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The Inwardly Rectifying Potassium Current of Embryonic Chick Hepatocytes

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Abstract. The single channel and whole-cell properties of an inward, rectifying potassium current in cultured embryonic chick hepatocytes were studied at 20°C. In cell-attached patches, channels open upon membrane hyperpolarization and are present in about 90% of cell-attached patches. With 145 mM potassium in the pipette, inward current has a slope conductance of 80 pS. The conductance is not a linear function of the external potassium concentration. Current saturates at high external potassium and has a Michaelis-Menten affinity constant of 275 mM potassium. Substitution of gluconate for chloride in the external solution has no significant effect on conductance, and the reversal potential shifts approximately 18 mV with a change in external potassium from 72.5 to 145 mM indicating potassium selectivity. Channel openings are characterized by multiple brief closures during a burst. The channel is inhibited by external cesium in a concentration-dependent manner. Block is characterized by an increased frequency of transient closures. Whole-cell dialysis with 145 mM CsCl of cells bathed in 145 mM KCl reveals time-independent inward currents that reverse at 0 mV in response to 200 msec-voltage steps. Although voltage ramps evoke currents that are 75% potassium dependent and cesium sensitive, the mean chord conductance (425 pS) indicates that less than five channels are open at any instant. We suggest that the inwardly rectifying potassium channel is partially inactivated in the dialysed hepatocyte.

Key words: Whole cell — Single channel — Cesium — Liver — Resting potential

Introduction

Resting cultured embryonic [14, 15] or neonate [23] chick hepatocytes have two currents, as judged by their time dependence, when measured at the whole-cell level. These are a slowly inactivating delayed rectifier (I_K), and a time-independent current (I_{TI}). A K^+-selective low conductance (7 pS) event that occurs at positive clamp potentials is thought to underlie I_K and be the sole single-channel type extant in these cells [23]. However, the resting membrane potential lies at least 40 mV positive to E_K and the time-independent current reverses between −15 and −25 mV with physiological gradients of K^+ and Na^+ and equimolar Cl^- [15], indicating that additional electrogenic pathway(s) are present.

Multiple types of K^+ channels have been reported in hepatocytes from different species in addition to the delayed rectifier described in embryonic and neonate chick hepatocytes. Two different groups have studied K^+ channels in the rat hepatocyte, reporting a 90 pS Ca^{2+}-activated channel [2], and a 44 pS inward rectifier [12]. A 20 pS Ca^{2+}-activated K^+ channel has been demonstrated in guinea pig hepatocytes [3]. Heterogeneity in function, known or hypothesized, is also apparent between species. The guinea pig whole-cell current is activated as a consequence of glycogenolytic hormone-stimulated increases in cytosolic Ca^{2+} [4] and the Ca^{2+}-sensitive channel in the rat may be involved in volume regulation [2]. No physiological function has been identified for the rat hepatocyte inward rectifier [12]. We have indirect evidence from whole-organ flux experiments in rats that K^+ conductance is increased following α-adrenoceptor activation, and that this increase is required for sustained glycogenolytic responses [13]. This conductance does not appear to be sensitive to cytosolic Ca^{2+}, so it may be carried, at least partially, by the inward rectifier already characterized by Henderson et al. [12].

The aim of the present report was to undertake a study of the single channel events in embryonic chick hepatocytes and to correlate these with the whole-cell currents. Here we report the identification and characterization of an inward rectifying K^+ channel in cell-

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attached patches. In contrast to the latter configuration, whole-cell dialysis suggests that a much smaller number of channels are open. The potential regulation by factors extrinsic to the channel protein is discussed.

Materials and Methods

Hepatocytes from the livers of 10-day old embryos of white Leghorn chickens (*Gallus gallus*) were isolated and cultured on glass coverslips as described previously [15]. Briefly, one liver was coarsely minced, followed by incubation in Hepes-buffered physiological saline containing 0.25% trypsin for a total of 31 min. The cells were filtered, collected and resuspended in HCO_3-buffered saline containing 4% (v/v) fetal bovine and 2% (v/v) horse sera, and 20% (v/v) M199 (GIBCO, Grand Island, NY). The cells were cultured on glass coverslips for 3–36 hr at 37°C in a humid atmosphere of 5% CO_2 and 95% air. More than 90% of the attached cells are hepatocytes based on their hexagonal morphology and only these cells were used for the experiments.

