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INWARD RECTIFICATION IN NEONATAL RAT SPINAL MOTONEURONES

BY TOMOYUKI TAKAHASHI

From the Department of Physiology, Kyoto University Faculty of Medicine, Kyoto 606, Japan

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SUMMARY

1. Inward rectifying currents were recorded, using tight-seal, whole-cell voltage-clamp methods, from motoneurones visually identified in thin slices of neonatal rat spinal cord.

2. When motoneurones were hyperpolarized from holding potentials near the resting potential (−60 to −70 mV), a slow inward-going current was recorded. After the hyperpolarizing command pulses, inward tail currents were recorded. Amplitudes of the inward current at the end of hyperpolarizing pulses as well as those of the tail current increased non-linearly with the membrane hyperpolarization, showing an inward rectification in the current–voltage relation.

3. Neither the amplitude nor the kinetics of the inward rectifying current (I_{IR}) was appreciably affected by replacement of extracellularly Ca^{2+} with Mg^{2+} combined with the application of tetrodotoxin (1 μM), tetraethylammonium (30 mM), and 4-aminopyridine (4 mM). The current was relatively resistant to Ba^{2+}, being only slightly suppressed at 2 but not at 0·2 mM. However, it was completely and reversibly abolished by Cs^+ (2 mM).

4. When the external K^+ concentration was raised, I_{IR} was augmented. However, the activation curve of I_{IR} constructed from relative tail current amplitudes in high K^+ solutions was indistinguishable from that in normal solution. The chord conductance of I_{IR} at various membrane potentials was similar for both normal and high K^+ solutions. Thus the whole-cell conductance of inward rectification in motoneurones depends on the membrane potential but not appreciably on the external K^+ concentration ($[K^+]_o$).

5. The reversal potential of I_{IR} was estimated by measuring the tail currents. In standard solution ($[K^+]_o = 3$ mM), the reversal potential was about −44 mV. Increasing $[K^+]_o$ shifted the reversal potential toward positive potentials by 22 mV for a tenfold change in potassium concentration

6. A fivefold reduction in the external Na^+ concentration shifted the reversal potential of I_{IR} in a negative direction by about 7 mV, suggesting that Na^+ may carry part of I_{IR}. A fivefold reduction in external Cl^- concentration shifted the reversal potential by about 2 mV but in a negative direction, the opposite of the expected shift in the Cl^- equilibrium potential.

7. When external Cl^- was substituted with isethionate or gluconate, I_{IR} was markedly and reversibly suppressed.

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8. It is concluded that in spinal motoneurones, I_{IR} is carried by both K^+ and Na^+ ions and that external Cl^- might be required to maintain the inward rectifier current.

INTRODUCTION

An asymmetrical membrane conductance, greater for inward than outward currents, was first described by Katz (1949) in skeletal muscle. This membrane behaviour, termed as anomalous rectification, was later found also in cardiac muscle (Hall, Hutter & Noble, 1963) and in eggs of marine animals (Hagiwara & Takahashi, 1974). It has been reported that the anomalous rectifier current is carried predominantly by K^+ ions and that its conductance varies as a function of the K^+ driving potential as well as of the extracellular K^+ concentration ($[K^+]_o$, Hagiwara & Takahashi, 1974; Leech & Stanfield, 1981).

More recently, another type of inward rectification carried by multiple cations has been found in cardiac muscle and termed I_h (Yanagihara & Irisawa, 1980) or I_f (Brown & DiFrancesco, 1980), while a similar current found in hippocampal neurones has been termed I_q (Halliwell & Adams, 1982). In addition, voltage-gated Cl^- currents ($I_{Cl(V)}$) showing inward rectification on hyperpolarization have been reported in *Aplysia* neurones (Chesnoy-Marchais, 1982), amphibian oocytes (Parker & Miledi, 1988) and in mammalian hippocampal neurones (Madison, Malenka & Nicoll, 1986).

In vertebrate neurones, all three types of inward rectifier have been reported: anomalous rectifier (Constanti & Galvan, 1983; Kaneko & Tachibana, 1985*b*; Stanfield, Nakajima & Yamaguchi, 1985; Williams, Colmers & Pan, 1988); I_h, I_f and I_q (Bader, Bertrand & Schwartz, 1982; Halliwell & Adams, 1982; Mayer & Westbrook, 1983; Kaneko & Tachibana, 1985*a*) and $I_{Cl(V)}$ (Madison *et al.* 1986). The membrane of spinal motoneurones is known to rectify inwardly upon hyperpolarization (Ito & Oshima, 1965; Nelson & Frank, 1967; Barrett, Barrett & Crill, 1980). However, as yet little is known about the ionic mechanism of this inward rectification. The aim of the present study was to characterize inward rectification in mammalian spinal motoneurones *in vitro* by whole-cell voltage-clamp recording.

METHODS

The preparation and recording methods were as described in the preceding paper (Takahashi, 1990). When Cl^- concentration were lowered, NaCl was substituted either with sodium isethionate or sodium gluconate. In Cl^--free solutions, KCl and $MgCl_2$ were also substituted with K_2SO_4 and $MgSO_4$. To gain better current resolution spontaneous inhibitory synaptic currents were abolished by adding strychnine (2 μM; Takahashi, 1984) unless otherwise noted. The standard pipette solution had the following composition (in mM): KCl, 18; potassium gluconate, 123; NaCl, 9; $MgCl_2$, 1; EGTA, 0·2; HEPES, 10; pH adjusted to 7·3 with KOH. In some early experiments, 140 mM-KCl was used instead of KCl and potassium gluconate (see legends). Membrane potential values given in the text were corrected for the liquid-junction potential between the perfusing solution and the pipette solution (8 mV for the standard pipette solution). When the external Cl^- concentration was changed, a 3 M-KCl–agar bridge, instead of a Ag–AgCl electrode, was used as the indifferent electrode. All experiments were carried out at room temperature (22–24 °C).

RESULTS

General properties of inward rectification

When motoneurones were hyperpolarized in steps from a holding potential of about −70 mV to various potentials, an instantaneous inward current jump was followed by a gradually developing inward current (Fig. 1*A*, control). The latter current approximately followed an exponential time course with an average time constant of about 2·4 s (five motoneurones) for command pulses to about −100 mV (Fig. 1*C* and *D*, ○). At the end of the command pulse, inward tail currents returned to control levels following an initial jump. The current–voltage (*I–V*) relation of the instantaneous current (I_0) was linear between −68 and −108 mV, suggesting that its slope represents the membrane leak conductance of the motoneurones at the holding potential (Fig. 1*B*, ○). The amplitude of the instantaneous current at the end of a hyperpolarizing command pulse (I_t, Fig. 1*B*, filled symbols) was larger than that at the beginning (I_0, Fig. 1*A* and *B*, control), indicating that the membrane conductance had increased during hyperpolarization. The slowly developing inward current component can be expressed as the difference between I_0 and I_t. The *I–V* plot of $I_0 - I_t$ showed clear inward rectification (Fig. 1*C*, ○).

