Permeation of Na\(^+\) through a Delayed Rectifier K\(^+\) Channel in Chick Dorsal Root Ganglion Neurons

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ABSTRACT In whole-cell patch clamp recordings from chick dorsal root ganglion neurons, removal of intracellular K\(^+\) resulted in the appearance of a large, voltage-dependent inward tail current (I\(_{\text{cat}}\)). I\(_{\text{cat}}\) was not Ca\(^2+\) dependent and was not blocked by Cd\(^2+\), but was blocked by Ba\(^2+\). The reversal potential for I\(_{\text{cat}}\) shifted with the Nernst potential for [Na\(^+\)]. The channel responsible for I\(_{\text{cat}}\) had a cation permeability sequence of Na\(^+\) \(\gg\) Li\(^+\) \(\gg\) TMA\(^+\) \(\gg\) NMG\(^+\) (P\(_{\text{Na}}\)/P\(_{\text{Na}}\) = 1:0.33:0.1:0) and was impermeable to Cl\(^-\). Addition of high intracellular concentrations of K\(^+\), Cs\(^+\), or Rb\(^+\) prevented the occurrence of I\(_{\text{cat}}\). Inhibition of I\(_{\text{cat}}\) by intracellular K\(^+\) was voltage dependent, with an IC\(_{50}\) that ranged from 3.0–8.9 mM at membrane potentials between -50 and -110 mV. This voltage-dependent shift in IC\(_{50}\) (e-fold per 52 mV) is consistent with a single cation binding site ~50% of the distance into the membrane field. I\(_{\text{cat}}\) displayed anomalous mole fraction behavior with respect to Na\(^+\) and K\(^+\); I\(_{\text{cat}}\) was inhibited by 5 mM extracellular K\(^+\) in the presence of 160 mM Na\(^+\) and potentiated by equimolar substitution of 80 mM K\(^+\) for Na\(^+\). The percent inhibition produced by both extracellular and intracellular K\(^+\) at 5 mM was identical. Reversal potential measurements revealed that K\(^+\) was 65–105 times more permeant than Na\(^+\) through the I\(_{\text{cat}}\) channel. I\(_{\text{cat}}\) exhibited the same voltage and time dependence of inactivation, the same voltage dependence of activation, and the same macroscopic conductance as the delayed rectifier K\(^+\) current in these neurons. We conclude that I\(_{\text{cat}}\) is a Na\(^+\) current that passes through a delayed rectifier K\(^+\) channel when intracellular K\(^+\) is reduced to below 30 mM. At intracellular K\(^+\) concentrations between 1 and 30 mM, P\(_{\text{K}}\)/P\(_{\text{Na}}\) remained constant while the conductance at -50 mV varied from 80 to 0% of maximum. These data suggest that the high selectivity of these channels for K\(^+\) over Na\(^+\) is due to the inability of Na\(^+\) to compete with K\(^+\) for an intracellular binding site, rather than a barrier that excludes Na\(^+\) from entry into the channel or a barrier such as a selectivity filter that prevents Na\(^+\) ions from passing through the channel.

INTRODUCTION

During the action potential, fast changes in membrane polarity depend upon the ionic selectivity of voltage-gated channels. Rapid depolarization requires the opening
of channels that are highly selective for ions with an inwardly directed driving force. Depending upon the cellular requirements, neurons utilize channels that are highly selective for either Na⁺ or Ca²⁺ for the upstroke of the action potential. Similarly, rapid repolarization via activation of delayed rectifier K⁺ channels requires that these channels have a high selectivity for K⁺ over Na⁺ and Ca²⁺.

Various cations show different permeability profiles through K⁺ channels (Latorre and Miller, 1983; Hille, 1992). Rb⁺ and Tl⁺ are quite permeant through most K⁺ channels, and conduct readily. Cs⁺ permeability can be either low or high for different K⁺ channels, but even where Cs⁺ permeability is relatively high, Cs⁺ conductance is quite low (Cukierman, Yellen, and Miller, 1985). Finally, Na⁺ and Ca²⁺, the only physiologically relevant cations that might compete for transport through K⁺ channels, have a low permeability and a negligible conductance in virtually all K⁺ channels examined. In some K⁺ channels, Na⁺ may enter the inner vestibule of the channel and act as an open channel blocker (Bezanilla and Armstrong, 1972; French and Wells, 1977). However, Na⁺ did not pass through these channels except with application of extreme depolarization (French and Wells, 1977).

We report here that a delayed rectifier K⁺ channel in chick dorsal root ganglion neurons displays novel permeation characteristics. Upon reduction of intracellular [K⁺] to under 30 mM, an Na⁺ conductance appears and becomes quite large when [K⁺] is reduced to below 3 mM. The selectivity mechanism of this channel for K⁺ over Na⁺ differed from that of other cations through K⁺ channels, in that Na⁺ had a very low permeability relative to K⁺, but displayed a large conductance through the channel when intracellular K⁺ was removed. This permeation behavior can be explained simply on the basis of the ability of Na⁺ and K⁺ to compete for an internal binding site in the channel, and is similar to the model of Na⁺ conductance through voltage-gated Ca²⁺ channels upon removal of extracellular Ca²⁺ (Almers and McCleskey, 1984; Hess and Tsien, 1984). A preliminary report of these data has been presented in abstract form (Callahan and Korn, 1993).

METH O D S

Cells
Dorsal root ganglion neurons were acutely isolated from thoracic and lumbar level ganglia of 14 d white leghorn chick embryos (University of Connecticut Poultry Farm). Ganglia were incubated in Tyrodes (in millimolar: 128 NaCl, 3 KCl, 2 CaCl₂, 1 MgCl₂, 27 NaHCO₃, 10 glucose, pH 7.3) containing 0.08% trypsin (610-5050PG Gibco Laboratories, Grand Island, NY) for 20 min at 37°C in a 10% CO₂ incubator. Cells were removed from the incubator and washed three times with Media 199 (Hyclone Laboratories, Inc., Logan, UT, B-1202-AX plus 3.1 g/liter NaHCO₃ ) plus 10% fetal bovine serum (FBS; A-1115-L, Hyclone Laboratories, Inc.). Cells were dissociated in Media 199 by trituration with a siliconized, fire-polished pasteur pipette and plated on polyornithine-coated 35-mm culture dishes. 30 min after plating, cells were fed with 2 ml of Media 199 plus FBS. Cells were used in experiments 1–4 h after plating.

Patch Clamp Recording
All recordings were made with the standard whole-cell patch clamp configuration (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981). Patch pipettes were fabricated from N51A glass (Garner Glass Co., Claremont, CA), coated with Sylgard and fire polished. Pipette resistance
varied from 0.5 to 1.5 MΩ. Series resistance was between 1 and 4 MΩ and was usually less than 3 MΩ (except in recordings made for Fig. 5 B [open circles]). The capacitive transient was electronically cancelled by membrane capacitance and series resistance neutralization; series resistance was occasionally compensated up to 80% but, due to such low series resistance, was invariably without effect (Dagan 3911A patch clamp amplifier, Dagan Corp., Minneapolis, MN). Membrane currents were filtered at 2 KHz (internal 3911A filter) and digitized at sample intervals of 50–1,000 μs. Experiments were performed at room temperature (20–24°C). Data were acquired and measured with Basic Fastlab (Indec Corp., Capitola, CA) and custom software. All curve fitting was done with SigmaPlot 4.02 (Jandel Scientific, Corte Madera, CA).

Solutions

Recordings were made from cells plated in 35 mm Nunc tissue culture dishes containing 1.5–2.0 ml of static bathing solution. Solutions bathing the cells were changed by manually lowering a large bore pipette that contained the desired test solution near the cell under study. Application of test solution was terminated by removal of the large bore pipette from the bath. In general, solutions bathing the cells were exchanged within 5 s. The solutions used in each experiment are listed in Table I.