The patch clamp recording technique [11] was used to observe membrane current under voltage clamp using the cell-attached patch or the whole cell configuration. Patch pipettes (2–5 MΩ resistance) were made from thin-walled (Kimax-51) borosilicate glass and were heat polished before use. For cell-attached patches the pipette solution (PS) was, in mmoles/liter; 145 KCl, 10 HEPES, 1 $MgSO_4$, 1 EGTA, 0.64 $CaCl_2$, pH 7.4. KCl was replaced with 145 mM K-gluconate in some experiments. For whole cell recording, the pipette solution was, in mmoles/liter; 145 CsCl, 10 HEPES, 1 $MgSO_4$, 1 EGTA, 0.64 $CaCl_2$, pH 7.4. The external solution (ES) contained, in mmoles/liter; 145 KCl, 1 $MgSO_4$, 1 $CaCl_2$, 10 HEPES, 5 glucose, pH 7.4. With this external solution, and following replacement of the CsCl in the whole-cell dialysate with 115 KCl and 30 NaCl, the membrane potential under current clamp was +3 ± 2 mV. We therefore assumed that the membrane potential across the patch in the cell-attached experiments was the inverse of the pipette potential. In some experiments, the KCl content of the external medium or the cell-attached pipette solution was decreased with an equimolar increase in NaCl.

Membrane currents were measured with an Axopatch-1C (Axon Instr, Foster City) amplifier in conjunction with pClamp 5.5 software (Axon Instruments), a Tecmar Labmaster TM40 A-D/D-A board and an Axon Instruments AL2000 event detector. In whole-cell experiments, membrane voltage was controlled by pClamp protocols. Whole-cell current records were filtered (low pass, 3 db cutoff at 0.5–2.5 kHz, Frequency Devices 902LPF) and digitized during offline analysis at 2.5 kHz. Single channel currents were low pass filtered (4 kHz) and sampled at 20 kHz prior to amplitude analysis using pClamp software. Whole cell currents were normalized to cell capacitance and data from identical experimental conditions were pooled. Current-voltage and other plots are derived from mean ± SEM of n separate cells and representative data traces are shown where appropriate.

Results

Single Channel Currents

Inward unitary currents are observed in the cell-attached patch configuration, with 145 KCl and 1 μM free Ca^{2+} in the pipette, when the membrane potential of cultured embryonic hepatocytes is held at values negative to –20 mV (Fig. 1*A*). At positive potentials, a much smaller

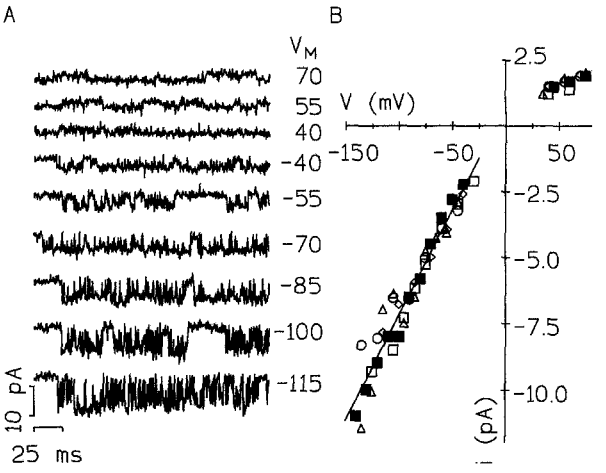

Fig. 1. Single channel currents in a cell-attached patch held at various membrane potentials (*A*) and the resulting current-voltage plot derived from five different cell-attached patches (*B*). Cells were bathed in 145 mM KCl ES, and the pipettes contained 145 mM KCl PS. Channel openings are represented by the downward deflections shown in (*A*). The inward currents were fit to a linear function and the line was extrapolated through the outward currents manually (*B*).