For further analysis, to separate inward rectifier currents from other voltage-gated currents, the perfusate was changed from the standard Krebs solution to a Ca^{2+}-free–Mg^{2+} (5 mM) solution containing tetrodotoxin (TTX, 1 μM), tetraethylammonium (TEA, 30 mM) and 4-aminopyridine (4-AP, 4 mM) in concentrations to suppress the fast inward Na^+ current (I_{Na}), the delayed K^+ current (I_K) and the A-current (I_A), respectively (Takahashi, 1990). The membrane leak conductance measured from the slope of I_0 under this condition was slightly decreased (Fig. 1*B*, △). However, the magnitude of the inward rectifying current was not affected (Fig. 1*C*, △). The time course of the inward rectifier in the presence of these channel blockers (Fig. 1*D*, ●) was similar to that in control (○). The following experiments were made in the presence of these blockers.

Barium and caesium

Low concentrations of barium are known to block the anomalous rectifying currents carried by K^+ ions (10–100 μM, Hagiwara, Miyazaki, Moody & Patlak, 1978; 500 μM, Constanti & Galvan, 1983). However, even at higher concentrations, Ba^{2+} has a little effect on I_h (5 mM-Ba^{2+}, Yanagihara & Irisawa, 1980) or no effect on I_q currents (1 mM-Ba^{2+}, Halliwell & Adams, 1982). On the other hand, extracellularly applied Cs^+, in millimolar concentrations, blocks both the anomalous rectifier (0·5–1 mM, Hagiwara, Miyazaki & Rosenthal, 1976) and I_h, I_f and I_q currents (20 mM, DiFrancesco & Ojeda, 1980; 0·5–3 mM, Halliwell & Adams, 1982) as well as other inward rectifiers (with a dissociation constant, K_d, of 3 mM; Quayle, Standen & Stanfield, 1988). Conversely, M-currents are abolished by Ba^{2+} (1 mM) but not by Cs^+ (3 mM, Halliwell & Adams, 1982).

As shown in Fig. 2, the magnitude of the inward rectifier was only slightly (by 20–35%, range of three motoneurones, hyperpolarization to −100 mV) suppressed by high concentrations of Ba^{2+} (2 mM, Fig. 2: *A*, middle panel; *C*, △). The membrane leak conductance was similarly reduced during Ba^{2+} application (Fig. 2*B*, △). Lower

concentrations of Ba^{2+} (0·2 mM) affected neither the leak conductance nor the inward rectifier (Fig. 2*D*).

When CsCl (2 mM) was added to the external solution, the inward rectifying current was markedly suppressed (Fig. 3*A*, middle panel; *C*, △). This effect was

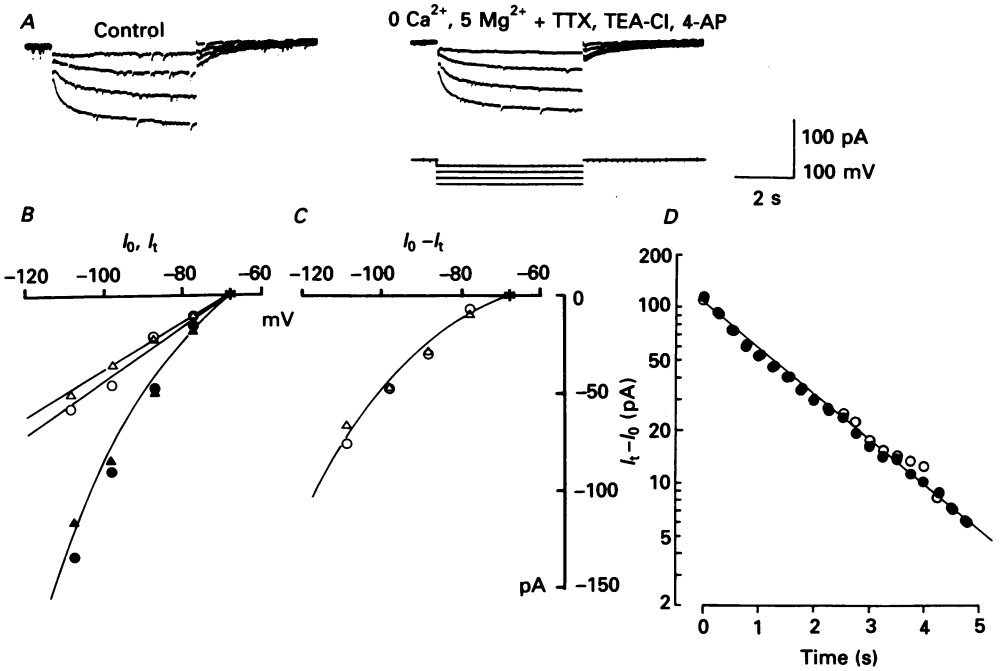

Fig. 1. Time- and voltage-dependent inward rectification evoked in a motoneurone by hyperpolarizing command pulses. *A*, currents in standard Krebs solution (control, left) and in Ca^{2+}-free–Mg^{2+} Krebs solution containing 1 μM-TTX, 30 mM-TEA-Cl and 4 mM-4-AP (right). Command voltage pulses used for these experiments are shown in the lower record. Holding potential was −68 mV (* in *B* and *C*) in this and following figures. *B*, *I–V* relations for the instantaneous current at the onset of the command pulses (I_0; open symbols) and the steady-state current at the end (I_t; filled symbols). *C*, *I–V* relation of the difference ($I_0 - I_t$). Circles, control; triangles, 0 mM-Ca^{2+}, 5 mM-Mg^{2+} plus antagonists. *D*, semilogarithmic plot of the time course of inward currents in control (○) and in 0 mM-Ca^{2+}, 5 mM-Mg^{2+} plus antagonists (●) solutions. Data points were derived from the average of five current recordings elicited by command voltage steps to −98 mV. The line was drawn by eye.

reversible (Fig. 3*A*, recovery; *C*, □). The membrane leak conductance was also reduced during Cs^+ application (Fig. 3*B*, △, cf. ○). Thus, the effects of Ba^{2+} and Cs^+ on the inward rectification of motoneurones are similar to those reported for I_h, I_f and I_q. Caesium seemed to block inward rectification only from the outside since inward rectification was well maintained when CsCl (140 mM) was present in the pipette solution (not shown).