RESULTS

Initially, intracellular and extracellular solutions were selected for isolation of Ca²⁺ and Ca²⁺-dependent Cl⁻ currents. N-methyl glucamine (NMG⁺) was the dominant intracellular cation, K⁺ was omitted from both solutions, 1 μM tetrodotoxin (TTX) was added to block TTX-sensitive Na⁺ channels, and 10 mM TEA was added extracellularly to block currents through TEA-sensitive channels. ATP, creatine phosphate and creatine kinase were added to the pipette solution to maintain phosphorylation of Ca²⁺ channels (Forscher and Oxford, 1985; Eckert, Chad, and Kalman, 1986), and leupeptin was added to prevent Ca²⁺ channel rundown due to proteolysis (Belles, Malecot, Hescheler, and Trautwein, 1988). Fig. 1 illustrates the membrane currents observed with these solutions. Within 2–3 s of obtaining the whole cell configuration, a voltage step from −80 to +10 mV evoked a mixed current, which contained a fast inward Ca²⁺ current and a delayed outward K⁺ current (Fig. 1, trace 1). The tail current (trace 1) recorded at −80 mV appeared to contain components from at least three different channel types: a fast Ca²⁺ tail current, a very slow, sustained inward current (this is a Ca²⁺-dependent Cl⁻ current; Mayer, 1985), and a small outward component superimposed on the slow inward current that appeared to have the timecourse of an outward K⁺ channel tail current. With successive stimuli, delivered at 10 s intervals, the outward current during the step to +10 mV was reduced and the inward current during the step increased. This reduction in outward current presumably occurred due to dilution of intracellular K⁺ by the pipette solution, and approximately reflects the timecourse of intracellular solution exchange in this cell (Pusch and Neher, 1988). Simultaneously with washout of the K⁺ current, a new inward tail current appeared and grew with successive stimuli until it reached a maximum (Fig. 1, traces 3–8). The slow, Ca²⁺-dependent Cl⁻ current remained relatively unchanged over this time period. The experiments described below were designed to determine the nature of this inward tail current (which we will call $I_{cat}$) that grew with successive stimuli. We addressed three
| Table 1: Bath solutions in millimolar | Pipette solutions in millimolar |
|--------------------------------------|---------------------------------|
| NaCl | CaCl₂ | MgCl₂ | Other | NaCl | MgCl₂ | Other | NaCl | MgCl₂ | Other |
| 150| 2| 0| 10 TEA-Cl | 140| 0| 0.5 | PO₄ system |
| 2 | 150| 2| 0| 140(c) | 150(a,b,d) | 0 | 0.5(a,b,d) | 10(c) | PO₄ system(a,b,d) |
| 3 | 166| 0| 0.03| 20| 120| 10 |
| 4 | 160| 0.5| 0| 140| 0| 10 | TEA-Cl, PO₄ system |
| 5a (Eₐ = 0 mV) | 155| 0| 0.03| 20| 130| 10 |
| 5a (Eₐ = -26 mV) | 155| 0| 0.03| 0| 50| 10 | 80 Na MeSO₄ |
| 5b (Eₐ = -29 mV) | 150| 0| 0.03| 0| 40| 10 |
| 5b (Eₐ = +13 mV) | 80| 0| 0.03| 0| 110| 10 |
| 6a, 14| 155| 2| 0| 150| 0| 0.5 | PO₄ system |
| 6c (Na) | 150| 0| 0.03| 0| 130| 10 |
| 6c (Li) | 25| 0| 0.03| 145 LiCl | 20| 120| 10 |
| 6c (NMG) | 25| 0| 0.03| 135 NMG-Cl | 30| 120| 10 |
| 6d (Eₐ = +54 mV) | 160| 0| 0.03| 140| 0| 10 |
| 6d (Eₐ = +18 mV) | 150| 0| 0.03| 80| 55| 10 |
| 6d (Eₐ = 0 mV) | 150| 0| 0.03| 0| 130| 10 |
| 6d (Eₐ = -44 mV) | 150| 0| 0.03| 135 NMG-Cl | 0| 130| 10 |
| 7a | 160| 0.5| 0| 0| 0| 140 KCl, 10 EGTA/K |
| 8 a,b | 160| 0| 0.03| 120-140| 0| 10 | 0-30 KCl |
| 8c | 160| 0| 0.03| 0| 100-130| 10 | 0-30 KCl |
| 8, 15 | 160| 2| 0| 150| 0| 0.5 | PO₄ system |
| 10-12 (Iₐ) | 155| 0| 0.03| 135 KCl | 0| 130| 10 |
| 10-12 (IK) | 150| 0.5| 0| 135 KCl | 0| 130| 10 |

*All bath solutions contained 1 MgCl₂, 10 HEPES, 10-20 glucose, and 1 μM TTX. pH = 7.34 (NaOH), osmolality = 300-315 mosm (osmolality of Fig. 5b bath solutions = 324/190).*

†All pipette solutions contained 4 MgCl₂, and 10 HEPES, unless otherwise noted. pH = 7.34 (NaOH), osmolality = 300-315 mosm (osmolality of Fig. 5a pipette solutions = 250/100).*

*4 ATPN₄H₂, 4 creatine phosphate, creatine kinase, leupeptin, NMG, N-methylglucamine; MeSO₄, methylsulfate.
questions: What ion species carried \( I_{cat} \)? What channel type did \( I_{cat} \) pass through? Did \( I_{cat} \) grow over time due to wash in of some substance from the pipette or wash out of some endogenous, intracellular substance?

**Divalent Dependence**

Removal of extracellular \( \text{Ca}^{2+} \) (Fig. 2A) or addition of 300 \( \mu \text{M} \) extracellular \( \text{Cd}^{2+} \) (Fig. 2B) blocked the inward \( \text{Ca}^{2+} \) current but did not affect \( I_{cat} \). However, replacement of extracellular \( \text{Ca}^{2+} \) by \( \text{Ba}^{2+} \) totally and reversibly prevented activation of \( I_{cat} \), even while producing a larger inward \( \text{Ca}^{2+} \) channel current (Fig. 2C). Addition of 1 \( \text{mM} \) extracellular \( \text{Ba}^{2+} \) in the presence of 2 \( \text{mM} \) \( \text{Ca}^{2+} \) reversibly inhibited \( I_{cat} \) (Fig. 2D). These data indicate that \( I_{cat} \) was not \( \text{Ca}^{2+} \) activated, did not pass through \( \text{Ca}^{2+} \) channels, and was blocked by \( \text{Ba}^{2+} \) by a mechanism unrelated to \( \text{Ca}^{2+} \).

**Fig. 1.** Wash in of tail current \( (I_{cat}) \) after break in. 100-ms depolarizing steps from -80 to +10 mV were made once every 10 s, starting 2–3 s after obtaining the whole cell configuration. The first, second, third, and eighth currents elicited during the step are labeled, the first and eighth tail currents are labeled. The early tail current grew progressively larger from stimulus 1 to stimulus 8. After stimulus 8, the currents remained unchanged. Unless otherwise stated, all figures below illustrate currents recorded after having washed in completely. (Dashed line) 0 current level. \( R_s = 3.5 \text{ M}\Omega \), \( C_m = 30 \text{ pF} \).

\( \text{Ca}^{2+} \). \( I_{cat} \) was qualitatively unaffected by addition or removal of intracellular \( \text{Mg}^{2+} \) (up to 5 \( \text{mM} \); not shown), EGTA (0.5–10 mM) or the ATP regenerating system plus leupeptin.

