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INWARD RECTIFICATION AND LOW THRESHOLD CALCIUM CONDUCTANCE IN RAT CEREBELLAR PURKINJE CELLS. AN *IN VITRO* STUDY

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SUMMARY

1. The bioelectrical properties of Purkinje cells were analysed in sagittal slices of adult rat cerebellum by the use of intracellular recordings performed at a somatic level in current or in voltage clamp.

2. The passive electrical constants of Purkinje cells were determined by measuring the time course and the amplitude of the voltage responses induced by hyperpolarizing current pulses. The mean value of input resistance was 21 ± 1 MΩ. Mean values of the membrane time constant and of the total electrotonic length of Purkinje cells were $19{\cdot}5 \pm 1{\cdot}7$ ms and $0{\cdot}59 \pm 0{\cdot}01$ ms respectively.

3. A time dependent inward rectification was present in all cells. In current-clamp experiments it appeared as a sag in hyperpolarizing voltage responses which were followed by well developed anodal breaks.

4. In voltage-clamped cells, the inward relaxation induced by hyperpolarizing commands fitted to a single exponential. It was already present near resting potential and could reach an amplitude of up to 4 nA for jumps near to -120 mV. This relaxation was provisionally termed I_h. Tail current relaxations also fitted to a single exponential when they were recorded in the presence of tetrodotoxin (TTX) and of Co.

5. The inward relaxation induced by hyperpolarizing commands was readily blocked by Cs, whereas it was unaffected when Ba replaced Ca in the bath, except near rest where it was strongly reduced.

6. The Ca channel blockers Cd, Co and D600 also markedly depressed or even suppressed the inward rectification near resting potential, and up to about -85 mV, whereas this blocking effect was much less apparent or even absent at more negative potentials.

7. I_h was clearly enhanced when the external K concentration was raised up to 20 mM.

8. In the presence of TTX and Co in the bath, inward relaxations induced by hyperpolarizing jumps were unaffected in Na-free solution, whereas the amplitude of tail currents was reduced. Furthermore, the reversal potential of I_h which ranged

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between −45 and −56 mV in the Co plus TTX containing solution, shifted toward more negative values in the Na-free medium.

9. In contrast, I_h remained unchanged in low Cl solution.

10. From these experiments, it is likely that K and Na are the main charge carriers of I_h. Furthermore, this current seems to be contaminated near resting potential by a Ca-dependent K current.

11. Anodal breaks following hyperpolarizing commands were slightly attenuated when Cd or TTX were added to the bath. In contrast they were markedly attenuated or totally abolished under TTX plus Cd. On the other hand, they were markedly enhanced by Ba. They appear therefore to be due at least in part to both Na and low threshold Ca currents de-inactivated by previous hyperpolarization of the cells.

INTRODUCTION

It is now well established that mammalian neurones display a rather large repertoire of ionic conductances, which underlies their specific bioelectrical properties (see Adams, 1982). Thus, cerebellar Purkinje cells, besides conductances responsible for the fast action potentials, exhibit a high threshold Ca conductance and a non-inactivating Ca conductance in their dendrites, as well as a non-inactivating Na conductance in their soma (Llinãs & Sugimori, 1980*a*, *b*; Crepel, Dupont & Gardette, 1984).

In vitro studies have also shown that Purkinje cells exhibit a well developed inward rectification below resting potential (Llinãs and Sugimori, 1980*a*, *b*; Crepel *et al.* 1984), the ionic basis of which was not investigated. It is known that in other neurones, as in striated muscles, heart cells and fish eggs, several types of inward rectifications have been described. Thus, at hyperpolarized levels of membrane potential, the instantaneous anomalous rectification primarily described in striated muscles by Katz (1949) as well as the time-dependent inward rectifications of marine fish eggs and olfactory cortex neurones are due to a pure K current (Constanti & Galvan, 1983; ref. in Hagiwara, 1983). In contrast, other types of inward rectifiers like I_h in spinal sensory ganglion neurones and rods, I_Q in hippocampal pyramidal cells, and I_h–I_f–I_{k2} current in heart cells are both Na and K dependent (Attwell & Wilson, 1980; Di Francesco, 1981*a*, *b*; Halliwell & Adams, 1982; Mayer & Westbrook, 1983; Bader & Bertrand, 1984). In *Aplysia* neurones, a Cl conductance activated by hyperpolarization of the cells also contributes to this rectification (Chesnoy-Marchais, 1983).

We have therefore analysed the ionic basis of the rectifying properties of Purkinje cells by the use of an *in vitro* slice preparation. A preliminary account of this work was previously published (Crepel & Penit-Soria, 1984).

METHODS

Experiments were carried out in sagittal (400 μm thick) cerebellar slices of adult Wistar rats maintained *in vitro*. The methods used for preparing the slices and the recording chamber were the same as previously described (Crepel, Dhanjal & Garthwaite, 1981). The composition of the bathing solutions is given in Table 1. They were continuously gassed with a mixture of O_2 (95%) and CO_2 (5%) and maintained at 35 °C. Intracellular recordings of Purkinje cells were made at a somatic level, under direct visualization of the cortical layers, with glass micro-electrodes filled with 3 M-KCl

(40–70 MΩ). Cells were either recorded under current clamp or they were voltage clamped by using a Wilson–Goldner switch clamp circuit (Wilson & Goldner, 1975) (Dagan 8100). In the current-clamp mode, currents were injected into the cells through the recording micro-electrode via a bridge circuit. In normal bathing medium, most cells exhibited a spontaneous firing of simple spikes at their resting potential ($-61 \pm 0{\cdot}6$ mV; $n = 23$) which would have made difficult the construction of current–voltage (I–V) plots (see Results). Therefore, they were maintained at -65 mV (i.e. below the firing threshold of these spikes) by passing a steady negative current through the micro-electrode when responses to depolarizing or hyperpolarizing current pulses were analysed (see Results). Furthermore, when manipulations of the composition of the bath led to a depolarization of the cells, the holding current was adjusted to bring back the transmembrane potential to -65 mV before studying I–V relationships of the neurones in these modified bathing mediums. In the single electrode voltage-clamp mode, the procedure used to clamp the cells was the same as previously described by Galvan & Adams (1982) and by Halliwell & Adams (1982). The switching

TABLE 1. Composition of the perfusing solutions (mM)