Potassium

When the external K^+ concentration was raised from 3 to 12 mM, the amplitudes of both the inward rectifying current (Fig. 4*A* and *C*) and the membrane leak

conductance (Fig. 4*B*) were markedly increased. The magnitude of I_{IR} ($I_0 - I_t$, Fig. 4*C*) at −100 mV in 12 mM $[K^+]_o$ was, on average, 3·1 times larger than that in 3 mM $[K^+]_o$ (range, 1·2–6·7, three motoneurones). Similarly, the magnitude in 24 mM $[K^+]_o$ was 2·4 (range; 1·7–4·1, four motoneurones) times larger than that in 6 mM $[K^+]_o$.

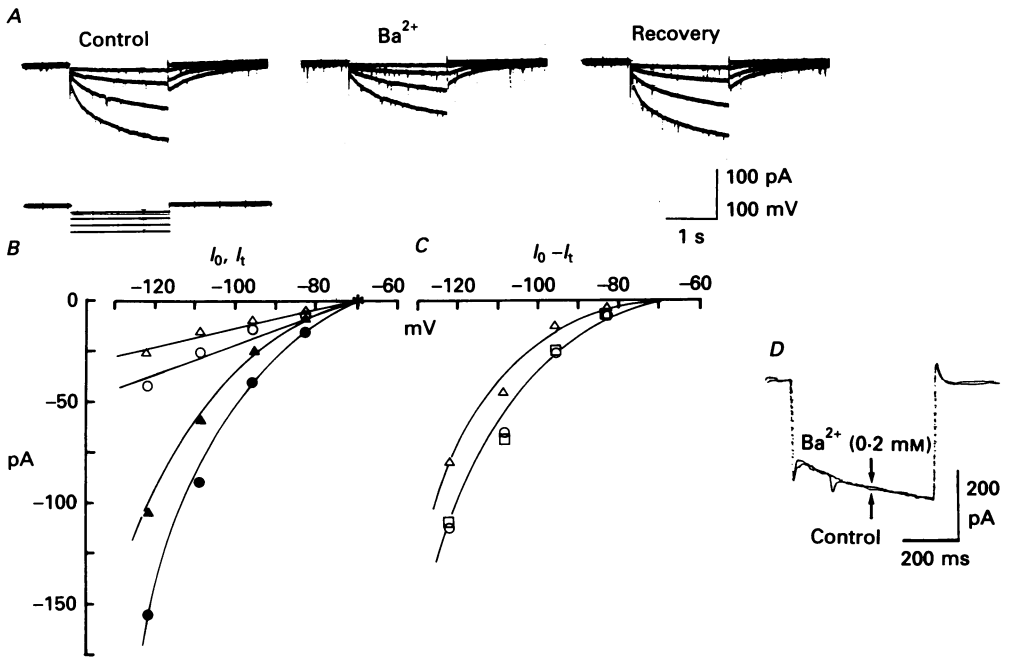

Fig. 2. Effects of Ba^{2+} on inward rectifying current. *A*, upper traces (left to right): currents in control, during application of 2 mM-$BaCl_2$ and after recovery from Ba^{2+}. Lower trace, command voltage pulses. *B*, *I–V* relation of I_0 (○, control; △, after application of Ba^{2+}) and I_t (●, control; ▲, during $BaCl_2$ application). *C*, *I–V* relation of $I_0 - I_t$ (○, △ and □ correspond to control, during Ba^{2+} application and recovery after washing out Ba^{2+}, respectively). Control solution is a standard Krebs solution, containing 5 mM-Mg^{2+}. Ba^{2+} was substituted for Ca^{2+}. *D*, effects of low concentration of $BaCl_2$ (0·2 mM) in another motoneurone. The currents were evoked by membrane hyperpolarization from a holding potential of −53 to −138 mV. Two traces in control and during Ba^{2+} application were superimposed. In this particular experiment, the pipette solution contained 140 mM-KCl without potassium gluconate. External solution, Ca^{2+}-free–Mg^{2+} Krebs solution containing TTX (1 μM) and TEA-Cl (30 mM).

Tail currents were recorded to study the ionic basis of inward rectification (Fig. 5). Following an initial hyperpolarizing pulse to −104 mV for 2 s, the membrane potential was stepped back to various levels by the second pulse (6 s in duration; the end of the second pulse is not shown in Fig. 5*A*). In standard Krebs solution, this protocol for analysing tail currents simultaneously evoked various voltage-gated currents such as inward Na^+ and Ca^{2+} currents and outward K^+ currents, including the A-current (Takahashi, 1990). Therefore the experiments were made in a Ca^{2+}-free–Mg^{2+} bathing solution containing TTX, TEA and 4-AP. The inward rectifying tail currents, which were obtained by subtraction of the current recorded without the hyperpolarizing pre-pulse from that evoked following the pre-pulse, reversed at −43·8 ± 1·9 mV (mean ± S.D., five motoneurones) in a solution containing 3 mM-K^+

(Fig. 5*A*, 3 mM-K^+; ● in *B*). This value was about 20 mV more positive than the resting potential and about 55 mV more positive than the K^+ equilibrium potential (E_K) estimated from the internal and external ionic compositions (cf. Fig. 7 Takahashi, 1990).