**I-V Relationships**

Fig. 3 illustrates current-voltage relationships of \( I_{cat} \) after its isolation from other currents. Currents carried by \( \text{Ca}^{2+} \) and \( \text{Ca}^{2+} \)-dependent channels were eliminated by removal of extracellular \( \text{Ca}^{2+} \) and addition of 30 \( \mu \text{M} \) extracellular \( \text{Cd}^{2+} \). The dominant intracellular and extracellular cation was \( \text{Na}^+ \). Fig. 3A illustrates currents evoked by 120-ms voltage steps to various potentials. Fig. 3B shows the magnitude of the step current \( (\text{open circles}) \) and tail current \( (\text{closed circles}) \) as a function of step potential. The step current reversed at 3.1 mV (calculated \( E_{Na} = 4.4 \text{ mV} \), calculated...
\( E_{Cl} = -3 \text{ mV} \) and the maximal whole cell conductance was \( \sim 14 \text{ nS} \). When measured using a tail \( I-V \) protocol in which currents were activated by a step to +65 mV (Fig. 3, C and D), both the reversal potential (extrapolated from the data between -60 and 0 mV) and the whole cell conductance of \( I_{cat} \) were virtually identical (3.0 mV and 13.5 nS, respectively).

Deactivation of the tail current was voltage dependent; currents decayed more rapidly with greater hyperpolarization (Fig. 4 A). This voltage dependence was qualitatively identical regardless of whether \( \text{Na}^+ \) or \( \text{NMG}^+ \) was the dominant intracellular cation. Tail currents were well fit by a single exponential between -130 and -90 mV (Fig. 4 B). However, at voltages between -80 and -60 mV, a small faster component was apparent (cf. -70 mV trace in Fig. 4 B). Whether this represented a \( \text{Ca}^{2+} \) tail current that slowed at more positive voltages (\( \text{Ca}^{2+} \) channels were not blocked in this experiment) or was a second decay component of \( I_{cat} \) could not be determined from these experiments. In cells recorded with \( \text{Ca}^{2+} \) channels blocked, tails displayed a small hook that precluded resolution of this issue. Nonetheless, at membrane potentials between -80 and -60 mV, all but the first 5–10 ms of the entire tail current was well described by a single exponential. These data suggest that most or all of the tail current involved a single population of channels. Fig. 4 C illustrates the dependence of decay time constant as a function of repolarization potential.
Anion Dependence

The previous results suggested that $I_{\text{cat}}$ may have been carried by either Na$^+$ or Cl$^-$. Two types of experiments were done to test for the possible contribution of an anion channel. In the first, currents were recorded from cells in which the intracellular solutions contained mixtures of Cl$^-$ and the presumably impermeant anion, methylsulfate (Fig. 5 A). One set of cells had a calculated Cl$^-$ equilibrium potential of 0 mV (closed circles) and one set of cells had a calculated Cl$^-$ equilibrium potential of −26 mV (open circles). The observed tail current reversal potentials in each condition were virtually identical, and were close to the calculated Na$^+$ equilibrium potential of 1 mV.

To control for the possibility that methylsulfate was permeant, similar experiments were performed using solutions of different ionic strength (without anion substitu-
substitution for both intracellular and extracellular Na\(^+\). Replacement of 97% of extracellular Na\(^+\) by Li\(^+\) nearly abolished I\(_{\text{cat}}\) (Fig. 6A,B). Fig. 6C illustrates three representative tail I-V curves, one each for cells in which the calculated Na\(^+\) equilibrium potential (E\(_{\text{Na}}\)) was 0 mV (squares), or E\(_{\text{Na}}\) was shifted to -40 mV by partial substitution of Li\(^+\) (diamonds) or NMG\(^+\) (circles) for extracellular Na\(^+\). The average reversal potentials and permeability ratios for several cells in each condition are shown in Table II. Of the ions tested, only NMG\(^+\) was totally impermeant. Li\(^+\) was apparently ~30% as permeant as Na\(^+\) (Table II), but carried very little current (Fig. 6, B and C). The Na\(^+\)-dependence of the I\(_{\text{cat}}\) reversal potential was studied more completely using both intracellular and extracellular NMG\(^+\) as a Na\(^+\) substitute (Fig. 6 D). The shift in reversal potential as a function of [Na\(^+\)] was exactly Nernstian; the slope of the regression line was 57.9 mV per decade change in [Na\(^+\)] ratio. The specificity for Na\(^+\) over Li\(^+\) indicates that I\(_{\text{cat}}\) passed through neither a TTX-insensitive Na\(^+\) channel nor a nonspecific cation channel.

The previously described results were obtained with either NMG\(^+\) or Na\(^+\) as the dominant intracellular cation. In contrast, when K\(^+\), Cs\(^+\), or Rb\(^+\) were used as the dominant intracellular cation, the growth of I\(_{\text{cat}}\) was not observed (Fig. 7). Two points are worth noting about these results. First, intracellular addition of cations that easily
enter K⁺ channels prevented the appearance of $I_{cat}$, whereas addition of cations that enter K⁺ channels poorly did not. Second, it was consistently observed that the kinetics of both tail current and step current were slightly slowed by Rb⁺. Both activation and deactivation of K⁺ channel currents are slower when Rb⁺ is used as a permeant ion (cf. Arhem, 1980; Matteson and Swenson, 1986).

**Figure 5.** Anion dependence of tail current. (A) Tail current magnitude as a function of repolarization potential from two different cells, one with a calculated chloride equilibrium potential ($E_{Cl}$) of 0 mV and one with a calculated $E_{Cl}$ of -26 mV (equimolar substitution of 80 mM methylsulfate for intracellular Cl⁻). Measured chloride reversal potentials were 6 and 8 mV, respectively. $R_s$ (in MΩ) = 3.2 ($E_{Cl} = -26$), 2.0 ($E_{Cl} = 0$); $C_m$ (in picofarads) = 16.6 ($E_{Cl} = -26$), 27.1 ($E_{Cl} = 0$). (B) Na⁺ and Cl⁻ equilibrium potentials were set on opposite sides of 0 mV by adjusting the ionic strength of the bath and pipette solutions, and making no ion substitutions. $R_s$ (in MΩ) = 11.0 ($E_{Cl} = -29$), 2.0 ($E_{Cl} = +13$); $C_m$ (in picofarads) = 19.0 ($E_{Cl} = -29$), 25.0 ($E_{Cl} = +13$). In both A and B, the voltage step protocol was identical to that in Fig. 3 C.

**Involvement of Intracellular K⁺ in $I_{cat}$ Development**

The observation that $I_{cat}$ was carried by Na⁺ but not significantly by Li⁺, together with the results that $I_{cat}$ did not appear in the presence of intracellular K⁺, suggested three possible hypotheses. The first possibility was that $I_{cat}$ was a Na⁺ conductance through a previously undescribed Na⁺ channel that was blocked by intracellular K⁺,
FIGURE 6. Monovalent cation selectivity of $I_{cat}$. Currents were elicited by incremental voltage steps in the presence of extracellular Na\(^+\) (A) and after 97% replacement of extracellular Na\(^+\) with Li\(^+\) (B). Currents were recorded in the presence of extracellular Ca\(^{2+}\), which accounts for the current observed during the depolarizing voltage step in B. (C) Representative tail-I-V plots for three different cells, in which the calculated Na\(^+\) equilibrium potential was set at 0 mV (Na\(^+\)), \(-40\) mV by substitution of extracellular Li\(^+\) for Na\(^+\) (Li\(^+\)), and \(-44\) mV by substitution of extracellular NMG\(^+\) for Na\(^+\) (NMG\(^+\)). Measured reversal potentials for these three cells were \(-1.9\), \(-17\), and \(-39.5\) mV, respectively. Average results for several cells in this experiment are given in Table II. Currents were evoked by a voltage pulse protocol identical to that of Fig. 3 C, and recorded in the absence of extracellular Ca\(^{2+}\) and presence of 30 \(\mu\)M extracellular Cd\(^{2+}\). (D) The Na\(^+\) equilibrium potential was varied by replacement of either intracellular or extracellular Na\(^+\) with NMG\(^+\). True reversal potentials were measured with a protocol similar to that in Fig. 3 C. Points represent mean ± SEM of reversal potentials measured from the number of cells in parentheses. $R_s = 2.4$ MΩ (A and B), $C_m = 23$ pF (A and B).