current was observed which we assume reflects outward current through the same channel since low conductance inward currents were not observed in the same patches. Channel activity identical to that illustrated was observed in 152 of the 170 patches surveyed. Currents measured in five different cells at potentials from +70 to –145 mV are shown in Fig. 1*B*. The mean slope conductance at potentials negative to –50 is 80 ± 3 pS, and the mean unit conductance at +55 mV is 25 ± 1 pS, demonstrating the inwardly rectifying properties of this channel. When 1 mM Ca^{2+} was included in the pipette the channel appeared to be blocked since each of 42 patches tested under these conditions showed no inward current. Excision of a patch to the inside-out configuration resulted in disappearance of the single channel events within 20 sec. For this reason all of the results described here are from cell-attached patches.

The channel was determined to be K^+-selective from the following observations. First, changing the pipette K^+ concentration to 72.5, 115, 217.5 or 300 mM causes positive shifts (relative to 72.5 mM) in the current-voltage plot (Fig. 2*A*). The data from the 145 and 300 mM K^+-containing pipettes are not shown due to their overlap with the conditions illustrated. The lines in Fig. 2*A* are extrapolated by eye through E_K assuming an intracellular K^+ concentration of 115 mM. The data obtained from pipettes containing 72.5 mM K^+ are fit by a straight line. The difference between the zero current potential with 72.5 and 145 mM K^+ containing solutions is 18 mV. Second, Fig. 2*B* shows that the single channel currents observed in the presence of K-gluconate rather than KCl are not significantly different, showing that this channel

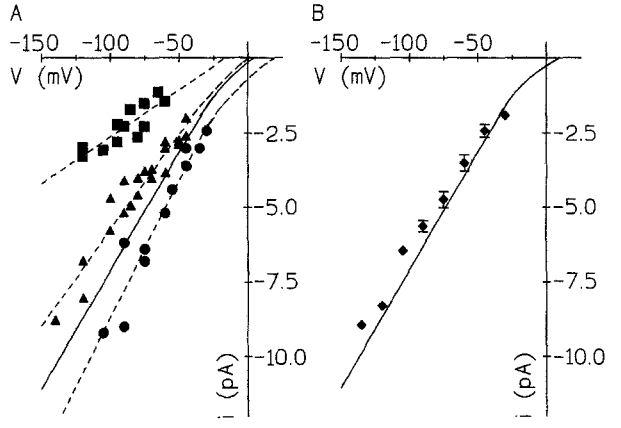

Fig. 2. K^+-selectivity of the inward current. Current-voltage plots showing the effects of PS prepared with different KCl concentrations (*A*), or with 145 mM K-gluconate (*B*). The continuous line is from Fig. 1*B* and the linear portions of the broken lines are from fits to the inward current data. Pipette solutions were 217 (●), 115 (▲) or 72.5 (■) mM KCl PS and the external solution was 145 KCl ES throughout. Data are from two (*B*) or three (*A*) cells for each condition.

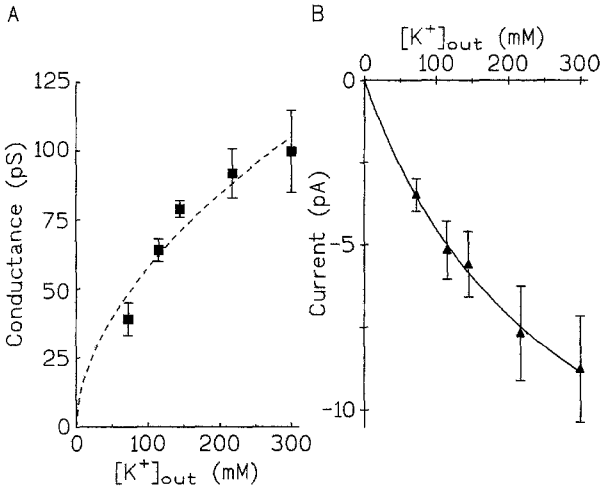

Fig. 3. Nonlinear dependence of slope conductance and current on external K^+ concentration. Mean conductance (*A*) or current amplitude at −100 mV (*B*) was determined from three patches for each PS containing between 72.5 and 300 mM KCl PS. The external solution was 145 mM KCl ES throughout. The data were fitted to the Michaelis-Menten function.

does not conduct Cl^- (Fig. 2*B*). Third, no inward currents were observed in six different patches when all of the KCl was replaced with NaCl (*not shown*).