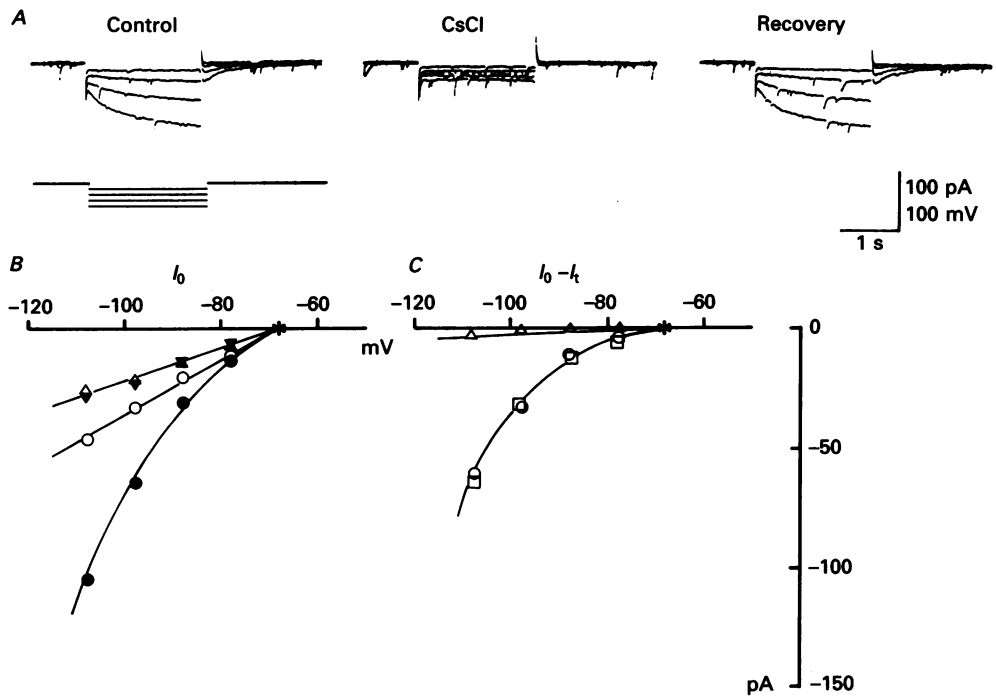

Fig. 3. Effect of Cs^+ on inward rectifier current. *A*, *B* and *C* as in previous figures. Control, standard Krebs solution. CsCl (2 mM) was added in substitution for $CaCl_2$ (1 mM). *B*, I_0 (○, control; △, with Cs^+) and I_t (●, control; ▼, with Cs^+). *C*: ○, △ and □ represent control, during Cs^+ application and recovery after washing out Cs^+, respectively. The asterisks in *B* and *C* represent the holding potential.

When $[K^+]_o$ was raised fourfold from 3 to 12 mM, the *I–V* plot of the peak tail currents shifted in a positive direction (Fig. 5*B*, ▼). The magnitude of the shift in reversal potential was $10{\cdot}6 \pm 2{\cdot}9$ mV (five motoneurones). Similarly, a fourfold change of $[K^+]_o$ from 6 to 24 mM caused a positive shift of the reversal potential by $12{\cdot}8 \pm 1{\cdot}5$ mV (four motoneurones).

When these reversal potentials were plotted against the logarithm of $[K^+]_o$ (Fig. 5*C*), the regression line had a slope of about 22 mV per 10-fold change in $[K^+]_o$ indicating that the inward rectifying currents are carried partly but not entirely by K^+ ions.

Another way of estimating the reversal potential of the inward rectifier is to compare the ohmic membrane currents at two potential levels well below the activation threshold for depolarization-induced currents. This difference would reflect the steady-state currents of inward rectification induced by membrane hyperpolarization. Under these conditions, the reversal potential of the inward rectification (E_{IR}) can be estimated from the intercept of the two *I–V* curves (e.g.

Mayer & Westbrook, 1983). This method is advantageous over the tail method in that the estimation of E_{IR} is uncontaminated by depolarization-induced currents. One disadvantage, however, is in possible errors caused by extrapolation.

The reversal potential of the inward rectifier was estimated by the two methods in

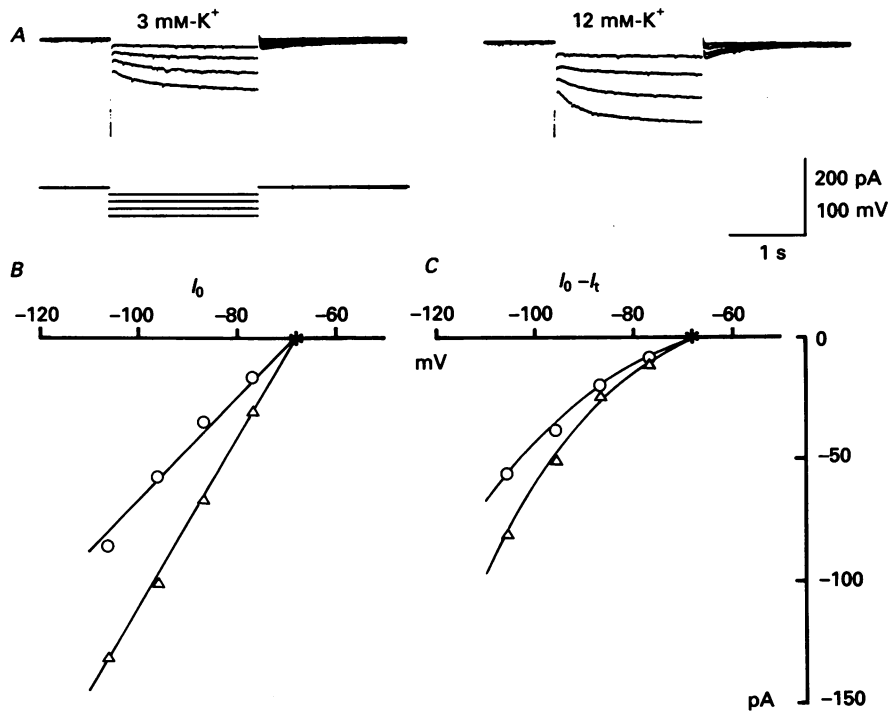

Fig. 4. Inward rectifier current in normal (3 mM) and high (12 mM) $[K^+]_o$. *A*, sample records in 3 and 12 mM-K^+ in response to command voltages indicated in the lower trace. *B* and *C*, *I–V* relations of I_0 and $I_0 - I_t$ respectively in 3 mM-K^+ (○) and 12 mM-K^+ (△) in Ca^{2+}-free–Mg^{2+} solution containing TTX, TEA-Cl and 4-AP. Asterisks represent the holding potential.

a motoneurone in 12 mM-K^+ solution (Fig. 6). Hyperpolarizing command pulses were given from a holding potential of −66 mV (Fig. 6*A a* and *A c*) and depolarizing pulses from −99 mV (*Ab*). The magnitude of the pulses was limited to the potential range in which the *I–V* relation was linear. The current amplitude were measured near the pulse onset just after the capacitative current stage subsided (50 ms after the onset; arrows in Fig. 6*A*) and were plotted against command voltages. The slopes of the linear regression lines throughout these points represent the membrane conductances at −66 and −99 mV, respectively. The extrapolated intercept of the two regression lines occurred at −34 mV (Fig. 6*B*). The reversal potential of tail currents recorded in the same motoneurone (Fig. 6*C*) was estimated by interpolation to be −30 mV (Fig. 6*D*). Thus, the reversal potentials of the inward rectifier estimated by the two independent methods were in good agreement.