Cs\(^+\), and Rb\(^+\) and extracellular Ba\(^{2+}\). The second possibility was that removal of intracellular K\(^+\) changed the selectivity of a K\(^+\) channel, such that structural barriers that normally prevented Na\(^+\) permeation or conductance were reduced in low [K\(^+\)]. The third possibility was that $I_{cat}$ was an Na\(^+\) conductance through a K\(^+\) channel that

### TABLE II

| Substituted cation | Calculated $E_{Na}$ | Observed $E_{m}$ | $n$ | $P_s/P_{Na}$ |
|--------------------|---------------------|-----------------|----|-------------|
| Na\(^+\)            | 0                   | 1.5 ± 2.8       | 3  | 1.0         |
| Li\(^+\)            | -40                 | 16.4 ± 3.4      | 4  | 0.31        |
| TMA\(^+\)           | -40                 | -30.6 ± 2.9     | 5  | 0.12        |
| NMG\(^+\)           | -44                 | -42.8 ± 1.4     | 4  | 0           |
normally had a small permeability to Na⁺. In this latter case, lack of Na⁺ conductance would not be due to barriers that prevented entry or conduction of Na⁺ but to the inability of Na⁺ to compete with K⁺ for a cation binding site within the channel. The experiments in Fig. 8 addressed these three hypotheses.

$I_{\text{cat}}$ was recorded in the presence of different concentrations of intracellular K⁺. Experiments were performed with both NMG⁺ (Fig. 8 A, B, and D) and Na⁺ (Fig. 8 C) as the dominant intracellular cation. Addition of 0.1 to 10 mM K⁺ to the intracellular solution resulted in a marked leftward shift in $I_{\text{cat}}$ reversal potential and a reduction of $I_{\text{cat}}$ magnitude. Fig. 8 A illustrates the tail current-voltage relationships of six cells recorded with NMG⁺ pipette solutions; three cells had 0 mM KCl added and three cells had 10 mM KCl added. As was characteristic of cells recorded with NMG⁺ solutions, the $I$-$V$ relationship inwardly rectified; outward currents, when they occurred at all, were very small. Although true reversal potentials could not always be obtained, it is clear from Fig. 8 A that addition of 10 mM K⁺ shifted the reversal potential of $I_{\text{cat}}$ from a large positive value to $\sim -20$ to $-40$ mV. In addition, the

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**Figure 7.** High intracellular concentrations of K⁺, Cs⁺, and Rb⁺ prevent development of $I_{\text{cat}}$. Voltage step stimuli were delivered every 3 s, starting 2–4 s after break in, and wash in of $I_{\text{cat}}$ was monitored. Voltage steps were from -80 to +65 mV except in C, in which the step was to +20 mV. Currents were recorded in the presence of extracellular Ca²⁺. Traces shown illustrate 6–10 currents evoked from four different cells, one each containing K⁺, Cs⁺, Rb⁺, or Na⁺ as the dominant cation in the pipette solution. Currents were recorded from immediately (2–4 s) after break in, for 64 s (A), 54 s (B), 39 s (C) and 66 s (D) after break in. With pipettes containing Na⁺ or NMG⁺, $I_{\text{cat}}$ always appeared within 12–25 s after break in. The outward currents in B are believed to be carried by Cs⁺ through Ca²⁺ channels. $R_s$ (in megaohms) = 1.5 (A), 4.0 (B), 2.0 (C), 2.0 (D). $C_m$ (in picofarads) = 22.0 (A), 8.4 (B), 22.0 (C), 18.4 (D).
absolute magnitude of the current, normalized for cell size, was reduced by addition of K⁺.

This inhibition of current magnitude was examined more carefully in Fig. 8 B. Inhibition was dependent upon intracellular [K⁺] between 0.1 and 30 mM, and was well fit by the equation,

$$I/I_0 = 1 - \left(\frac{[K^+]_i}{[K^+]_i + IC_{50}^n}\right)$$  

where $IC_{50}$ is the concentration at which current was inhibited by 50% and $n$ is the Hill coefficient that provides an indication of cooperative interactions between ligand and substrate.

The $IC_{50}$ for inhibition of $I_{cat}$ by K⁺ ranged from 3.0–8.9 mM and was voltage dependent; at more positive membrane potentials, inhibition occurred at a lower
[K+] (Fig. 8 B). Fig. 8 D plots the values of the parameters of Eq. 1 as a function of membrane potential. The value of n was 1 at all membrane potentials, which suggests that the block by K+ involved a simple, noncooperative interaction of a single K+ with each binding site. The IC50 for block by K+ shifted e-fold for 52 mV, which would place a single binding site ~50% of the distance into the membrane field according to the equation (Danko, Smith-Maxwell, McKinney, and Begenisich, 1986):

$$K_d = K_0 \exp \left( -\frac{zdV}{RT} \right)$$

where $K_d$ is the dissociation constant, $K_0$ is the dissociation constant at $V = 0$, $d$ is electrical distance into membrane field, $z$ is valence, $V$ is voltage, $F$, $R$, and $T$ are the usual constants. $K_0 = 1.0$ for the data plotted in Fig. 8 D.

Tail current $I-V$ relationships were relatively linear when intracellular Na+ was used as the dominant internal cation (cf. Figs. 3 D, 6 C), and true reversal potentials could be obtained. Addition of 0.1 to 30 mM K+ to the intracellular solution resulted in a concentration-dependent decrease in cat conductance (plotted as the current at -110 mV) and a negative shift in cat reversal potential (Fig. 8 C). The permeability ratio for K+ and Na+ was determined from the reversal potentials obtained from the data in Fig. 8 C. At four concentrations between 1 and 30 mM K+, $P_K/P_Na$ ranged from 65 to 107 (Fig. 9). Between 1 and 30 mM, there was no change in $P_K/P_Na$. Taken together, the data in Figs. 7–9 argue against the first hypothesis above, namely that $I_{cat}$ is a Na+ channel conductance normally blocked by internal K+. These data support the hypothesis that $I_{cat}$ represents a Na+ conductance through a K+ channel that is revealed by reduction of intracellular K+. Furthermore, the data in Fig. 9 support the third hypothesis over the second, namely, that this channel is normally 60–100-fold more permeable to K+ than Na+ and does not change its permeability characteristics as a function of [K+]i. Experiments described below address the identity of this K+ channel.

**Channel Identity: Activation and Inactivation Parameters**

Dorsal root ganglion neurons isolated from chick embryos have several electrophysiologically documented K+ channels, including an A-type current and more than one
type of delayed rectifier (Florio, Westbrook, Vasko, Bauer, and Kenyon, 1990; Westbrook and Kenyon, 1990). The activation and inactivation characteristics of $I_{cat}$ observed in previous experiments were similar in appearance to that of a delayed rectifier $K^+$ channel. The following experiments more closely examined the kinetic characteristics of $I_{cat}$ and compared them to those of the delayed rectifier current observed with a high intracellular concentration of $K^+$.

Fig. 10A illustrates the time course of activation of $I_{cat}$. Voltage steps to +20 mV were presented at 6-s intervals for durations of 2–76 ms, incremented by 2 ms/stimulus. Tail currents were recorded at a repolarization potential of −80 mV. The peak tail current is plotted as a function of voltage step duration in Fig. 10B (circles). For comparison, a $K^+$ channel current that contains an A-type current component and a delayed rectifier component is superimposed. The activation of $I_{cat}$ did not display this fast transient component, which suggests that no part of $I_{cat}$ involved conductance through an A-type channel. Indeed, subtraction of $I_{cat}$ from $I_k$ (not shown) yielded a current with virtually identical activation and inactivation kinetics as the early transient (A-type) $K^+$ current previously described in chick DRGs (Florio et al., 1990). $I_{cat}$ and the sustained $I_k$ followed the same time course from ~40

![Image](image_url)
ms onward. Fig. 10 C illustrates the inactivation time course of $I_{\text{cat}}$ from the same cell; currents were evoked by stimuli that were varied in duration from 100–1500 ms by 100-ms increments. $I_K$ from Fig. 10 B was digitized at identical time points and plotted in Fig. 10 D, superimposed on the measured current amplitudes from $I_{\text{cat}}$ in Fig. 10 C. Over this time scale, the rate of inactivation of $I_{\text{cat}}$ and $I_K$ were nearly identical.