The slope conductances of the lines shown in Fig. 2*A* are plotted as functions of the pipette K^+ concentration in Fig. 3*A*. The data are not linearly related to the external K^+ concentration but are well fitted to a rectangular hyperbola, or Michaelis-Menten function, indicating a tendency toward saturation of the channel conductance at high external K^+ concentrations. The data were not well fitted to a square root function if K^+ concentrations above 145 mM were included in the analysis (*not shown*). Single channel current also shows a nonlinear relationship with the external K^+ concentration, indicative of binding sites for K^+ in the channel. Fig. 3*B* shows current at −100 mV measured at five different K^+ concentrations. The data have been fit to a Michaelis-Menten function,

$$i = \frac{i_{max}}{1 + (K_m/[K^+]_{out})} \quad (1)$$

where i_{max} is −17 pA and the K_m is 275 mM K^+.

The bursting behavior of the channel (*see* Fig. 1) indicates that there exist a minimum of two closed states in addition to the open state. The mean intraburst closed times could not be resolved by our recording system (sampling frequency 41 kHz, filtering 8 kHz) but from inspection of the recordings these are less than 100 μsec. Consequently we did not undertake an open time analysis since the resulting time constant would have been underestimated.

Inward rectifying K^+ channels are sensitive to external blocking ions such as Ba^{2+} and Cs^+ [8, 19, 22, 25, 27]. Like these earlier reports, Cs^+ inhibition of the hepatocyte channel is both concentration dependent and characterized by an increase in the number of closing transitions per burst (Fig. 4*A*). Although the mean current amplitudes at −75 and −90 mV are lower with 0.5 mM Cs^+ compared with the control current, these decreased currents likely result from the inability to resolve very short transitions between the closed (blocked) and open states (Fig. 4*B*). Because of this limitation we did not attempt a dwell time analysis.

Whole Cell Currents

Whole cell currents were evoked from cells dialyzed with Cs^+ as the major cation in order to maximize the voltage range over which the inward rectifier could be observed, and to inhibit outward currents through the delayed rectifying K^+ channels. The time dependence of the inward current was investigated by applying a series of voltage steps to the cells for a duration of 200 msec each. The membrane potential was held at 0 mV both prior to and between each step (for 30 sec) in order to inactivate any time-dependent current due to delayed rectifier (I_K) activity. One complete series was run 30 sec, 8.5 min and where possible 20 min following the onset of intracellular dialysis. The resulting currents were identical, indicating that current run down, if present, occurs within 30 sec of attaining the whole cell configuration. Fig. 5*A* shows a representative series beginning at 8.5 min. Each trace has been offset 40 pA from the others so that none of the records overlap each

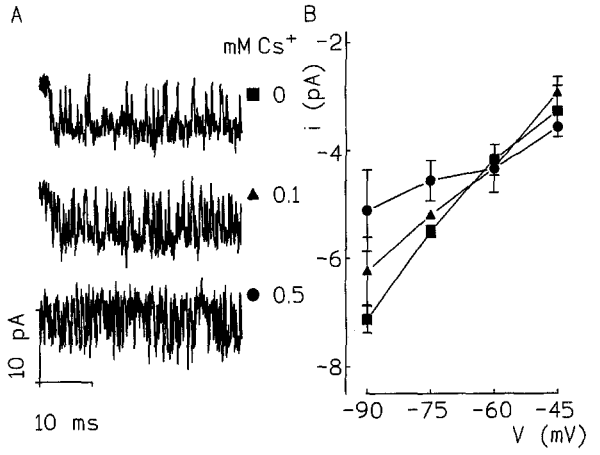

Fig. 4. Effect of external Cs^+ on the current-voltage relationship. The left hand panel (*A*) shows records of inward current at −75 mV in the absence (upper trace) or presence of 0.1 (middle) or 0.5 (bottom) mM CsCl in 145 KCl PS and 145 KCl ES. The data are from three different cells. (*B*) Current-voltage plot shows the mean current (±SEM) obtained from 40 sec records and measured in the absence (■) or presence of 0.1 (▲) or 0.5 (●) Cs^+. Data are from three cells for each PS.