Voltage-dependent activation of the inward rectifier was studied by measuring the tail currents evoked by hyperpolarization (Fig. 7). As the intensity of the hyperpolarizing command pulses was increased by steps (lower trace in inset), the

magnitude of the tail current increased till it reached a plateau at hyperpolarizing steps beyond −120 mV. When $[K^+]_o$ was raised from 3 to 12 mM, the amplitudes of the tail currents increased (Fig. 7, inset; 12 *vs.* 3 mM-K^+). This is consistent with the larger steady-state inward current observed in higher K^+ solution (Fig. 4*C*). When

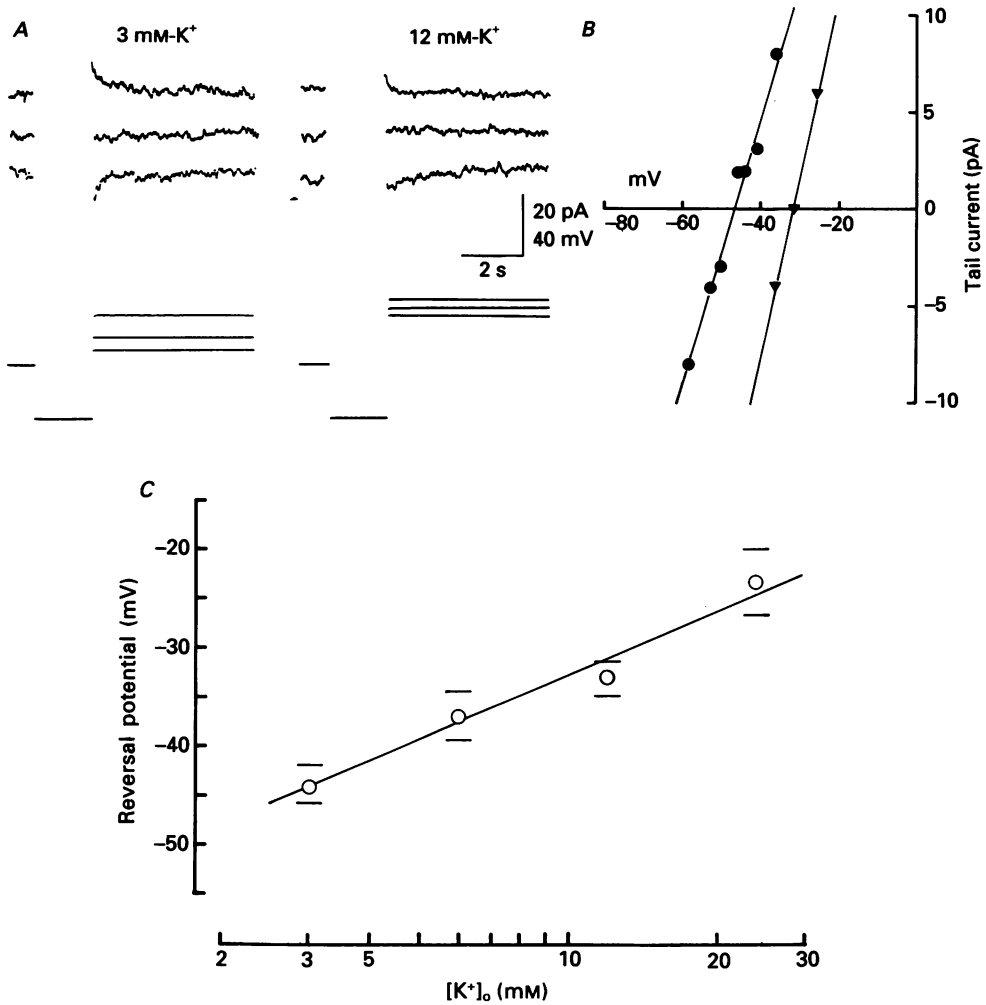

Fig. 5. Reversal potential of the inward rectifier in the same motoneurone as shown in Fig. 4. *A*, tail currents were evoked in 3 and 12 mM-K^+ using the command voltage pulses shown in the lower traces. Holding potential −68 mV. *B*, peak amplitudes of the tail currents plotted against membrane potential in normal (3 mM, ●) and high $[K^+]_o$ (12 mM, ▼) solutions. *C*, reversal potentials of the inward rectifier tail current in $[K^+]_o$ of 3, 6, 12 and 24 mM. Data points and error bars indicate means and S.D.s derived from five to seven motoneurones. Continuous line was drawn by the least-squares method.

amplitudes of tail currents (I) evoked by hyperpolarizing steps to different membrane potentials were normalized against the maximum tail current amplitude (I_{max}), the activation curve of the relative inward rectifier tail current (I) thus obtained was

S-shaped (Fig. 7). The activation curves looked similar in both 3 mM (△) and 12 mM (○) $[K^+]_o$ and approximately fitted the equation

$$I/I_{max} = (1+\exp(V-V_h)/k)^{-1},$$

where V is membrane potential and V_h the potential at which I equals 50% of I_{max}.