It is clear from Fig. 10 that the time course of inactivation of $I_{\text{cat}}$ and $I_K$ were both slow and quite similar. To examine this further, twin pulse protocols were used to examine the voltage and time dependence of inactivation of the sustained $I_K$ and $I_{\text{cat}}$. Fig. 11, A and B, illustrates the voltage dependence of inactivation of the sustained $I_K$ produced by a 10-s prepulse. Similar experiments were also performed using a prepulse duration of 0.5 s (traces not shown). With each prepulse duration, both the voltage and time dependence of the sustained $I_K$ and $I_{\text{cat}}$ were nearly identical (Fig. 11 C). Thus, the channels through which $I_{\text{cat}}$ flows have the same voltage and time dependence of inactivation as the sustained delayed rectifier $K^+$ channels in these cells.

We more closely compared the activation parameters of $I_{\text{cat}}$ with the total delayed rectifier current, recorded with a high $K^+$ intracellular solution. A typical family of delayed rectifier currents is illustrated in Fig. 12 A. The $I-V$ relationships for $I_K$ (open circles) and $I_{\text{cat}}$ (closed circles, taken from Fig. 3 A) are plotted in Fig. 12 B for

![Image](image-url)
comparison. The minimum depolarizations necessary for activation of $I_K$ and $I_{cat}$ were identical. However, there appeared to be a slight negative shift in the voltage dependence of activation (Fig. 12D). In cells treated with up to 8 mM 4-aminopyridine (4-AP), which inhibits the transient, A-type current in rat DRGs, (Mayer and Sugiyama, 1988), $I_K$ still activated more quickly than $I_{cat}$ (Fig. 12C). However, transient outward currents in chick DRGs are quite insensitive to 4-AP (Westbrook and Kenyon, 1990), and may not, in fact, be classical A-type currents. Nonetheless, the data in Figs. 10–12 demonstrate that $I_{cat}$ displays the characteristics of the sustained delayed rectifier in these cells, and does not display either the activation or the inactivation kinetics of the faster, transient outward current.

**TEA Sensitivity**

$I_{cat}$ was totally insensitive to extracellular application of 10 mM TEA (Fig. 13A). It was therefore logical to hypothesize that $I_{cat}$ passed through a TEA-insensitive delayed rectifier. TEA (10 mM) inhibited $I_K$ by 70.5 ± 3.4% at +10 mV ($n = 4$; Fig. 13, B1 and C). Because the TEA-insensitive $I_K$ had a nearly identical kinetic profile to
the total current (Fig. 13 B2), comparison of the activation time course of \( I_{\text{cat}} \) to that of the TEA-insensitive \( I_K \) gave essentially the same results as seen in Fig. 12 C. A typical \( I-V \) relationship for the TEA-insensitive \( I_K \) is shown in Fig. 13 C. There is very little current at potentials more negative than +40 mV. The \( I-V \) relationship for the TEA-insensitive \( I_K \) turned upward near +40 mV, which suggests that currents at these potentials were not carried exclusively by TEA-insensitive channels but also by TEA-sensitive channels in which TEA was driven out by strong depolarization. At +30 mV, the calculated conductance (which is 85–90% of the maximum conductance) for the TEA-insensitive \( I_K \) was 0.18 nS/pF \((n = 2)\), which was much smaller than the average \( I_{\text{cat}} \) conductance. The macroscopic conductance of \( I_{\text{cat}} \) was not significantly different from that of the total delayed rectifier K\(^+\) current \((0.69 \pm 0.16 \text{ nS/pF and } 0.93 \pm 0.12 \text{ nS/pF, respectively; } n = 4)\).

**Wash In Versus Wash Out**

It seemed clear from the previous data that the appearance of \( I_{\text{cat}} \) resulted from wash out of intracellular K\(^+\). Nonetheless, we further tested this hypothesis with the following analysis. Fig. 1 illustrates that \( I_{\text{cat}} \) appeared simultaneously with the reduction of outward K\(^+\) current. We examined the relationship between outward current reduction and development of \( I_{\text{cat}} \) in two cells that had a clear outward tail current on the first trace and did not display contaminating Ca\(^{2+}\)-dependent Cl\(^-\) currents. One of these cells is illustrated in Fig. 14 A. Wash in was recorded as described previously, and we made the following calculations to estimate the intracellular [K\(^+\)] at which the inward \( I_{\text{cat}} \) tail current appeared.

Given the known extracellular ion concentrations, \( P_K/P_Na \) from Fig. 9, and estimates of the initial intracellular [K\(^+\)] and [Na\(^+\)], we used the Goldman-Hodgkin-Katz (GHK) equation to estimate the reversal potential for \( I_K \) at the time of the first stimulus. This estimated reversal potential \((V_{\text{rev}})\), together with the known membrane potential \((V_m)\) and current magnitude \((I)\), was then used to calculate the macroscopic \( I_K \) conductance \((g_K)\) from the equation, \( g_K = I/(V_m - V_{\text{rev}}) \). Given the known \( V_m \) and the calculated \( g_K \), a new reversal potential was then calculated for the second measured outward current, \( I_K \). The intracellular [K\(^+\)] at the time of the second trace was then calculated using the GHK equation with this new reversal potential and the constant permeability ratio and extracellular ion concentrations. These calculations were repeated to obtain an estimate of the intracellular [K\(^+\)] at the time of each current trace. Varying the initial estimate of intracellular [Na\(^+\)] between 0 and 20 mM had virtually no effect on the results. The one critical assumption for these calculations was the estimate of intracellular [K\(^+\)] at the time the first trace was recorded. Clearly, some dilution of intracellular [K\(^+\)] may have occurred before we were able to record the first current. Therefore, two sets of calculations were made. One utilized an initial [K\(^+\)] of 140 mM (Fig. 14 B, open symbols), which approximates [K\(^+\)] in the intact cell. The second utilized an initial [K\(^+\)], of 35 mM (filled symbols), which presumed a 75% reduction of intracellular [K\(^+\)] by the time of the first measured current. Fig. 14 B plots the tail current magnitude as a function of calculated [K\(^+\)], using a \( P_K/P_Na \) of 65. Using these widely spaced estimates of initial [K\(^+\)], this analysis suggests that the inward tail current \((I_{\text{cat}})\) appeared when [K\(^+\)] was between 6 and 12 mM.
Figure 13. Sensitivity of $I_{ca}$ and $I_K$ to 10 mM TEA. (A) $I_{ca}$, evoked by a stimulus to +65 mV from −80 mV, before and after application of 10 mM TEA. Repolarization potential was −80 mV. (B1) $I_K$, evoked by depolarization to +60 mV, before (Cont.), during (TEA), and 2 min after removal (Recov.) of 10 mM TEA from the external solution. (B2) Control and post TEA currents from B1 were normalized to the peak to compare time courses. (C) Current magnitude, plotted as a function of depolarizing step potential, for $I_{ca}$ (inward currents) and $I_K$ (outward currents). $I_K$ magnitude was measured at the end of the depolarizing step. Rs (in megaohms) = 2.9 (A), 2.0 (B). Cm (in picofarads) = 13.8 (A), 15.5 (B).