	Standard Krebs	Ca-free			Ca- and Na-free	
		Cd	Co	Ba	Choline	Tris-HCl
NaCl	124	130	130	130	—	—
KCl	5	6·20	6·20	6·20	6·20	6·20
KH_2PO_4	1·15	—	—	—	—	—
$MgSO_4$	1·15	—	—	—	—	—
$MgCl_2$	—	1·10	1·10	1·10	1·10	1·10
$CaCl_2$	2·50	—	—	—	—	—
$BaCl_2$	—	—	—	2·50	—	—
$CdCl_2$	—	1	—	—	—	—
$CoCl_2$	—	—	2	—	—	—
Tris-HCl pH 7·4	—	25	25	25	25	130
Choline	—	—	—	—	130	—
$NaHCO_3$	25	—	—	—	—	—
Glucose	10	10	10	10	10	10

frequency was 3–5 kHz with a duty cycle of 25 or 50 % and the maximum gain which could be achieved was 2000 to 3500, depending on electrical properties of the micro-electrodes. Since in these conditions, simple spikes and Ca spikes (Llinãs & Sugimori, 1980*a*, *b*) could not be adequately clamped, the holding potential was maintained at -60 or -65 mV, depending on experiments, i.e. below the firing level of the cells. Even so, many cells were not perfectly voltage clamped as small sags or overshoots were seen in the voltage records when hyperpolarizing commands were applied, probably because the gain was still lower then the value required to ensure an adequate voltage clamp. Furthermore, the rather long membrane time constant of Purkinje cells and their rather short electrotonic length (see Results) prevented cells to be isopotential, especially during the 30–50 ms which followed the voltage step as already shown by Johnston & Brown (1983) on hippocampal neurones. However, this did not seem to grossly distort the current relaxations we were interested in, as their time course was much slower. In particular, they appeared monotonic in most cells (see Results), which allowed us to extrapolate them back to zero time, to reach an estimate of the instantaneous I–V relationship. These extrapolations, as well as I–V plots, determination of membrane time constant and electrotonic length of Purkinje cells were performed on line on a Goupil 3 microcomputer, following procedures described in the Results with a sampling frequency of 1 or 5 kHz. Data were also stored on tape for further analysis (5 kHz band width).

RESULTS

Cable properties of Purkinje cells

The passive electrical constants of Purkinje cells were determined, in the current-clamp mode, by measuring the transmembrane voltage changes elicited by prolonged

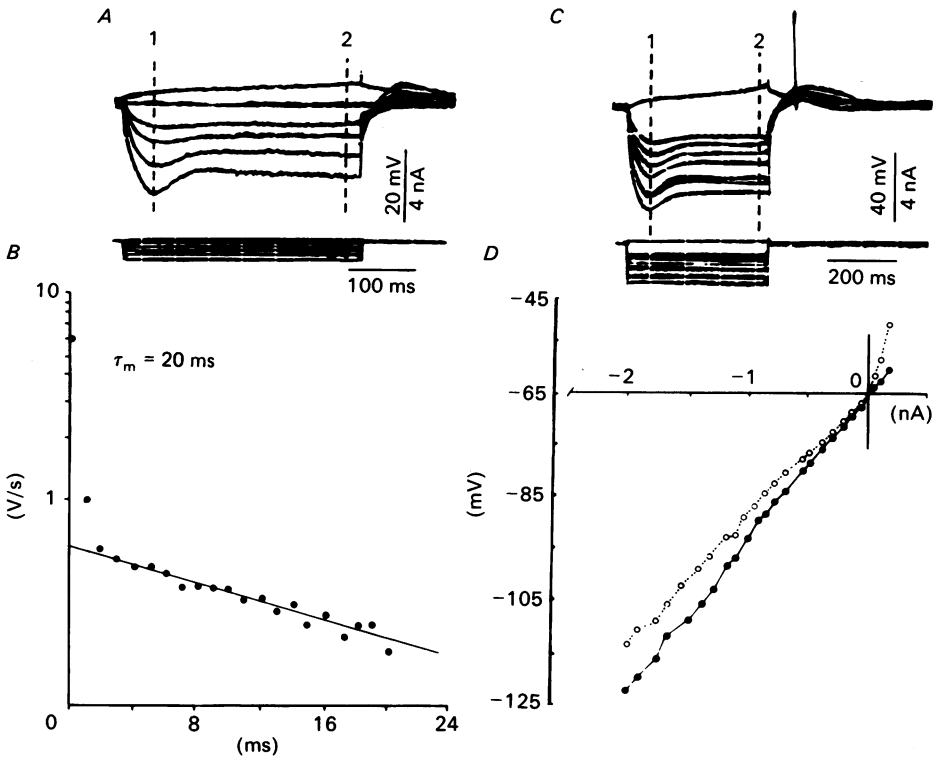

Fig. 1. Responses of Purkinje cells to depolarizing and hyperpolarizing current pulses in the current-clamp mode. *A*, transmembrane voltage changes (upper traces) induced by the current pulses shown in the lower trace. *B*, semilog plot of the slope of the voltage transient (dV/dT) *versus* time after onset of a hyperpolarizing current step of 0·4 nA for the same cell as in *A*. The straight line was obtained as explained in the results by applying the least square criterion. The membrane time constant (τ_m), the first equalizing time constant (τ_1), and the electrotonic length of this Purkinje cell were 20 ms, 0·6 ms and 0·55 ms respectively. Only one point out of every five has been drawn on the semilog plot. *C*, same experiments as in *A* in another cell. *D*, current–voltage (*I–V*) plots of the cell illustrated in *C*. In this and in Fig. 5 the curve with filled circles and continuous line represents the *I–V* plot taken at the time indicated by 1 in *A* and *C*, whereas the curve with open circles and dotted line was taken at the steady state (2 in *A* and *C*).

hyperpolarizing current pulses applied through the recording micro-electrode (Fig. 1). The influence of the inward rectification of these cells (see next section) on measurements of their input resistances was minimized by determining them from the slope of the initial portion of the *I–V* plots produced by small current pulses of less than 0·4 nA, at a time indicated by 1 in Fig. 1 *A* and *C*, i.e. near the end of the initial transients (see below). In the nineteen cells tested, the mean input resistance was 21 MΩ (S.E. of mean = 1; range: 12–30 MΩ).

Similarly, membrane time constants and electrotonic lengths of Purkinje cells were obtained by analysing the time course of the voltage transients produced by current pulses of less than 0·4 nA. Between 5 and 30–40 ms after the onset of the current step, the slope of the voltage transient (dV/dT) *versus* time closely fitted a single

exponential (Fig. 1*B*), the time constant of which is considered as representing the membrane time constant τ, according to Rall's model (Rall, 1969). At earlier times the experimental points deviated markedly from this extrapolated exponential, and the difference between the two curves also fitted a single exponential, the time constant of which is the first equalizing time constant τ_1 that governs equalization of the membrane potential over the length of the processes (Rall, 1969). These two parameters allowed us to calculate for each cell the total electrotonic length L for a theoretical equivalent cylinder, with $L = (\tau/\tau_1 - 1) - \frac{1}{2}$ (Rall, 1969). Thus, in standard Ringer solution, mean values of τ and of L ($n = 14$) were 19·5 ms (S.E. of mean = 1·7; range: 13–26 ms) and 0·59 (S.E. of mean = 0·01; range: 0·50–0·82 ms) respectively.