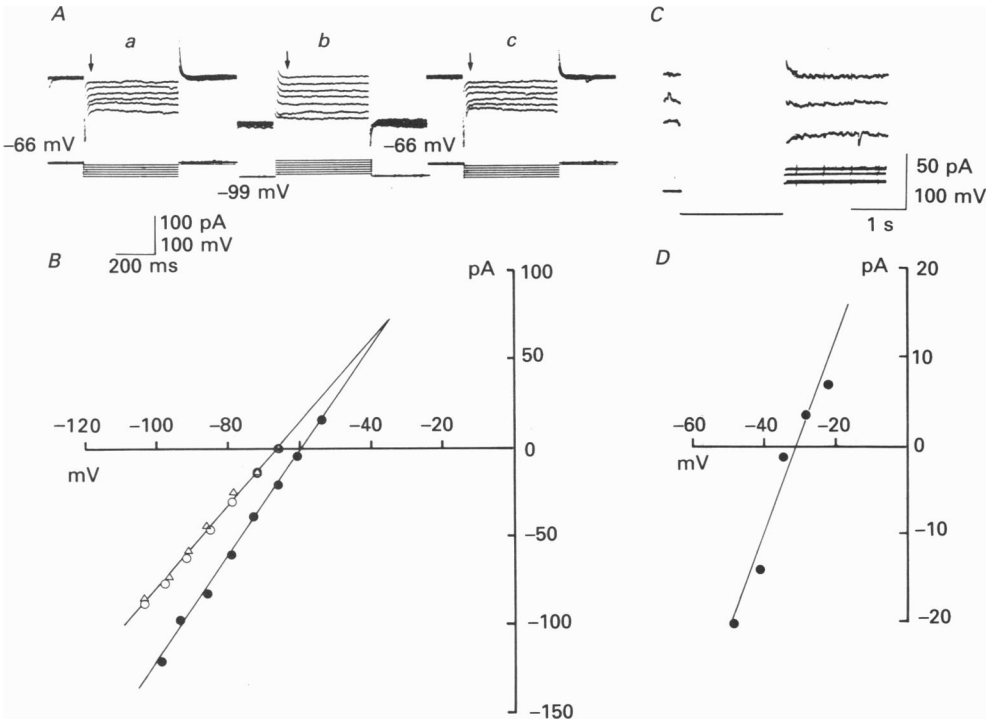

Fig. 6. Estimation of the reversal potential of the inward rectifier by two different methods (*A*, *B* and *C*, *D*) in a motoneurone. *A*; currents (upper traces) evoked by voltage command steps (lower traces) from holding potentials of −66 mV (*a* and *c*) and −99 mV (*b*). Current amplitudes for each voltage step were measured 50 ms after the pulse onset (arrows) when the capacitative current surge had practically subsided. *B*; current amplitude plotted against membrane potential at a holding potential of −66 mV (○ and △ corresponding to *Aa* and *Ac*, respectively) and at −99 mV (●, *Ab*). Linear regression lines for data points at −66 and −99 mV crossed at −34 mV when extrapolated. *C*, sample records of tail currents (upper traces) evoked by the voltage commands shown in lower traces. *D*, tail currents plotted against membrane potential as in Fig. 5. External solution was Ca^{2+}-free–Mg^{2+} Krebs containing 12 mM-K^+ and TTX, TEA-Cl and 4-AP (same concentrations as Fig. 1); no strychnine present.

The best-fitted curve was approximated with $V_h = -95$ mV and a slope factor, $k = 13{\cdot}5$ mV (Fig. 7). These results indicate that the conductance of the inward rectifier in motoneurones depends on the membrane potential but not on the driving force for K^+ ions. Thus the activation profile of inward rectification in motoneurones contrasts with those of the anomalous rectification observed in marine eggs (Hagiwara & Takahashi, 1974) and skeletal muscle fibres (Leech & Stanfield, 1981),

but agrees well with those of I_h observed in sensory neurones, retinal cells and cortical cells (Mayer & Westbrook, 1983; Kaneko & Tachibana, 1985*a*; Spain, Schwindt & Crill, 1987; but see Bader & Bertrand, 1984).

The chord conductance (G) of the inward rectifier can be expressed as

$$G = (I_t - I_0)/(V_r - V),$$

where V and V_r are the membrane potential and the reversal potential of the inward rectifier, respectively.

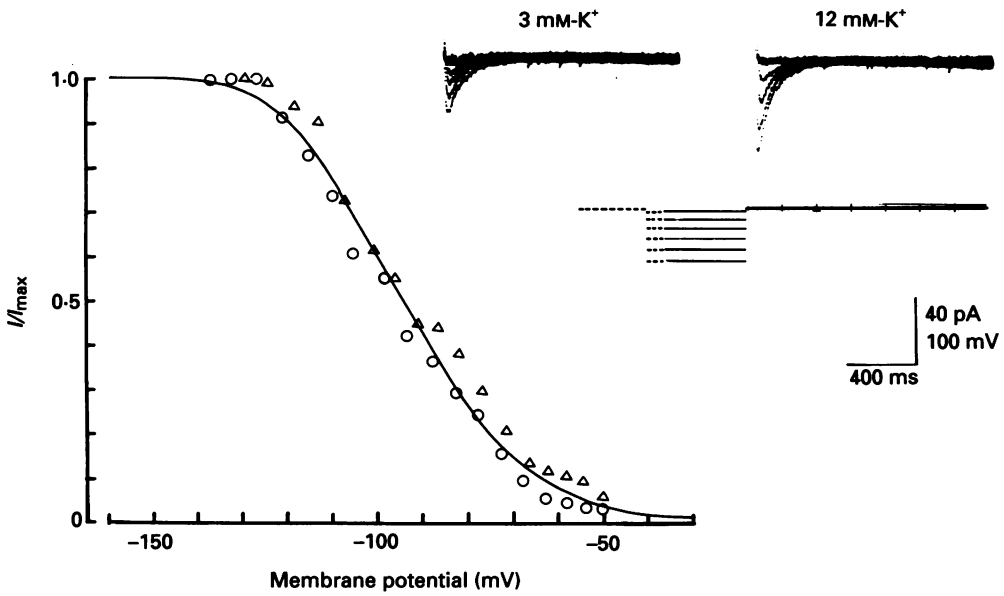

Fig. 7. Activation curve of the inward rectifier tail current of a motoneurone in two different $[K^+]_o$ (3 and 12 mM). Tail currents were evoked by hyperpolarizing command voltage steps as shown in the lower traces of the inset (dashed lines indicate the baseline and the 2 s pulse steps truncated in the original pictures). Six superimposed tail currents in solutions with 3 and 12 mM $[K^+]_o$ are shown in upper traces. Tail currents (I) evoked by various magnitudes of hyperpolarization were normalized to the maximum tail current amplitude (I_{max}) and I/I_{max} plotted against membrane potential. △ and ○ each correspond to the data points at the external K^+ concentrations of 3 and 12 mM, respectively. Holding potential −46 mV. External solution contained TTX, TEA-Cl and 4-AP as in Figs 1, 4, 5 and 6. The curve was drawn according to the equation $I/I_{max} = (1+\exp(V-95)/13{\cdot}5)^{-1}$.

As shown in Fig. 8, the chord conductance of inward rectification increased as the membrane was hyperpolarized. For different $[K^+]_o$s (3 mM, ○ and 12 mM, △) the chord conductance was similar. This is further evidence that the conductance of the inward rectifier depends entirely on membrane potential but not on $[K^+]_o$.

Sodium

Since the reversal potential of the tail currents is far more positive than E_K and depends only partly upon $[K^+]_o$ (Fig. 4*C*), it is suggested that inward rectification in motoneurones involves ions other than just K^+. When $[Na^+]_o$ was reduced about

fivefold from 139 to 26 mM, the reversal potential of the tail currents (Fig. 9, ▲) shifted in a negative direction by 6·7 ± 1·8 mV (six motoneurones). The shift in reversal potential corresponded to about one-sixth of that expected from the Nernst equation for a Na^+ electrode. The slope of the tail current *I–V* relation was also less steep in the low Na^+ solution. It is suggested that Na^+ participates in carrying the inward current but that its contribution as a carrier is less than that of K^+.