Figure 14. Calculated intracellular [K+] during wash in of $I_{ca}$. (A) Experimental protocol as in Fig. 1. Starting within 2−4 s after break-in, currents were evoked by voltage steps from −80 to +20 mV, which were delivered every 6 s. 10 records are shown. The first shown (1; largest outward step current, outward tail current), was recorded 14−16 s after break-in, the last (10; smallest outward step current, largest inward tail current) was recorded 104−106 s after break-in. Rs = 4.8 MΩ, Cm = 27.4 pF. (B) Tail current magnitude as a function of calculated intracellular [K+]. Intracellular [K+] was calculated as described in text. The two curves illustrate the calculated [K+] if intracellular [K+] was assumed to be 140 mM (open circles) or 35 mM (closed circles) at the time of the first stimulus after break in. $P_K/P_{Na}$ was assumed to be 65. (C) Effect of $P_K/P_{Na}$ on calculated [K+]. Curves denoted by circles are from $B$; curves denoted by triangles represent the same calculations using $P_K/P_{Na}$ of 100.
$P_K/P_{Na}$ values calculated from the data in Fig. 8 ranged from $\sim 65$ to $\sim 100$ (Fig. 9). In Fig. 14 C, we examined the dependence of our calculations on the value of $P_K/P_{Na}$. The triangles in Fig. 14 C illustrate the results of the calculation for a permeability ratio of 100; the values from Fig. 14 B are replotted for comparison. This alteration in $P_K/P_{Na}$ values shifted the estimate of $[K^+]_i$ at the appearance of inward tail current by just 1–2 mM, and shows that this calculation is relatively unaffected by possible variations in calculated permeability ratio. Similar calculations made from the cell illustrated in Fig. 7 D suggested that the $[K^+]_i$ at the appearance of inward tail current was between 14 and 45 mM ($P_K/P_{Na} = 65$). These calculations support the hypothesis that the appearance of $I_{cat}$ coincides with the reduction of $[K^+]_i$ to below 30 mM.

**Figure 15.** Effects of extracellular $K^+$. (A) Extracellular addition of 5 mM $K^+$ in the presence of 160 mM Na$^+$ inhibited $I_{cat}$ by 59 ± 7.9% ($n = 3$) at $-80$ mV. (B) Equimolar replacement of 80 mM extracellular Na$^+$ with $K^+$ markedly potentiated both the inward step current and $I_{cat}$. When normalized, the tail currents superimpose, which suggests that they are passing through the same population of ion channels. Currents in B are leak-subtracted. Rs (in megaohms) = 1.9 (A), 2.0 (B). Cm (in picofarads) = 19.1 (A), 16.0 (B).

**Effects of External $K^+$**

Since both $K^+$ and Na$^+$ flow through the delayed rectifier channel in both directions, it might be expected that the Na$^+$ conductance would be inhibited by extracellular $K^+$ at a similar concentration as observed with intracellular $K^+$. Furthermore, one might expect to observe anomalous mole fraction behavior for this channel. The data in Fig. 15 illustrate that both of these phenomena occurred. Application of 5 mM extracellular $K^+$ inhibited $I_{cat}$ by 59 ± 7.9% ($n = 3$) at $-80$ mV (Fig. 15 A). This compares with 50% inhibition by 5 mM intracellular $K^+$ predicted by the data in Fig. 8 B. Equimolar replacement of 80 mM extracellular Na$^+$ with $K^+$ greatly potentiated $I_{cat}$ (Fig. 15 B). When normalized, the tail currents carried by Na$^+$ and $K^+$ in Fig. 15 B superimposed (not illustrated).

**DISCUSSION**

Delayed rectifier $K^+$ channels in squid axon (Bezanilla and Armstrong, 1972), frog node (Hille, 1973), snail neurons (Reuter and Stevens, 1980) and frog skeletal muscle (Gay and Stanfield, 1978) are all quite impermeable to Na$^+$, with $P_{Na}/P_K$ values that
range from 0.01 to 0.07. In all preparations, Na⁺ had a negligible conductance through K⁺ channels at physiological levels of depolarization. However, with depolarization to greater than +160 mV, Na⁺ began to conduct (French and Wells, 1977). French and Wells suggested that the conduction of Na⁺ with large depolarizations, but not at more physiological potentials, was due to an alteration in the permeability of K⁺ channels to Na⁺ at very positive potentials. Our results reported here demonstrate that in embryonic chick DRGs, delayed rectifier K⁺ channels also have a very low relative permeability to Na⁺, with a $P_{Na}/P_K$ value of ~0.01. In stark contrast to the previous results, however, Na⁺ was able to produce a large conductance through the DRG delayed rectifier K⁺ channel at physiological potentials, despite the low $P_{Na}/P_K$. The ability of Na⁺ to conduct through this K⁺ channel was [K⁺]-dependent; the Na⁺ conductance occurred when intracellular [K⁺] was reduced to less than 30 mM and became more prominent as intracellular [K⁺] was reduced further.

Pore Structure

K⁺ channels have been modeled as multi-ion pores, with multiple cation binding sites in the conduction pathway (Hille and Schwarz, 1978; French and Shoukimas, 1985; Wagoner and Oxford, 1987). Eyring rate models of K⁺ channel permeation pathways typically include two or more energy wells that represent cation binding sites and three or more energy barriers over which ions must traverse to enter and pass through the channel. The relative permeability, $P_X/P_Y$, represents the ability of two ions, X and Y, to compete for entry into the channel conduction pathway, whereas the conductance represents the ease with which an ion travels completely through the pore once it enters. In single-occupancy channels, the permeability of an ion in a pore depends upon the height of energy barriers that control access to the pore (Hille, 1992). In multi-ion pores, the permeability ratio for two ions may also depend upon their relative affinities for binding sites within the channel (Almers and McCleskey, 1984; Hess and Tsien, 1984; Hille, 1992). Na⁺ is relatively impermeable in the K⁺ channel, which suggests that either a very high energy barrier prevents entry of Na⁺ into the channel or that Na⁺ competes poorly against K⁺ for a binding site within the channel. The data in Fig. 9 indicate that $P_{Na}/P_K$ doesn't change as a function of intracellular [K⁺]. This observation, together with the observation that Na⁺ conducts well at low [K⁺], suggests that the low Na⁺ permeability does not result from a barrier for entry but is due to the inability of Na⁺ to compete with K⁺ for a binding site. Once inside the pore, the energy barrier for passage of Na⁺ completely through the channel is relatively low, which gives Na⁺ its high conductance. Thus, ion selectivity in this channel does not appear to result from a selectivity filter that prevents Na⁺ from conducting (Latorre and Miller, 1983) but rather from the inability of Na⁺ to compete with K⁺ for entry into the pore. The observation of anomalous mole fraction behavior observed by us and by Zhu and Ikeda (1993) for an apparently similar phenomenon (see below) is consistent with this model. Our results produce a picture for Na⁺ permeation in a K⁺ channel that is much like that proposed for the permeation of Na⁺ in Ca²⁺ channels (Almers and McCleskey, 1984; Hess and Tsien, 1984). This model predicts that the change in Ca²⁺ channel selectivity is brought on by a change in the competitive environment for
Na⁺. Indeed, the relative permeability of Ca²⁺ channels for Na⁺ and Ca²⁺ may not change. However, the very high affinity of Ca²⁺ for the Ca²⁺ channel binding site ($K_d = 0.3 \mu M$) precludes the ability to examine permeability ratios from reversal potential measurements; micromolar concentrations of Ca²⁺ prevent Na⁺ entry into the pore but are not enough to produce a measurable current. Determination of permeability ratios for $I_{cat}$ was possible because the relevant cation binding site in chick DRG delayed rectifier K⁺ channels has a $K_d$ for K⁺ between 1 and 10 mM (Fig. 8); this concentration produces a measurable K⁺ current and shift in $I_{cat}$ reversal potential.