These values were clearly greater and smaller respectively than those determined in Purkinje cells in previous *in vivo* experiments (Crepel & Delhaye-Bouchaud, 1979), probably because cells recorded *in vivo* were more depolarized on the average than those studied here. In the present experiments, the inward rectification induced by hyperpolarization of the cells and which was already present near resting potential (see next section) might have contributed to increase the speed of the transmembrane voltage changes induced by hyperpolarizing current pulses and thus led to a shortening of τ. Since Ca-channel blockers markedly attenuated the rectification near resting potential (see next section), τ and L were determined in five cells bathed with a Ca-free medium containing 1 mM-Cd. Mean values of τ and of L ($17{\cdot}4 \pm 1{\cdot}4$ and $0{\cdot}62 \pm 0{\cdot}06$ ms respectively) were not significantly different from those in standard medium which suggests that measurements of the passive electrical constants of Purkinje cells were minimally affected by the inward rectification near rest in standard medium.

Inward rectification of Purkinje cells

Current-clamp experiments. When prolonged hyperpolarizing current pulses were applied to the cells ($n = 23$) through the recording micro-electrodes, a well developed time-dependent inward rectification appeared in the voltage records as a sag in the transmembrane potential changes (Fig. 1*A* and *C*). In most cells, this sag started 50–70 ms after the onset of the current pulse. Current–voltage plots derived from these records allowed us to reach an estimate of the magnitude of this inward rectification when comparing *I–V* curves obtained at the peak of the hyperpolarizing responses and at the steady state, near the end of the pulse (times indicated 1 and 2 respectively in Fig. 1*A* and *C*). Thus, in most cells, this inward rectification was already present near resting potential, and still persisted in hyperpolarizing responses of more than 60 mV, thus at levels of membrane potential near to −120 mV (Fig. 1*D*).

At depolarized levels of membrane potential, subthreshold to firing level, a slow depolarization of the neurones occurred when prolonged depolarizing currents were injected through the micro-electrode (Fig. 1*A* and *C*). It also appeared in the *I–V* plots as a divergence between the curves obtained 50–70 ms after onset of the current pulse and near its end respectively (Fig. 1*D*). Although the ionic basis of this slow depolarization was not investigated in the present experiments, it is likely that it was mainly due to the slow Na current previously described in Purkinje cells (Llinãs &

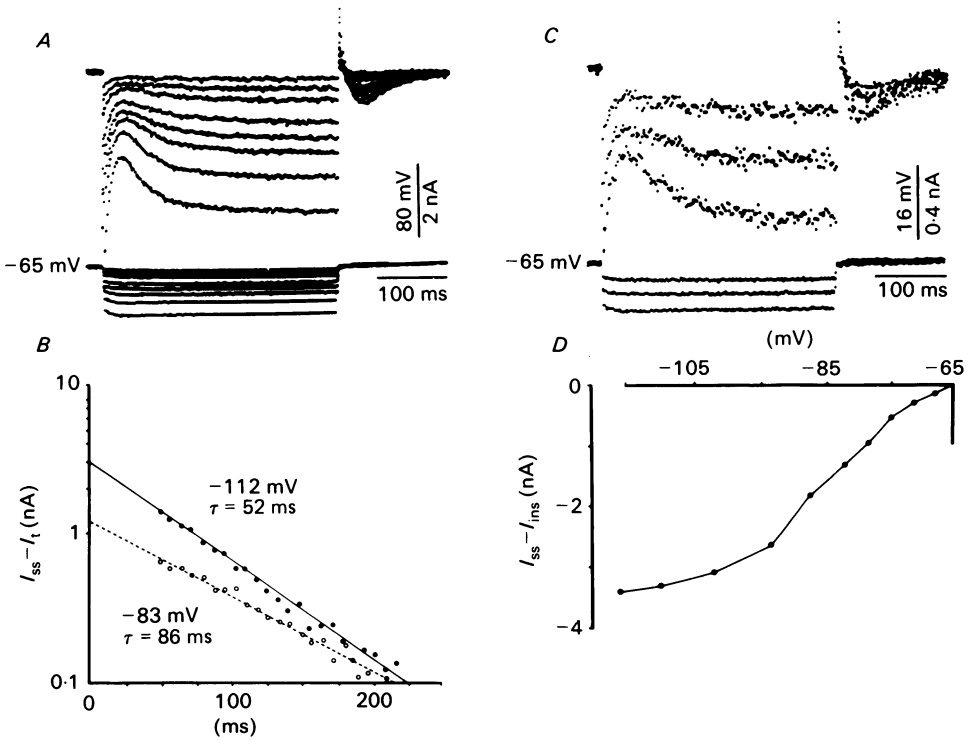

Fig. 2. Responses of a Purkinje cell to hyperpolarizing voltage jumps in the voltage-clamp mode. *A*, current relaxations (upper traces) induced by the voltage commands shown in the lower trace. Holding potential: −65 mV (full explanation in text). *B*, semilog plots of the 'on' relaxation *versus* time induced by hyperpolarizing jumps from −65 to −112 mV (continuous line and filled circles), and from −65 to −83 mV (dotted line and open circles). Each point on the two curves represents the difference between the steady-state current, I_{ss}, and the current at any given time after onset of the voltage jump, I_t. The two straight lines were obtained by applying the least square criterion to the corresponding data points. Their intercept with zero time gives the amplitude of the two 'on' relaxations. The time constants, τ, of the relaxations were 86 ms and 52 ms for jumps to −83 mV and −112 mV respectively. *C*, high gain recording of the current relaxations (upper traces) induced by the voltage jumps shown in the lower trace. Holding potential, −65 mV. Note that 'on' and 'off' relaxations were already induced by hyperpolarizing commands as small as 4 mV. Ten sweeps were averaged for each trace in *A* and *C*. *D*, current–voltage relationship of the 'on' relaxation for this neurone. Current values represent the difference between the instantaneous (I_{ins}) and the steady-state (I_{ss}) currents.

Sugimori, 1980*a*, *b*; Crepel *et al.* 1984) since it persisted in a Ca-free medium containing 1 mM of the Ca blocker Cd (see Fig. 5*A* and *B*).

Finally, well developed anodal breaks followed hyperpolarizing responses in most cells (Fig. 1*A* and *C*).

Voltage-clamp experiments. In the sixty-six successfully voltage-clamped Purkinje cells, jumps from the holding potential of −65 mV to more negative potentials always elicited a well developed time and voltage dependent inward relaxation ('on' relaxation) following the initial transients (Fig. 2*A*).