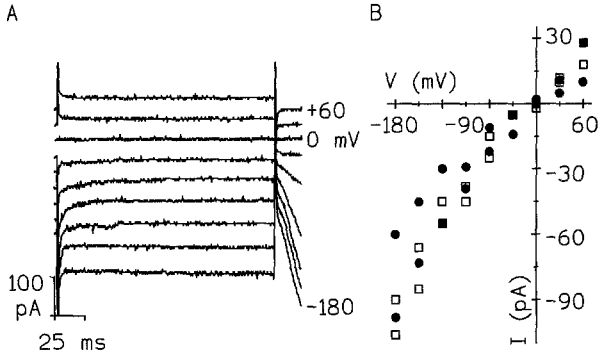

Fig. 5. Time-independence of inward currents of Cs^+-dialysed embryonic hepatocytes. Currents were evoked from consecutive 30 mV voltage steps between −180 and +60 mV, holding potential = 0 mV, interpulse duration = 30 sec, initiated 8.5 min following whole-cell attainment. Cells were dialysed with 145 CsCl PS and incubated in 145 KCl PS. (*A*) Representative records from one cell, capacitance 9.5 pF, are offset by 40 pA from each other. (*B*) Current at 175 msec of the voltage step plotted as a function of the step potential from four cells, two dialysed with 145 CsCl PS (●, 8.0 and 11.5 pF) and two with 145 KCl PS (□, 9.5 and 11 pF).

other. Following the capacitance artifact the resulting current traces do not show either time-dependent activation or inactivation. The time-dependent tail currents appearing following return to 0 mV is inward current through I_K channels that open at 0 mV following removal of inactivation at the hyperpolarizing step potentials (−60 mV and greater). We know this because E_K is infinitely positive in Cs^+-dialysed cells, and, I_K inactivates fully when the membrane potential is held at 0 mV for 20 sec and activates with an increasing rate as the membrane is held at −50 mV and successively more negative potentials [15]. Fig. 5*B* shows the relationship between current measured at the end of the voltage steps and the step potential. Cells were dialyzed with 145 mM of either CsCl or KCl in duplicate and each data point is plotted. Under both conditions, the reversal potential is close to 0 mV indicating that the time-independent current is not very K^+ selective.

Time-dependent activation of some inward rectifiers is observed only following closure of the channels at depolarizing potentials. To determine if such a mechanism is operating in the chick hepatocyte the membrane potential was held at 0, +20, +40 or +60 mV prior to applying a 200 msec step to −120 mV. The resulting currents evoked at −120 mV were identical under each condition and showed no time-dependent activities (*data not shown*). The combined results from these experiments allowed us to generate current-voltage relationships from voltage ramps. The much shorter duration of the ramp protocols also permitted us to accumulate data from a larger number of cells. The latter were used to determine the K^+-selectivity and Cs^+-sensitivity of the inward current.

Voltage ramps from −180 to +60 mV generated inward currents having a mean slope conductance of 92 pS/pF when the cells were incubated in 145 mM K^+ (Fig. 6). Replacing all of the external K^+ with Na^+, or addition of 5 mM Cs^+ to the K^+-containing incubation medium, decreased the mean conductance to 46 and 23 pS/pF respectively and shifted the zero current potential about −15 mV. These results demonstrate that a Cs^+-sensitive K^+ conductance of approximately 46 pS/pF provides about 50 percent of the total inward current under the defined experimental conditions. The mean capacitance of these cells was 9.2 ± 1.5 pF (n = 12), resulting in a mean whole cell conductance due to the Cs^+-sensitive channel of 425 pS. Therefore only five channels are open at any instant in the dialyzed cells. The small negative shift in the zero current potential is presumably due to a block of the inward current through the residual K^+-selective inward rectifier channels since Cs^+ has no significant effect on the currents generated in the presence of NaCl (*not shown*). The combined results from the whole-cell experiments indicate that, in contrast to the cell-attached configuration, dialysis results in a condition that inhibits the activity of the Cs^+-sensitive K^+ channel. The inward current appearing at −20 mV is the opening of residual delayed rectifier channels that have been activated in response to ramping the membrane voltage from negative potentials.