**TEA Insensitivity**

$I_{cat}$ was completely insensitive to extracellular application of 10 mM TEA (Fig. 12A). Delayed rectifiers vary considerably in their sensitivity to extracellular TEA (Stuhmer, Ruppersberg, Schroter, Sakmann, Stocker, Giese, Perschke, Baumann, and Pongs, 1989; Hille, 1992), with those in squid giant axon and several cloned rat brain K⁺ channels being quite insensitive (Tasaki and Hagiwara, 1957; Stuhmer et al., 1989). The most obvious interpretation of these findings is that $I_{cat}$ passes through a TEA-insensitive K⁺ channel. Indeed, the inactivation profile of $I_{cat}$, together with the low sensitivity to TEA, bears a striking similarity to the RCK5 cloned K⁺ channel (Stuhmer et al., 1989). However, another possibility is that the TEA-sensitivity is changed when the permeant ion is switched from K⁺ to Na⁺. This less obvious interpretation appears to be a reasonable possibility, since there was little TEA-insensitive K⁺ current in our cell population (Fig. 13). Indeed, the whole cell conductance for $I_{cat}$ was comparable to the whole cell conductance for the total delayed rectifier K⁺ current, and 5–10 times that of the TEA-insensitive K⁺ current. TEA apparently binds to sites within the K⁺ channel pore (MacKinnon and Yellen, 1990; Taglialatela, Vandongen, Drewe, Joho, Brown, and Kirsch, 1991). These binding sites may be intimately related to the conduction pathway, which would make an interaction between conducting ion and TEA plausible. Resolution of this question awaits the use of cloned channels to determine the exact identity of the K⁺ channel through which $I_{cat}$ flows.

**Tail Hook**

$I_{cat}$ tail currents often displayed a hook on the tail; after repolarization, the tail grew briefly before decaying (cf. Figs. 1, 2, 14). Hooks were usually observed with NMG⁺ as the dominant intracellular cation, and were voltage dependent; they were more prominent at positive repolarization potentials and often disappeared at repolarization potentials of $-100$ mV or more. Hooks similar to these are produced by quaternary ammonium ions in squid axon K⁺ channels, and are explained as a transient blocking effect following depolarization-induced entry of a nonconducting ion into the pore (Armstrong, 1969). Whether NMG⁺ produces a similar, transient block remains to be examined.

**Channel Identification**

Clearly, the kinetic characteristics of $I_{cat}$ closely resemble those of a delayed rectifier present in these cells. The alternative, that $I_{cat}$ flows through an A-type channel,
appears unlikely. The most compelling argument in favor of \( I_{\text{cat}} \) being a delayed rectifier relates to its rate of inactivation: 50% of the current remains after 1.5 s at +20 mV (Fig. 10) and 20% of the current remains after a 10-s prepulse to −40 mV (Fig. 11). In contrast, the A-type current in rat DRGs inactivates with time constants of 8 and 43 ms at +20 mV (Mayer and Sugiyama, 1988; the slower time constant may be derived from a delayed rectifier) and the A-type current in chick DRGs inactivates with a time constant of ~30 ms (Florio et al., 1990). Four other observations also argue that \( I_{\text{cat}} \) flows through delayed rectifier channels: (a) activation of \( I_{\text{cat}} \) is slower than the transient currents observed in our cells and other preparations, (b) the kinetic and voltage-dependent characteristics of \( I_{\text{cat}} \) were quite similar to those of the delayed rectifier in these cells, (c) the macroscopic current density of \( I_{\text{cat}} \) and the total delayed rectifier in these cells is statistically identical, and (d) \( I_{\text{cat}} \) was observed in all cells examined, but the fast transient current was not. It must be stressed that this identification is based on phenotype, and does not address the molecular identity of the channel. Studies of cloned channels have produced a more complicated picture of channel identity, that of one or more large families of K⁺ channels with kinetic characteristics that range from A-like to delayed rectifier-like (Stuhmer et al., 1989; Papazian, Timpe, Jan, and Jan, 1991; Salkoff, Baker, Butler, Covarrubias, Pak, and Wei, 1992; Chabala, Bakry, and Covarrubias, 1993; Rettig, Heinemann, Wunder, Lorra, Parcej, Dolly, and Pongs, 1994). It is therefore possible that the \( I_{\text{cat}} \) channel belongs to a molecular family that includes the A-type channel. It is also plausible that the inactivation kinetics of an A-type channel could change dramatically with the change in permeant ion. However, the data in Fig. 14, wherein a large \( I_{\text{cat}} \) appeared in a cell that was originally devoid of a fast transient outward K⁺ current, argue against this latter possibility.

Channel Sightings

The infrequency of observing \( I_{\text{cat}} \) may not be related so much to the infrequent presence of this particular delayed rectifier K⁺ channel but to the experimental conditions used to perform patch clamp recordings. In particular, most patch clamp investigators use Cs⁺ in pipette solutions to block K⁺ channels when K⁺ channels are not being investigated. Recently, Zhu and Ikeda (1993) reported an anomalous Na⁺ conductance \( (I_u) \) in rat superior cervical ganglion neurons that may be identical to \( I_{\text{cat}} \). Zhu and Ikeda (1993) attributed the occurrence of \( I_u \) to removal of extracellular K⁺, whereas we found that \( I_{\text{cat}} \) resulted from removal of intracellular K⁺. However, Zhu and Ikeda (1993) also found that addition of intracellular K⁺ blocked \( I_u \) and we observed that addition of 5 mM extracellular K⁺ blocked \( I_{\text{cat}} \) to the same extent as 5 mM intracellular K⁺. It would be expected that K⁺ would have access to the cation binding sites in the channel from both sides of the pore. However, our results differed from those of Zhu and Ikeda (1993) in one very important respect. Whereas \( P_{K}/P_{Na} \) ranged from 60–100 for \( I_{\text{cat}} \), Zhu and Ikeda (1993) found \( P_{K}/P_{Na} \) to be just 2.5 for \( I_u \). Whether this difference is due to a difference in the channel responsible for these currents or to the significantly different experimental techniques used to obtain permeability ratios is presently unclear. However, a 2.5-fold difference in permeability ratio would not be expected to preclude a significant Na⁺ component in \( I_K \) in the presence of K⁺. If selectivity were based simply on competition for entry into
the channel, a permeability ratio of 2.5 would produce potassium current reversal potentials of ~-20 mV in normal physiological solutions. Consequently, the results of Zhu and Ikeda suggest a totally different permeation model for $I_a$ than $I_{cat}$. The observation that $I_a$ has a $P_K/P_{Na}$ of 2.5, together with the low Na$^+$ conductance at high [K$^+$], requires that removal of K$^+$ alters either a barrier that resists Na$^+$ entry into the channel or a barrier that prevents Na$^+$ conductance (such as from a selectivity filter) after removal of K$^+$. Our results with $I_{cat}$ are best explained by a model in which neither an exclusion barrier nor a conductance barrier are altered by K$^+$.

Other investigators have described novel or unidentified inward currents that were similar in many ways to $I_{cat}$. Patch clamp recordings from CA1 hippocampal pyramidal cells revealed a tail current, called $I_x$ (Kay and Wong, 1987), that had many of the same characteristics of $I_{cat}$. It was voltage activated, blocked by intracellular Cs$^+$, and insensitive to Ca$^{2+}$ and Cd$^{2+}$. Although experimental details were not completely provided, it apparently was not blocked by Ba$^{2+}$ as would be expected if it were $I_{cat}$. Two other Na$^+$ conductances that appear to be quite similar to $I_{cat}$ were reported recently in patch clamp recordings from central neurons. To the extent that similar types of experiments were done, a slow inward tail current observed in patch clamp recordings from dorsal raphe neurons (Pennington and Kelly, 1993) was similar in all respects to $I_{cat}$. It was observed in cells recorded without intracellular K$^+$ and was blocked by Cs$^+$. Finally, Hoehn, Watson, and MacVicar (1993) described a TTX-insensitive Na$^+$ current in striatal and hippocampal neurons with the kinetic and pharmacologic profile of $I_{cat}$.

**Significance**

Studies that combine molecular techniques with biophysical studies have led to enormous leaps in our understanding of ion channel structure and function. The ability of some but not other delayed rectifiers to permit a large Na$^+$ conductance in the absence of K$^+$ suggests the presence of a subtle difference in channel structure that alters the permeation profile of the channel. Two points are of immediate interest. First, it will be important to determine the relationship between $I_{cat}$ and TEA sensitivity, which is associated with structural sites in the channel pore, some of which may be involved in channel inactivation (MacKinnon and Yellen, 1990; Choi, Aldrich, and Yellen, 1991; Taglialatela et al., 1991; Yellen, Jurman, Abramson, and MacKinnon, 1991). One of two possibilities will prevail. Either $I_{cat}$ flows through only TEA-insensitive channels, or channels lose their sensitivity to TEA when Na$^+$ is the permeant cation. Second, identification of structural differences between delayed rectifiers that permit Na$^+$ to conduct and those that don't will provide important information about the structural determinants of cation binding sites and conductance through voltage-gated channels.