In most cells, this 'on' relaxation was quite monotonic, i.e. the difference between the current value at the steady state (I_{ss}) and that at any other time (I_t) fitted to a single exponential (Fig. 2*B*), the extrapolation of which at zero time allowed us to determine the instantaneous current value (I_{ins}). Furthermore, the time constant (τ) of the 'on' relaxation was faster for large hyperpolarizing jumps than for smaller ones. For instance, in the cell illustrated in Fig. 2, τ was 86 ms for a voltage step from -65 to -83 mV, whereas it was only 52 ms for a jump to -112 mV. Very similar values were obtained in the other tested cells, i.e. the time constant of the 'on' relaxation was generally 3–4 times longer than the membrane time constant for any given cell, depending on the magnitude of the voltage jump. In the other neurones, the 'on' relaxation poorly fitted a single exponential. In this case, the current value at the end of the initial transients was taken as a crude estimate of I_{ins}.

In all cells, direct examination of the records (Fig. 2*C*) as well as the comparison of the instantaneous and steady-state *I–V* curves (Fig. 3*C*) showed that the inward rectification was already present near -65 mV and increased to reach values of 0·8 to 4 nA depending on cells, for jumps to about -120 mV (Fig. 2*D*).

For convenience, the current responsible for this 'on' relaxation will be provisionally termed I_h in the following sections.

'Off' relaxations were not analysed in standard Ringer, especially because they were likely to be contaminated by the currents responsible for the anodal breaks following hyperpolarizing commands (see last section). When this complication was avoided by adding 5×10^{-6} M-TTX and substituting Ca by 2·5 mM-Co in the bath, 'off' relaxations also fitted to a single exponential, the time constant of which was generally different from that of the 'on' relaxation. Under these circumstances, extrapolation of the 'on' and 'off' relaxations back to their times of onset showed that the ohmic step at the onset of the voltage jump was smaller than at its end (Fig. 9). This indicated that the 'on' relaxation is likely to be due to a time- and voltage-dependent inward current activated by hyperpolarization of the cells, rather than due to the closure of M channels (Brown & Adams, 1980; Halliwell & Adams, 1982). However, this conclusion might not hold for small voltage jumps in standard medium (see Discussion).

Ionic basis of the inward rectification

Effect of Cs. Since in striated muscles (Gay & Stanfield, 1977), heart cells (Di Francesco, 1981*a*, *b*, 1982), fish eggs (ref. in Hagiwara, 1983), and nerve cells (Halliwell & Adams, 1982; Constanti & Galvan, 1983; Mayer & Westbrook, 1983; Bader & Bertrand, 1984), the inward rectification at hyperpolarized levels of membrane potential is blocked by Cs, we tested the effect of this impermeant ion on I_h in eight voltage-clamped Purkinje cells.

In the typical experiment illustrated in Fig. 3, the 'on' relaxation was nearly completely abolished for any hyperpolarizing jump tested when 10 mM-Cs was added to the bath. In contrast, tail currents were still present, although they were markedly attenuated. Therefore, they are certainly the inward currents responsible for the anodal breaks (see last section). Very similar results are obtained in the seven other cells.

In the cell illustrated in Fig. 3, and in five other cells, Cs also decreased the slope

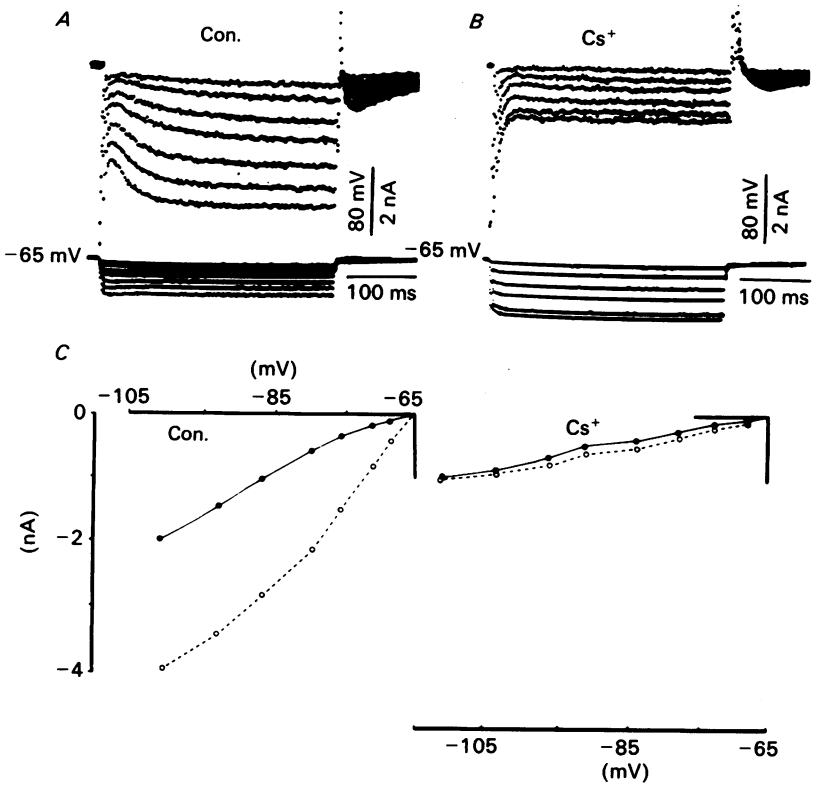

Fig. 3. Effects of Cs on the inward relaxation of a Purkinje cell under voltage clamp. *A*, control (Con.) responses to various voltage commands in standard Ringer solution. *B*, *idem* as in *A* under 10 mM-Cs^+ in the bath. *C*, current–voltage relationships of the 'on' relaxation of this neurone in standard solution (left graph) and under Cs (right graph). In this and in Figs. 4, 6, 7 and 11, the curves with filled circles and continuous lines represent the instantaneous current–voltage plots computed by extrapolating the 'on' relaxations back to zero time (full explanation in the text), and the curves with open circles and dotted line represent the steady-state current–voltage relationships. Ten sweeps were averaged for each trace in *A* and *B*.

of the instantaneous *I–V* curves. As previously discussed (Hagiwara, Miyazaki & Rosenthal, 1976; Halliwell & Adams, 1982; Mayer & Westbrook, 1983), the effect of Cs on the instantaneous current might be due to the fact that a fraction of the channels responsible for the inward rectification were already opened at rest in these cells. This hypothesis might also explain the instantaneous rectification which appeared as a small curvature in the instantaneous *I–V* plots in many cells (see Figs. 3*C*, 4*C*, 6*C* and 11*C*), assuming that the individual channels involved also show inward rectification. The fact that raising the external K concentration markedly enhanced the instantaneous rectification in all tested cells (see Fig. 7) strengthens this hypothesis. Finally, only small (less than 0·3 nA) and inconsistent variations in the holding current were seen under Cs.