Discussion

The report shows that inward rectifying K^+ channels ($I_{K(IR)}$) are expressed in embryonic chick hepatocytes,

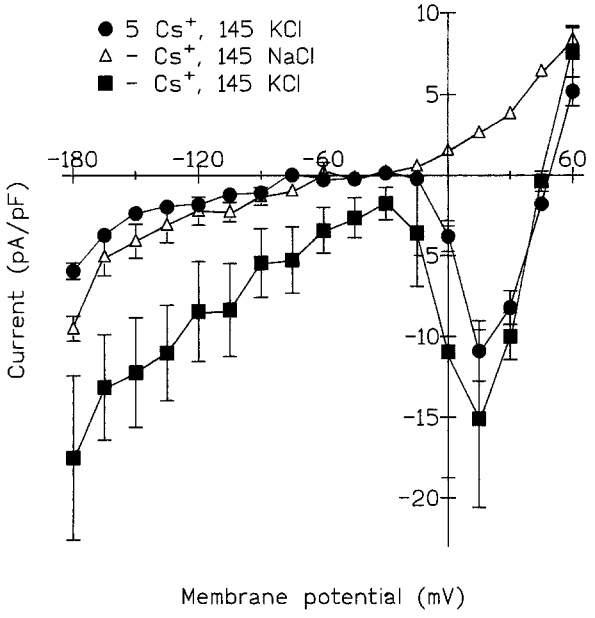

Fig. 6. K^+- and Cs^+-dependence of inward whole-cell ramp currents in Cs^+-dialysed embryonic hepatocytes. Current ramps were evoked from 2 sec voltage ramps between −180 and +60 mV, holding potential = 0 mV. Ramps were digitized at 15 mV intervals and data from four cells (±SEM) for each condition were normalized to cell capacitance (mean = 9.2 ± 1.5 pF, n = 12) pooled and plotted. Cells were dialysed with 145 CsCl PS, and incubated in PS containing 145 KCl (■), 145 NaCl (△), or 145 KCl + 5 CsCl (●).

and the single channel properties are similar to those identified in other excitable and nonexcitable cells. The channels were present in 90 percent of patches surveyed. However, whole-cell analysis indicates that only residual activity from these channels remains during dialysis. This discussion focuses on the existence, properties and functions of inward rectifiers in hepatocytes, and speculates on the mechanisms underlying the differences in activity seen between the intact and dialyzed cells.

Inward rectifying K^+ channels have been demonstrated in rat hepatocytes [12] in addition to the studies on embryonic chick cells reported here. However, other studies of these hepatocytes, or cells isolated from other species, do not report the presence of $I_{K(IR)}$, or conclude that only other types of K^+ channels exist. One difference between the studies demonstrating $I_{K(IR)}$ and those others is the concentration of Ca^{2+} in the external solutions. In our experiments, we never saw $I_{K(IR)}$ channels in cell-attached patches when 1 mM Ca^{2+} was included in the pipette solution, yet 90 percent of patches had active channels when the Ca^{2+} was buffered to 1 μM or less. A more circumstantial correlation exists in the literature between the absence of external Ca^{2+} and the appearance of inward currents through $I_{K(IR)}$ channels. For example, the study identifying $I_{K(IR)}$ in rat hepatocytes used an external solution containing 2 mM Mg^{2+}, 1 mM EGTA and no added Ca^{2+} [12] whereas the other two studies routinely included millimolar levels of Mg^{2+} and Ca^{2+} [3, 31]. Similarly, $I_{K(IR)}$ was not identified in neonate chick hepatocytes, a study in which the external solutions contained millimolar Mg^{2+} and Ca^{2+} [23]. Other nonexcitable cell types in which $I_{K(IR)}$ has been identified include human macrophages [10], endothelial cells [28] and parathyroid cells [7], Ehrlich ascites tumor cells [6], MDCK renal epithelioid cells [9], bullfrog oxyntic cells [26], and two cloned channels from mouse macrophages (IRK1) [22] and rat kidney (ROMK1) [17]. All of these studies used external solutions either with no added divalent cations, or Ca^{2+}-free solutions containing Mg^{2+} with or without EGTA. One interpretation is that the presence of physiological concentrations of Ca^{2+} at the external mouth of inward rectifying channels inhibits inward current flow. Clarification of such a mechanism in the embryonic hepatocytes is confounded by our inability to measure currents in either excised patches or dialzyed cells.