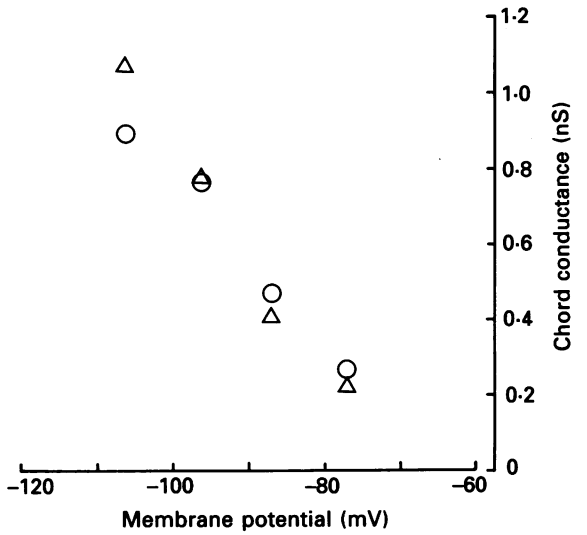

Fig. 8. Relationship between membrane potential and the chord conductance of the inward rectifier. Chord conductance at each membrane potential was calculated from the current amplitude ($I_0 - I_t$, Fig. 4*C*) and reversal potentials estimated in Fig. 5, according to the equation described in the text. in normal (3 mM, ○) and high (12 mM, △) $[K^+]_o$.

Chloride

When the external Cl^- concentration ($[Cl^-]_o$) was reduced about fivefold from 156 to 30 mM by replacement with the impermeant ion isethionate, the reversal potential of the inward rectifier tail current (Fig. 9, △) was slightly more negative than control (○) by 1·8 ± 1·1 mV (four motoneurones). However, this shift in reversal potential was the opposite to that expected if Cl^- ions carry the inward rectifier current. It is thus unlikely that the inward rectifier current is carried significantly by Cl^-.

When external Cl^- was totally replaced by the impermeant anions gluconate (Fig. 10) or isethionate (not shown), inward rectification was markedly suppressed (Fig. 10*A*, middle panel; *C*, △, cf. ○) with almost complete recovery on returning to the control solution (Fig. 10*A*, right panel; *C*, □). The leak conductance of the motoneurone measured by hyperpolarizing pulses did not appreciably change in a Cl^--free solution (Fig. 10*B*). Since the intracellular Cl^- concentration, $[Cl^-]_i$, was maintained by the pipette solution, the present results cannot be attributed to redistribution of intracellular Cl^- secondary to removal of external Cl^-. Also, since external Cl^- does not carry inward current, the suppression of the inward rectifier current in the absence of external Cl^- indicates that it may somehow be maintained by external Cl^-.

DISCUSSION

The present study has demonstrated that a slowly developing inward current can be evoked in motoneurones by hyperpolarization. This current displayed inward rectification in a manner similar to the anomalous rectification in skeletal muscle

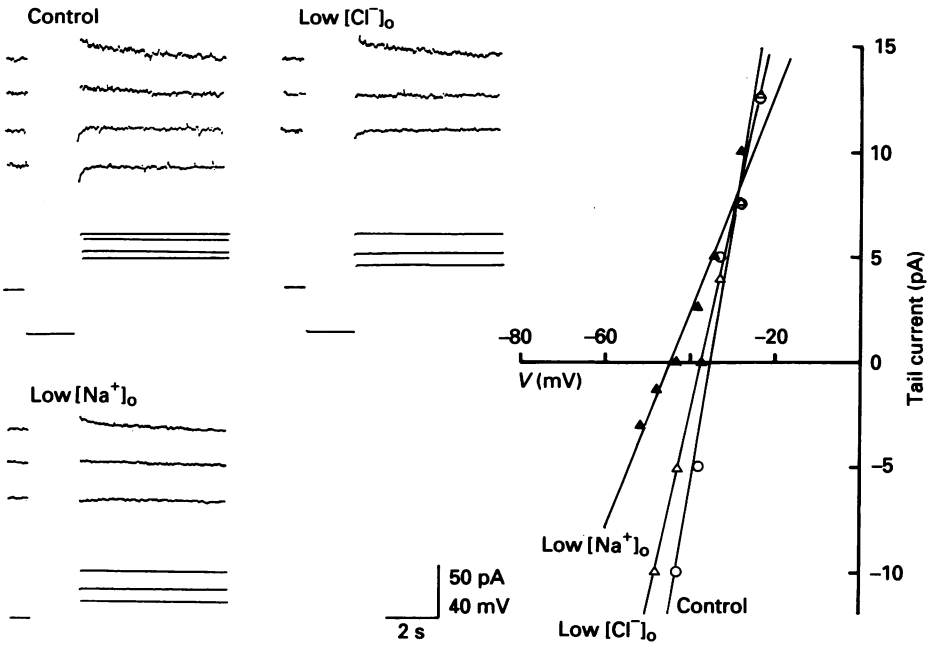

Fig. 9. Effects of varying extracellular Na^+ and Cl^- concentrations on the reversal potential of the inward rectifying tail currents. Sample records and command voltages are as in Figs 5 and 6. Tail current amplitudes in low external Na^+ (26 mM, ▲), in low external Cl^- (30 mM, △) are plotted against membrane potential. Perfusate, a Ca^{2+}-free–Mg^{2+} solution containing 12 mM-K^+, TTX, TEA-Cl and 4-AP.

(Katz, 1949; Leech & Stanfield, 1981) and marine oocytes (Hagiwara & Takahashi, 1974). However, many of the characteristics of inward rectification in motoneurones were different from those of the classical anomalous rectification. First, the activation kinetics were slower. Second, the activation voltage was more positive. Third, the reversal potential did not coincide with the potassium equilibrium potential. Fourth, the inward rectifying currents involved Na^+ in addition to K^+. Fifth, externally applied Ba^{2+} did not block the inward rectifying current. Sixth, the whole-cell conductance of the inward rectification was independent of external potassium concentrations.