Effect of Ba. Ba is a cation known to block the K-dependent anomalous rectification (Constanti & Galvan, 1983; see also ref. in Hagiwara, 1983), whereas it does not affect

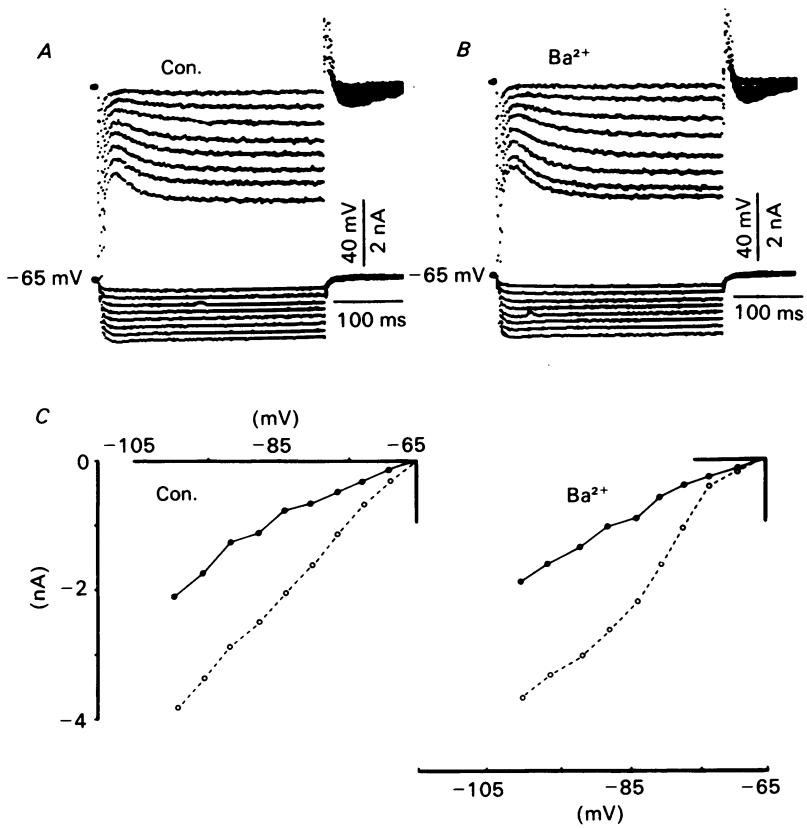

Fig. 4. Effects of Ba on the inward rectification of a Purkinje cell under voltage clamp. *A*, control responses to various current pulses in standard solution. *B*, *idem* as in *A* after replacement of Ca by Ba in the bath. *C*, current–voltage relationship in standard solution (Con.) and under Ba. Same symbols as in Fig. 3. Note that the 'on' relaxation was not affected by Ba, except near rest where it was markedly reduced.

I_h, I_Q and I_h–I_f–I_{k2} (Brown & Di Francesco, 1980; Di Francesco, 1981*a*, *b*, 1982; Halliwell & Adams, 1982; Mayer & Westbrook, 1983). In voltage-clamp experiments ($n = 6$), the inward rectification at hyperpolarized levels of membrane potential was not affected by replacing Ca by Ba in the bath, except near rest (up to -85 mV) where it was consistently attenuated (Fig. 4). With the exception of this effect of Ba near rest which will be interpreted later on (see Discussion), the present results are consistent with the interpretation that the inward rectification of Purkinje cells is mainly due to I_h.

Effect of Ca-channel blockers. Preliminary current-clamp experiments on the ionic basis of rebounds of depolarization showed that the inward rectification of Purkinje cells was reduced near rest by Ca-channel blockers (Crepel & Penit-Soria, 1984). This was investigated further in the present experiments.

In current-clamp experiments, replacement of Ca by 1 mM-Cd in the bath markedly decreased or even suppressed the inward rectification of Purkinje cells ($n = 7$) near

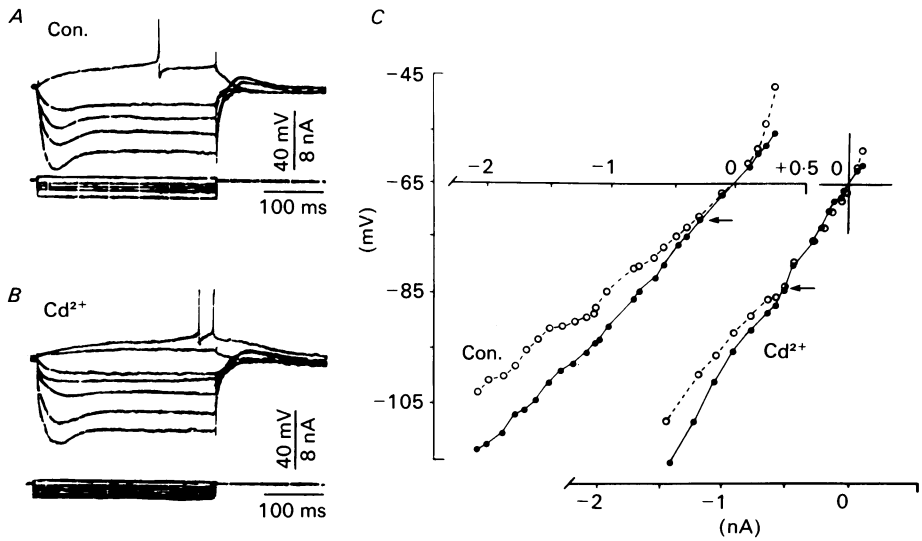

Fig. 5. Effects of Cd on the inward rectification of a Purkinje cell under current clamp. *A*, control responses to various current pulses in standard solution, *B*, *idem* as in *A* in a Ca-free solution containing 1 mM-Cd. Note that both the slow depolarization induced by depolarizing currents and the rebounds of depolarization following hyperpolarization of the cell were still present under Cd. *C*, current–voltage relationship in standard solution (Con.) and under Cd. Note that Cd abolished the inward rectification of this cell near resting potential in the hyperpolarizing direction. Same symbols as in Fig. 1.

resting potential, and up to about −85 mV. At more negative membrane potentials, the inward rectification was much less attenuated (Fig. 5).