The single channel properties of the inward rectifier described here are similar, in general, to those reported from a variety of excitable and nonexcitable cell types. More specifically, inward rectification, nonlinear relationships between either inward current or channel conductance and external K^+ concentration, and open channel transient block by external Cs+ are common features of a group of proteins having similar electrophysiological and structural properties. Nevertheless, there is considerable quantitative heterogeneity within this group with respect to conductance, time dependence, and modulation by intracellular Mg^{2+} and second messengers such as ATP or G-proteins. The cloned channels represent the spectrum of properties observed in vivo. For example, the classical inward rectifier represented by IRK1 has a low conductance (23 pS), no outward current in cell-attached patches and inactivates with sustained hyperpolarization [22]. In contrast, the cloned ATP-sensitive inward rectifiers, ROMK1 [17] and rcK_{ATP} [1], have higher conductances (45 and 70 pS respectively), pass outward current even in the presence of cytosolic Mg^{2+} and ROMK1 shows very little time dependence at the whole-cell level (not reported for rcK_{ATP}). Generation of a chimeric protein using IRK1 and ROMK1 shows that conductance and Mg^{2+}-sensitivity is conferred upon these members of the inward rectifier family by the cytosolic carboxy terminus [32]. The remaining cloned member of this family is GIRK1. This channel has an intermediate conductance (43 pS) and an intermediate sensitivity to intracellular Mg^{2+}, and a distinctive slow activation [29]. It is apparent that the inward rectifier family of channels consist of a heterogeneous group with different degrees of overlap of their properties. This is reflected in the embryonic chick hepatocyte inward rectifier whose conductance (80 pS) is at the high end and which can pass outward current in the presence of cytosolic Mg^{2+}.

Nonlinear relationships between external K^+ concentration and channel conductance or current imply deviation from independent transit of the ions through the channel and pore saturation [16]. Using a theoretical description, we found that conductance can be related to $[K]^+_{out}$ by a Michaelis-Menten, or saturation binding, function. A similar relationship was used to describe conductance in lens epithelial cells [8]. The half maximal saturation concentration for K^+ in the latter cells of 53 mM is significantly lower than the 198 mM we determined for the chick hepatocyte, suggesting a difference in affinity for K^+ between the two different channels. Michaelis-Menten analysis of current-$[K^+]_{out}$ relationships result in K_M values from the embryonic hepatocyte or cardiac ventricular cells [30] that are forty- to fifty-fold greater than plasma K^+ levels, indicative of saturable binding site(s) within the channel pore.