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REFERENCES

Almers, W., and E. W. McCleskey. 1984. Non-selective conductance in calcium channels of frog muscle: calcium selectivity in a single-file pore. Journal of Physiology. 353:585–608.

Arhem, P. 1980. Effects of rubidium, caesium, strontium, barium and lanthanum on ionic currents in myelinated nerve fibers from Xenopus laevis. Acta Physiologica Scandinavica. 108:7–16.

Armstrong, C. M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. Journal of General Physiology. 50:553–575.

Bezanilla, F., and C. M. Armstrong. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. Journal of General Physiology. 60:588–608.

Belles, B., C. O. Malecot, J. Hescheler, and W. Trautwein. 1988. "Run-down" of the Ca current during long whole cell recordings in guinea pig heart cells: role of phosphorylation and intracellular calcium. Pfliigers Archiv. 411:353–360.

Callahan, M. J., and S. J. Korn. 1993. Removal of intracellular potassium alters K+ channel selectivity in chick dorsal root ganglion neurons. Society for Neuroscience Letters. 19:1337.

Chabala, L. D., N. Bakry, and M. Covarrubias. 1993. Low molecular weight poly(A)+ mRNA species encode factors that modulate gating of a non-Shaker A-type K+ channel. Journal of General Physiology. 102:713–728.

Choi, K. L., R. W. Aldrich, and G. Yellen. 1991. Tetraethylammonium block distinguishes two inactivation mechanisms in voltage-activated K+ channels. Proceedings of the National Academy of Sciences, USA. 88:5092–5095.

Cukierman, S., G. Yellen, and C. Miller. 1985. The K+ channel of sarcoplasmic reticulum. A new look at Cs+ block. Biophysical Journal. 48:477–484.

Danko, M., C. Smith-Maxwell, L. McKinney, and T. Begenisich. 1986. Block of sodium channels by internal mono- and divalent guanidinium analogues. Modulation by sodium ion concentration. Biophysical Journal. 49:509–519.

Eckert, R., J. E. Chad, and D. Kalman. 1986. Enzymatic regulation of calcium current in dialyzed and intact molluscan neurons. Journal de Physiologie. 81:518–524.

Florio, S. K., C. D. Westbrook, M. R. Vasko, R. J. Bauer, and J. L. Kenyon. 1990. Transient potassium currents in avian sensory neurons. Journal of Neurophysiology. 63:725–737.

Forscher, P., and G. S. Oxford. 1985. Modulation of calcium channels by norepinephrine in internally dialyzed avian sensory neurons. Journal of General Physiology. 85:743–763.

French, R. J., and J. J. Shoukimas. 1985. An ion’s view of the potassium channel. The structure of the permeation pathway as sensed by a variety of blocking ions. Journal of General Physiology. 85:669–678.

French, R. J., and J. B. Wells. 1977. Sodium ions as blocking agents and charge carriers in the potassium channel of the squid giant axon. Journal of General Physiology. 70:707–724.

Gay, L. A., and P. R. Stanfield. 1978. The selectivity of the delayed potassium conductance of frog skeletal muscle fibers. Pfliigers Archiv. 378:177–179.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfliigers Archiv. 381:85–100.

Hess, P., and R. W. Tsien. 1984. Mechanism of ion permeation through calcium channels. Nature. 309:453–456.

Hille, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. Journal of General Physiology. 61:669–686.

Hille, B. 1992. Ionic Channels in Excitable Membranes. Second edition. Sinauer Associates Inc., Sunderland, MA.
Hille, B., and W. Schwarz. 1978. Potassium channels as multi-ion single-file pores. *Journal of General Physiology.* 72:409–442.

Hoehn, K., T. W. J. Watson, and B. A. MacVicar. 1993. A novel tetrodotoxin-insensitive, slow sodium current in striatal and hippocampal neurons. *Neuron.* 10:543–552.

Ikeda, S. R., and G. G. Schofield. 1987. Tetrodotoxin-resistant sodium current of rat nodose neurons: monovalent cation selectivity and divalent cation block. *Journal of Physiology.* 389:255–270.

Kay, A. R., and R. K. S. Wong. 1987. Calcium current activation kinetics in isolated pyramidal neurons of the CA1 region of the hippocampus. *Journal of Physiology.* 392:605–616.

Latorre, R., and C. Miller. 1983. Conduction and selectivity in potassium channels. *Journal of Membrane Biology.* 71:11–30.

MacKinnon, R., and G. Yellen. 1990. Mutations affecting TEA blockade and ion permeation in voltage-activated K⁺ channels. *Science.* 250:276–279.

Matuson, D. R., and R. P. Swenson, Jr. 1986. External monovalent cations that impede the closing of K channels. *Journal of General Physiology.* 87:795–816.

Mayer, M. L. 1985. A calcium-activated chloride current generates the after-depolarization of rat sensory neurones in culture. *Journal of Physiology.* 364:217–239.

Mayer, M. L., and K. Sugiyama. 1988. A modulatory action of divalent cations on transient outward current in cultured rat sensory neurones. *Journal of Physiology.* 396:417–433.

Papazian, D. M., L. C. Timpe, Y-N. Jan, and L. Y. Jan. 1991. Alteration of voltage-dependence of *Shaker* potassium channel by mutations in the S4 sequence. *Nature.* 349:305–310.

Pennington, N. J., and J. S. Kelly. 1993. Ionic dependence of a slow inward tail current in rat dorsal raphe neurones. *Journal of Physiology.* 464:33–48.

Pusch, M., and E. Neher. 1988. Rates of diffusional exchange between small cells and a measuring pipette. *Pflügers Archiv.* 411:204–211.

Reuter, H., and C. F. Stevens. 1980. Ion conductance and ion selectivity of potassium channels in snail neurones. *Journal of Membrane Biology.* 57:103–118.

Salkoff, L., K. Baker, A. Butler, M. Covarrubias, M. D. Pak, and A. Wei. 1992. An essential ‘set’ of K⁺ channels conserved in flies, mice and humans. *Trends in Neurosciences.* 15:161–166.

Stuhmer, W., J. F. Ruppersberg, K. H. Schrotter, B. Sakmann, M. Stocker, K. P. Giese, A. Perschke, A. Baumann, and O. Pongs. 1989. Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. *European Molecular Biology Organization Journal.* 8:3235–3244.

Taglialatela, M., A. M. J. Vandongen, J. A. Drewe, R. H. Joho, A. M. Brown, and G. E. Kirsch. 1991. Patterns of internal and external tetraethylammonium block in four homologous K⁺ channels. *Molecular Pharmacology.* 40:299–307.

Tasaki, I., and S. Hagiwara. 1957. Demonstration of two stable potential states in the squid giant axon under tetraethylammonium chloride. *Journal of General Physiology.* 40:859–885.

Wagoner, P. K., and G. S. Oxford. 1987. Cation permeation through the voltage-dependent potassium channel in the squid axon. Characteristics and mechanisms. *Journal of General Physiology.* 90:261–290.

Westbrook, C. D., and J. L. Kenyon. 1990. Potassium currents in chick sensory neurons change with development. *Developmental Brain Research.* 54:1–10.

Yellen, G., M. E. Jurman, T. Abramson, and R. MacKinnon. 1991. Mutations affecting internal TEA blockade identify the probable pore-forming region of a K⁺ channel. *Science.* 251:999–941.

Zhu, Y., and S. R. Ikeda. 1993. Anomalous permeation of Na⁺ through a putative K⁺ channel in rat superior cervical ganglion neurones. *Journal of Physiology.* 468:441–461.