The effects of Co and D600 on I_h were studied in twenty voltage-clamped cells. As for Cd, replacement of Ca by Co in the bath markedly decreased or suppressed I_h for hyperpolarizing jumps from −65 mV to −75, −85 mV depending on cells ($n = 12$), without significantly affecting the values of the holding currents. At more hyperpolarized levels of membrane potential, the rectification was left unaffected (Fig. 6). Very similar results were obtained by adding 5×10^{-5} M-D600 to the standard Ringer (not illustrated). Finally, Ca-channel blockers often increased the membrane resistance of the cells, as revealed for instance by the increase of the slopes of the *I–V* plots in current-clamp experiments (Fig. 5). From these experiments, it is therefore very likely that, near resting potential, I_h is contaminated by a Ca-dependent current. Because this current was also blocked by Cs (see before), K ions are likely to be its main charge carrier, suggesting that it might correspond to a Ca-dependent K current ($I_{K\text{-}Ca}$) (see Discussion).

In other types of excitable cells, one knows that I_h, like I_Q and I_h–I_f–I_{k2} is due to a mixed K and Na current (Attwell & Wilson, 1980; Di Francesco & Ojeda, 1980; Halliwell & Adams, 1982; Mayer & Westbrook, 1983; Bader & Bertrand, 1984). So, we studied the effect of varying the external K and Na concentration on the inward rectification of Purkinje cells.

Effect of external K concentration. The effect of raising the external K concentration

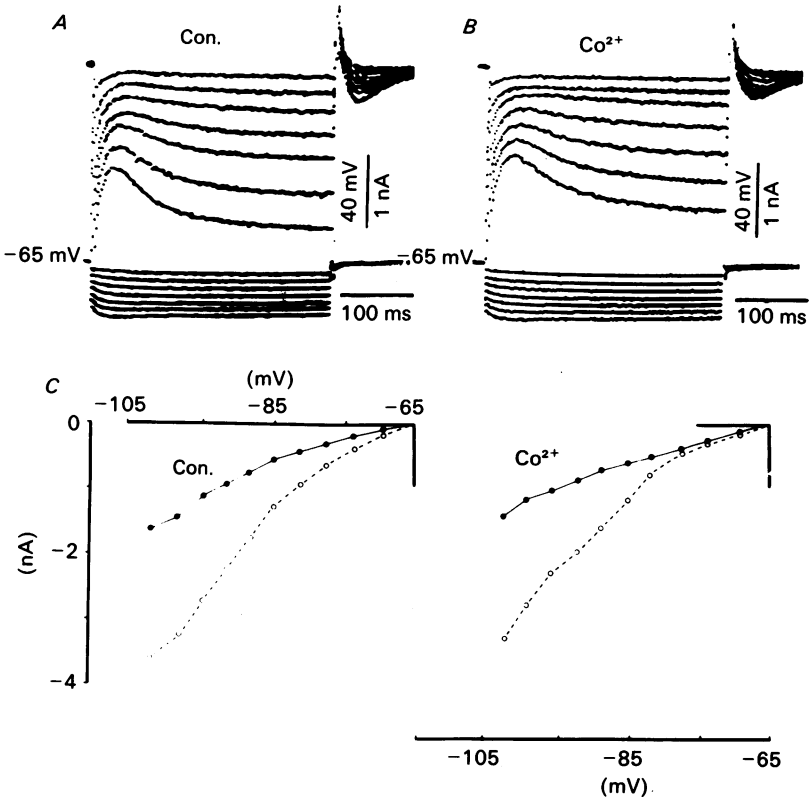

Fig. 6. Effect of Co on the inward relaxation of a Purkinje cell under voltage clamp. *A*, 'on' and 'off' relaxations and corresponding voltage commands in standard solution. Holding potential: −65 mV. *B*, *idem* as in *A* in a Ca-free medium containing 2·5 mM-Co. Ten sweeps averaged for each trace in *A* and *B*. *C*, instantaneous and steady-state *I–V* plots in standard Ringer (Con.) and under Co. Same symbols as in Fig. 3. Note that Co almost completely abolished the 'on' relaxation near the holding potential of −65 mV whereas it left it rather unaffected for larger hyperpolarizing jumps.

up to 20 mM was studied in three cells in the current-clamp mode and in three other voltage-clamped neurones.

In all these cells, this led to a pronounced increase of I_h (Fig. 7*A* and *B*) and to an increase in the curvature of the instantaneous *I–V* curves (Fig. 7*C*). These results fit well with the known properties of I_h in other neurones, since raising the external K concentration up to 20 mM shifted the equilibrium potential for K (E_k) from about −82 mV in standard Ringer to about −40 mV in the high-K solution, if we assume an internal K concentration in Purkinje cells of 130 mM.

The 'off' relaxations were also markedly enhanced in the high-K solution (Fig. 7*B*).

Effect of external Na concentration. In preliminary current-clamp experiments, we could not detect any change in the rectifying properties of Purkinje cells at hyperpolarized levels of membrane potential when Na was replaced by choline in the bath (Crepel & Penit-Soria, 1984). Because in Purkinje cells I_h is likely to be contaminated by a Ca-dependent current near rest, the present experiments were

