfish egg (34). In R15, the resting current observed when K\(^+\) is replaced by Tl\(^+\) is similar to that in controls and, in Tl\(^+\) medium, serotonin evokes a current that must be both activated and conducted by Tl\(^+\) (Fig. 2D). The serotonin-evoked current is inwardly rectifying but not so much as in K\(^+\) medium. The E\(_K\) is approximately −80 mV, not significantly different from the E\(_K\) in normal (10 mM) K\(^+\).

Fig. 1. Steady-state current vs. voltage relationship showing effects of change in K\(_r\) on resting (●) and serotonin-evoked (○) K\(^+\) currents. All current values in this and subsequent figures are negative. K\(^+\) concentrations were 2 mM (A), 4.6 mM (B), 10 mM (C), and 20 mM (D). Each pair of current vs. voltage curves is from a different cell, but the curves in control conditions (10 mM K\(_r\)) were the same for all four cells. The serotonin concentration was 1 μM and the cells were exposed to bath-applied serotonin for 20 min, after which there was no further increase in evoked current. The steady-state current was defined as the membrane current measured at the end of a 0.75-sec clamp command step from a holding potential of −80 mV. Arrows indicate theoretical E\(_K\) values obtained from the Nernst equation assuming K\(_r\) = 290 mM and a 58-mV change in membrane potential per decade change in K\(_r\) (22). The outward K\(^+\) current was sometimes obscured due to the instability of the Na\(^+\)/Ca\(^{2+}\) current evoked by steps to membrane potentials near the spike threshold (−35 mV).

DISCUSSION

Previous work has shown that the serotonin response in R15 is mediated by cAMP (23) and that cAMP-dependent protein phosphorylation is a necessary step in the process leading to the increase in K\(^+\) conductance (36). Furthermore, application of serotonin to R15 results in changes in the phosphorylation of specific proteins within this cell (37). Protein phosphorylation also alters the electrical activity of several other molluscan neurons (38–40), apparently by affecting specific K\(^+\) channels. The observations reported here show that the K\(^+\) channel regulated by phosphorylation in R15 is an anomalous rectifier that is already partially activated in the absence of exogenous serotonin.

By decreasing the K\(_r\) and thus shifting the E\(_K\) to hyperpolarized potentials, we have been able to separate the I\(_A\) from a "classical" anomalous rectifier, from the "anomalous rectification" arising from the voltage- and time-dependent activation of an inward current probably carried by Ca\(^{2+}\) (41). Furthermore, the I\(_A\) can be measured in the presence of Cd\(^{2+}\), which blocks inward Ca\(^{2+}\) currents. We also have shown that the I\(_A\) is distinct from the I\(_K\). Our data show that the I\(_A\) [as well as the delayed K\(^+\) current (I\(_K\)) and the Ca\(^{2+}\)-activated K\(^+\) current (I\(_{Ca}\); unpublished data] is not affected by serotonin in R15 and that only the channels conducting the I\(_A\) are influenced by sero-
Fig. 2. Steady-state current vs. voltage relationships showing the blocking actions of Ba\(^{2+}\) (A), Cs\(^+\) (B), and Rb\(^+\) (C), together with the resting and serotonin-evoked currents in Tl\(^+\) (0 K\(^+\)) medium (D). Each set of curves is from a different cell. The steady-state currents were measured as for Fig. 1, and arrows indicate the theoretical \(E_K\). The serotonin concentration was 1 \(\mu M\), and it was applied for 20 min, after which the blocking ions were added in the presence of 10 nM (normal) K\(^+\). The steady-state current was measured after 5 min of perfusion, when no further decrease in current could be detected. Concentrations: Ba\(^{2+}\), 10 mM; Cs\(^+\), 10 mM; Rb\(^+\), 5 mM; Tl\(^+\), 10 mM. In D no K\(^+\) was present, all Cl\(^-\) ions were replaced by NO\(_3\)\(^-\), and clamp electrodes contained 3 M KNO\(_3\). A similar electrode was used as a bath ground. (A–C) \(\bullet\), Resting current; \(\circ\), serotonin-evoked current; \(\triangle\), current in the presence of the blocking ion. (D) \(\bullet\), Resting current; \(\circ\), serotonin-evoked current.

In muscle fibers, there are two possible precedents for neurotransmitter modulation of an anomalously rectifying K\(^+\) current. In frog atrial muscle and in sheep cardiac Purkinje fibers, the time-independent background K\(^+\) current, \(i_{\text{K}}\), which has an anomalously rectifying component (42), appears to be modulated by acetylcholine (43, 44).

Ohmori et al. (45) have shown that anomalously rectifying K\(^+\) channels in myotubes individually increase in conductance when exposed to higher K\(^+\). In view of this finding, it is of interest to ask whether serotonin might not act similarly to increased K\(^+\) by increasing the single channel conductance of the \(i_{\text{K}}\) channels. Noise and patch clamp analyses are approaches that may answer this question.

This work was supported in part by Grant NS17910 to I.B.L. from the National Institute of Neurological and Communicative Disorders and Stroke.