Block by external Cs^+ of inward current through inward rectifying K^+ channels from various cell types is both voltage- and concentration-dependent [8, 20, 22, 25, 27]. At the whole cell level Cs^+ inhibits $I_{K(IR)}$ in parotid cells, lens epithelia, or cloned from macrophages or brain tissue, with an ID_{50} of approximately 100 μM [8, 19, 22, 27]. Although we did not determine the concentration-dependence of Cs^+ block of the K^+-selective whole-cell current, we found that 5 mM Cs^+ fully blocked this component and yet had little effect on inward current through the delayed rectifier. Cs^+, at submillimolar levels, blocks unitary currents in ventricular cells [25] and the cloned mouse brain channel [27] by increasing the number of transient closures during a burst. This property is augmented with hyperpolarization, and with increased concentrations of Cs^+ [25, 27]. Kinetic analysis of Cs^+ inhibition shows that both the blocking and unblocking rates are voltage dependent, whereas only the blocking rate is concentration dependent [25]. We found that Cs^+ block of the hepatocyte inward rectifier has qualitatively similar properties, since visual inspection of the records show that the open time decreases with both an increase in Cs^+ concentration and membrane potential whereas the intraburst closed time is less sensitive to voltage and Cs^+. However, in contrast to the ventricular cell [25], the embryonic hepatocyte channel has an intraburst closed time less than 0.1 msec (i.e., minimally 10–20-fold lower), and does not have subconductance levels. The meaning of the relatively short closed times estimated from the chick hepatocyte records awaits quantitative analysis of the dwell times with a higher resolution acquisition and recording system. The subconductance phenomenon could be induced in heart cells by external Cs^+ or internal Mg^{2+} [24, 25], or is present in other multiconductive state channels in cell-attached patches and has been proposed to indicate a multibarrelled structure [18]. Since our studies were performed under conditions that should induce subconductance levels (continuous exposure to internal Mg^{2+} in the absence or presence of external Cs^+) and yet did not, it is probable that the hepatocyte channels are not multibarrelled structures.

We approached the question of a role(s) for the inward rectifier in embryonic chick hepatocytes by assessing its contribution to the total membrane conductance at potentials where the delayed rectifier would be inactive. We found that a Cs^+- and K^+-dependent inward current contributes 75 percent of the total membrane conductance. From the single channel conductance (80 pS) these results suggest that only a small number of channels are open in the dialyzed cell. Similarly, whole cell patch-clamped rat hepatocytes also have a small Ba^{2+}-sensitive whole cell conductance that would be contributed to by 30 to 40 open inward rectifying channels based on their unitary conductance [12]. The authors suggested that channel "run down," which occurs in excised patches, could happen in dialyzed cells. We also found that inward rectifying events disappeared within seconds of excision to the inside-out configuration, whether in Ca^{2+}-free, Ca^{2+}-containing, or 2 mM ATP-containing solutions, indicating that internal dialysis could account for the small whole cell conductance. Since our whole cell and single channel experiments were carried out in the same K^+ solutions, it seems most probable that the rapid loss of an intracellular activator during dialysis results in block of $I_{K(IR)}$ during whole cell voltage clamp. Recent sequence data from two different cloned inward rectifiers show that numerous phosphorylation sites are present in these proteins [17, 22]. The activities of other members of the same structural family are modified by intracellular nucleotide di- and triphosphates [1, 17], or GTP-binding proteins [29]. These results suggest that either modification of the channel phosphorylation state, or loss of activated G-protein subunits or nucleotide diphosphates during dialysis or excised patch configuration could underlie channel inactivation in the embryonic chick hepatocytes. Until the disparity between the whole cell and unitary conductances seen in cell-attached patches is understood it will be difficult to identify further details of the role of this channel in the physiology of the hepatocyte.

Speculative roles for inward rectifying K^+ channels in other secretory epithelia include uptake of K^+ during stimulated secretion of parotid cells [20]. However, our results suggest that embryonic chick hepatocyte inward current through $I_{K(IR)}$ would be blocked by external Ca^{2+}. It seems more likely that outward current through this channel would serve to counter as yet undefined depolarizing influences, thereby providing a significant force in setting the resting membrane potential, as has been proposed for other epithelial cells [8].

In summary, this report shows that, in addition to the delayed rectifier already identified in embryonic chick hepatocytes, these cells have an inward rectifying K^+ channel with properties similar to those of other excitable and nonexitable cells. Further, our data suggest that

inward current through this channel is sensitive to sub-physiological concentrations of external Ca^{2+}. We suggest that this latter property, along with the apparent rapid run down of the channel during whole-cell dialysis, may explain the discrepancies in the literature concerning the identification of this channel in other hepatocyte preparations.

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