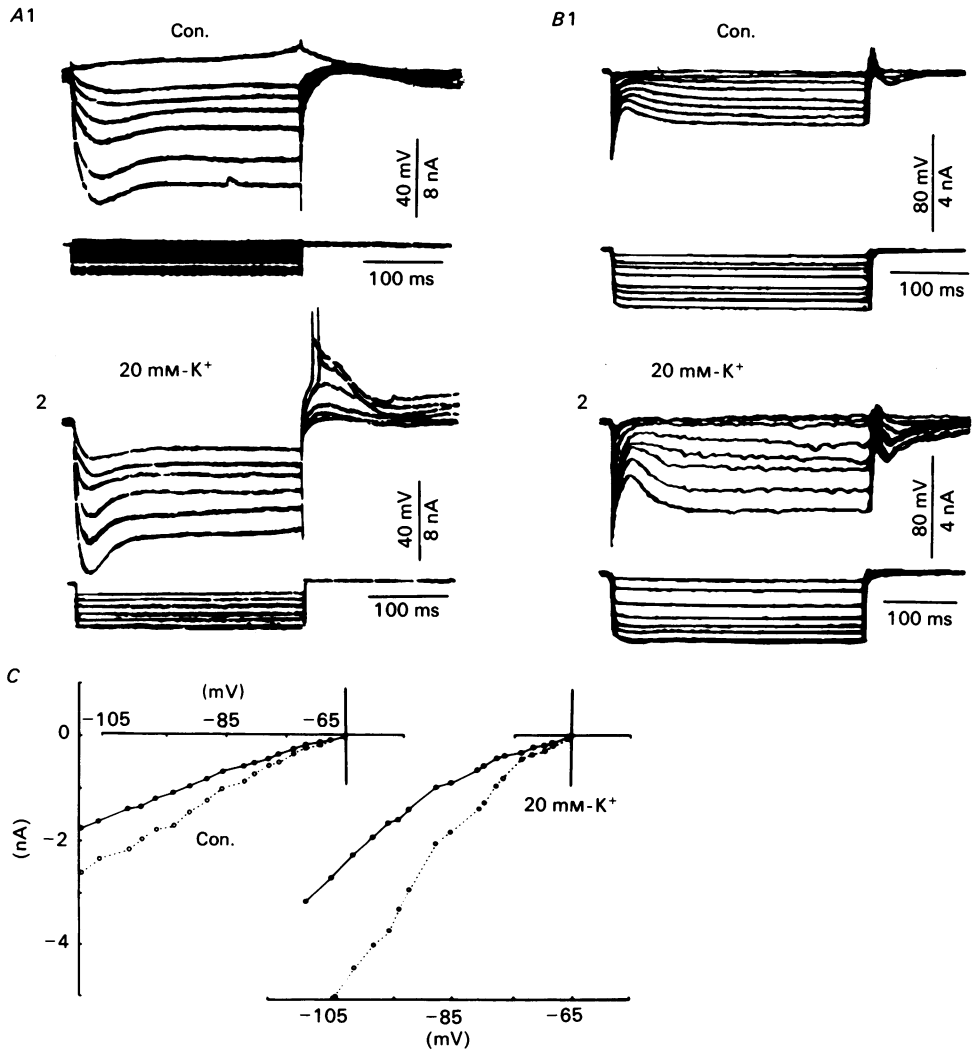

Fig. 7. Effects of external K on rectifying properties of Purkinje cells under current clamp (*A*) and voltage clamp (*B*, *C*). *A*1, control responses (Con.) to depolarizing and hyperpolarizing currents in standard Ringer. Specimen records in *A*2 were taken from the same cell as in *A*1 after increasing the concentration of K in the bath to 20 mM. *B*, *idem* as in *A*1, 2 respectively, in another cell under voltage clamp. *C*, instantaneous and steady-state *I–V* plots of the 'on' relaxation in standard Ringer (Con.) and under 20 mM-K in the bath, for the cell illustrated in *B*. Same symbols as in Fig. 3.

performed in a Ca-free solution containing 2·5 mM-Co to avoid this complication, and TTX was also added to the bath to allow the analysis of tail currents uncontaminated by the currents responsible for the anodal breaks.

In a first set of experiments, we compared the amplitudes of the 'on' and 'off' relaxations in media with and without Na. In three of the four cells studied, the 'on' and 'off' relaxations induced by rectangular hyperpolarizing jumps from the holding

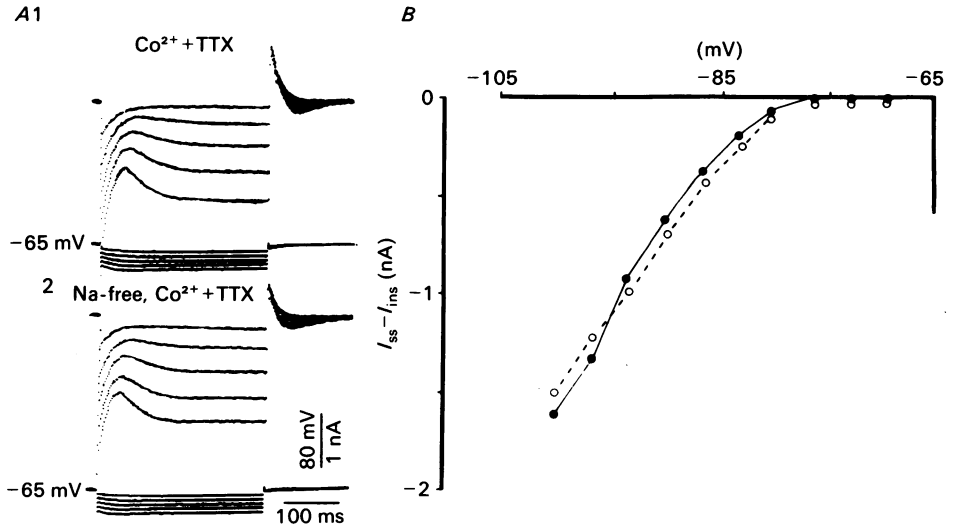

Fig. 8. Effects of external Na on the inward rectification of a Purkinje cell under voltage clamp. *A*1, 'on' and 'off' relaxations and corresponding voltage commands in a Ca-free medium containing 2·5 mM-Co and 5×10^{-6} mM-TTX. Holding potential: −65 mV. *A*2, *idem* as in *A*1 when Na was replaced by choline in the bath. Ten sweeps averaged for each trace in *A*1, 2. *B*, current–voltage relationships of the 'on' relaxations in media with (filled circles and continuous line) and without Na (open circles and dotted line). Current values represent I ($V_H \rightarrow V$), i.e. the difference between the instantaneous and the steady-state currents. Full explanations in the text. Note the absence of any inward relaxation near rest, due to the presence of Co in the bath, and also note the absence of effect of external Na concentration on the 'on' relaxation.

potential V_H (−65 mV) to various potentials V, were monotonic (see Fig. 9), which allowed us to extrapolate them back to their times of onset to obtain their amplitude, I ($V_H \rightarrow V$) and I ($V \rightarrow V_H$) respectively. In these three cells, only minor changes, if any, were seen in the amplitude of the time-dependent inward current, I ($V_H \rightarrow V$), at any potential tested when Na was replaced by choline in the bath (Figs. 8 and 9), thus in agreement with previous current-clamp experiments (Crepel & Penit-Soria, 1984). Furthermore, the time constants of the inward relaxations were unchanged or were only slightly modified in the Na-free medium (Fig. 9). However, the amplitude of tail currents, I ($V \rightarrow V_H$), was reduced whereas their time constant was increased in the absence of Na ions as illustrated in Figs. 8 and 9. The latter effects were observed for hyperpolarizing jumps up to about −100 mV in the three cells.

In a second set of experiments, we attempted to compare the reversal potential of I_h in TTX plus Co perfusing solutions with and without Na. This was done first by the use of the indirect method previously described by Chesnoy-Marchais (1983) since it is only required to determine the ratio I ($V_H \rightarrow V$)/I ($V \rightarrow V_H$) for a series of rectangular hyperpolarizing jumps, the reversal potential being the potential at which this ratio changes its sign. In two of the three cells studied, this ratio well fitted a linear function of V (Fig. 10*C*), which allowed us to measure the reversal potential in medium with and without Na by linear extrapolation.