The characteristics of the motoneurone inward rectifier, I_{IR}, agree generally with those of the currents termed as I_h or I_f in cardiac muscle (Yanagihara & Irisawa, 1980; Brown & DiFrancesco, 1980) and I_q in hippocampal neurones (Halliwell & Adams, 1982). Inwardly rectifying currents with similar characteristics have been observed in sensory ganglion neurones (Mayer & Westbrook, 1983), retinal cells (Bader *et al.* 1982; Kaneko & Tachibana, 1985*a*) and neurones in cerebellar and

cerebral cortices (Crepel & Penit-Soria, 1986; Spain *et al.* 1987). However, there appear to be some quantitative differences in properties depending upon cell types. For example, the reversal potential of the inward rectifier, E_{IR}, in motoneurones was about −44 mV in normal Krebs solution. This figure is more positive than that

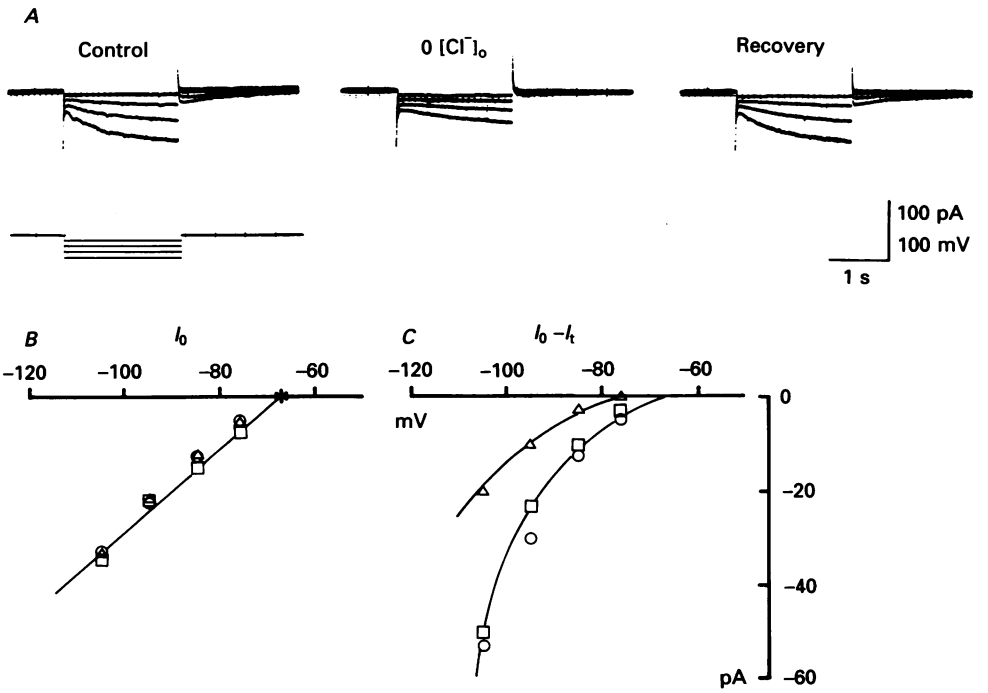

Fig. 10. Inward rectifier in a Cl^--free solution. *A*, inward currents evoked by hyperpolarizing command pulses (lower trace) in the following solutions: control, Cl^--free ($0[Cl^-]_o$) and after returning to control (recovery). *B*, leakage (I_0) and *C*, inward rectifying current components ($I_0 - I_t$) plotted against the membrane potential. ○, control; △, Cl^--free; □, recovery. In the Cl^--free solution, NaCl was replaced by sodium gluconate, KCl by K_2SO_4 and $MgCl_2$ by $MgSO_4$, respectively. Perfusate, Ca^{2+}-free–Mg^{2+} Krebs solution.

reported for I_q in the hippocampus (−54 mV, Halliwell & Adams, 1982) but more negative than that for I_h in cardiac muscle (−25 mV, Yanagihara & Irisawa, 1980), sensory ganglion cells (−30 mV, Mayer & Westbrook, 1983) and retinal cells (about −30 mV, Bader, *et al.* 1982; Bader & Bertrand, 1984). E_{IR} shifted by 22 mV for a 10-fold change in $[K^+]_o$. This compares with the reversal potential reported for I_h in cardiac and retinal cells (26 mV, DiFrancesco, 1981; Bader *et al.* 1982). However, for a fivefold change of $[Na^+]_o$, E_{IR} in motoneurones shifted by about 7 mV, corresponding to about 9 mV for a tenfold change. Thus, the $[Na^+]_o$ dependence of E_{IR} is less than that in cardiac cells (29–34 mV for tenfold change, DiFrancesco, 1981). The smaller contribution of Na^+ conductance to the inward rectification in motoneurones may partly explain why the reversal potential is more negative than that of I_h in cardiac cells. The more negative value of E_K (−98 mV) in the present

study compared to others (−74 mV, Bader & Bertrand, 1984) may be an additional reason.

It has been reported that removal of external Cl^- resulted in marked reduction in I_h (Seyma, 1979; Yanagihara & Irisawa, 1980; Mayer & Westbrook, 1983). This was indeed the case for the I_{IR} recorded from motoneurones with the whole-cell method. In the previous intracellular studies, two explanations seemed possible. I_{IR} could be suppressed if the intracellular Cl^- carrying the inward current is depleted by redistribution following the removal of extracellular Cl^-. Alternatively, the external Cl^- may be essential for maintaining the conductance of I_{IR}. The present results favour the latter possibility because $[Cl^-]_i$ in the present conditions was maintained by the pipette solution. Also, E_{IR} in different $[Cl^-]_o$ did not indicate involvement of Cl^- in I_{IR} (Fig. 9). Replacement of external Cl^- with either gluconate or isethionate suppressed the inward rectifier in the present study. Replacement of Cl^- with acetate (Seyama, 1979; Yanagihara & Irisawa, 1980) also caused suppression of a similar current, I_h. This suggests that the presence of external Cl^- is essential for the operation of the inward rectifiers, rather than non-specific blocking effects by the anion substitute as has been suggested by Mayer & Westbrook (1983).

The activation curve of the inward rectifier tail current (Fig. 7) demonstrated that the conductance of I_{IR} is partially activated at the resting potential (about −65 mV, Takahashi, 1990). I_{IR} at −65 mV corresponding to about 20% of that at −100 mV. Since chord conductance of I_{IR} at −100 mV was about 0·8 nS (Fig. 5), the value at the resting potential would be about 0·16 nS, corresponding to about 4·7% of the input conductance of motoneurones (3·4 nS, Takahashi, 1990). It has been estimated in newborn rat motoneurones that resting Na^+ conductance constitutes about 12% of total input conductance (Forsythe & Redman, 1988).

Since E_{IR} is more positive than the resting potential, this conductance must contribute to the maintenance of the resting membrane potential at levels positive to E_K. This might favour neuronal excitation (Bader *et al.* 1982). Another important aspect of the inward rectifier in motoneurones as in other cells would be its possible activation or suppression by transmitters or modulators (see Nicoll, 1988). Because of the E_{IR} positive to the resting potential, activation of the I_{IR} would increase excitability of neurones, whereas its suppression would damp their excitability. An excitatory action of serotonin on motoneurones by activation of a conductance similar to that of the inward rectifier is described in the following paper (Takahashi & Berger, 1990).

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