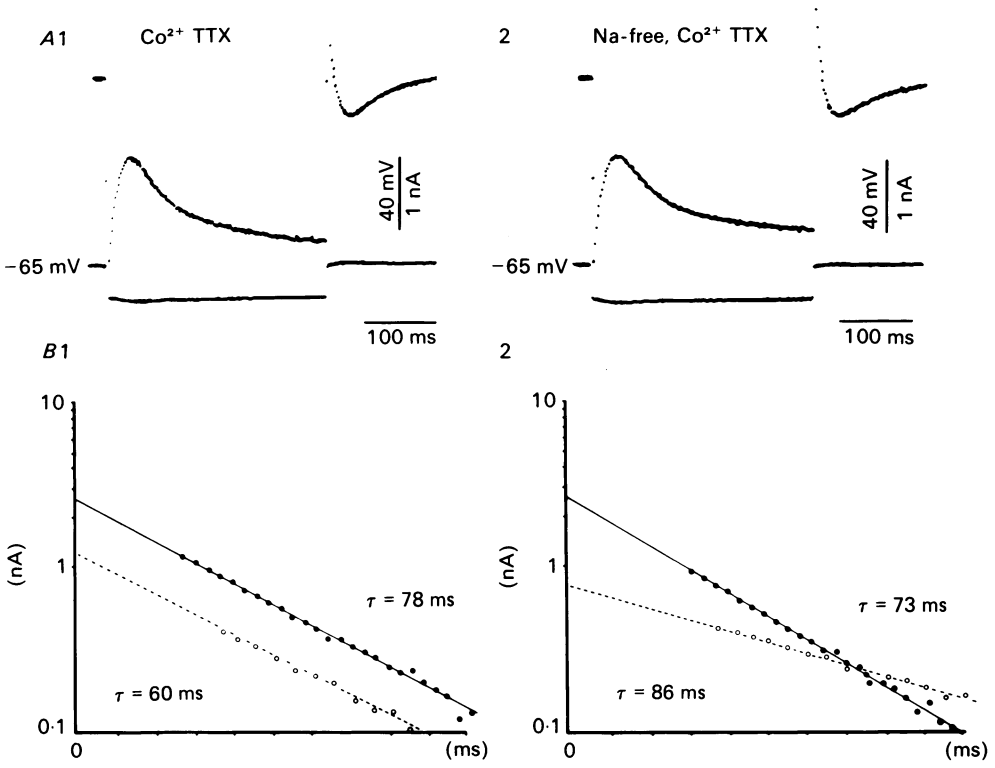

Fig. 9. Effect of external Na on the current tail relaxations of I_h in a Purkinje cell. *A*1, example of the current relaxations (upper trace) induced by the voltage jump shown in the lower trace in a Ca-free medium containing 2·5 mM-Co and 5×10^{-6} M-TTX. Holding potential: −65 mV; *A*2, *idem* as in *A*1 when Na was replaced by choline in the bath. Ten sweeps were averaged in each case. *B*1, 2, semilog plots of the 'on' relaxations (filled circles and continuous lines) and of the current tail relaxations (open circles and dotted lines) beyond capacity transients for the records shown in *A*1 and 2 respectively. The time scale represents 250 ms and 150 ms for the plots of the 'on' relaxations and of the tail currents respectively. Data points for the 'on' relaxation were calculated as in Fig. 2. Each point of the tail current curves represents the difference between the current at any given time after the end of the voltage jump and the steady-state current corresponding to the holding potential. The straight lines were obtained by applying the least square criterion to the data points. Their intercept with zero time gives the amplitude of the 'on' relaxations I ($V_H \rightarrow V$), and of the tail currents, I ($V \rightarrow V_H$) in the media with and without Na respectively. Full explanation in the text. Note that I ($V_H \rightarrow V$) was not modified when Na was replaced by choline in the bath whereas I ($V \rightarrow V_H$) was clearly reduced.

In both cases, the reversal potential shifted from −52 mV and from −56 mV in the TTX plus Co control solution to −60 mV and −63 mV respectively in the Na-free medium (Fig. 10*C*). In the remaining cell, the ratio did not fit a linear function of V, probably because the current rectified in this range of potentials.

However, because we could not preclude that the current also rectified above −80 mV in the two cells where an estimate of the reversal potential of I_h was achieved by this indirect method (see above), we also attempted to measure it directly by

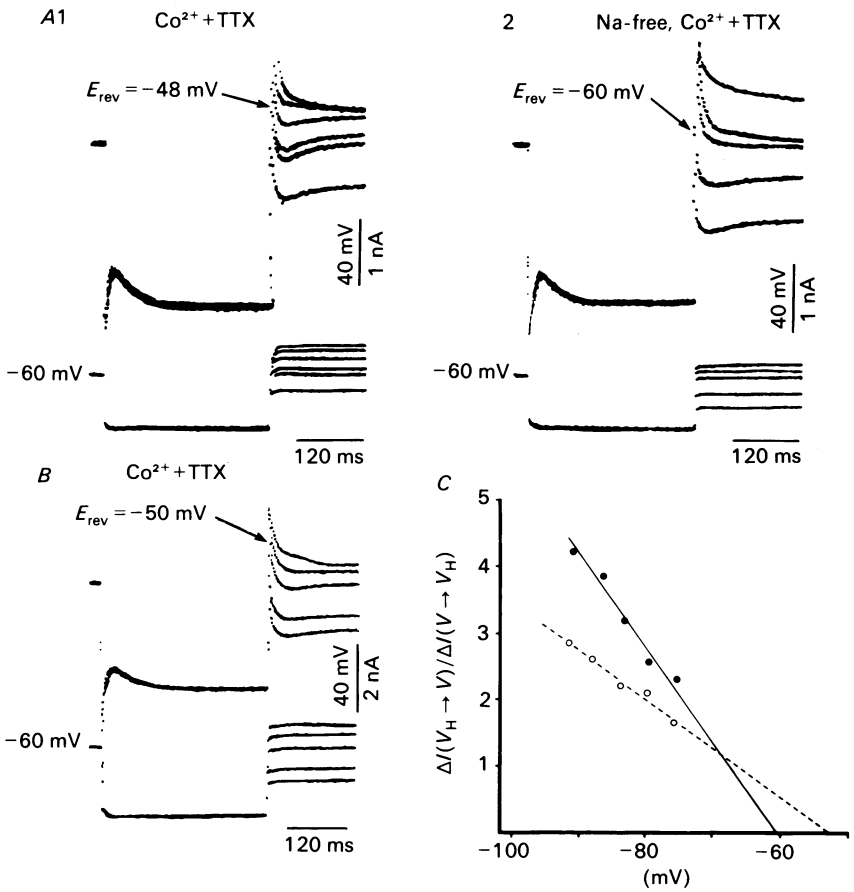

Fig. 10. Effect of external Na on I_h current reversal. *A*1, 2, inward relaxation and tail currents (upper traces) induced in a Purkinje cell by applying a constant voltage jump from a holding potential of −60 mV to −100 mV and stepping back the membrane potential to various levels (lower traces) in a superfusing medium with and without Na respectively. In both cases, the solution contained 5×10^{-6} M-TTX and Ca was replaced by 2·5 mM-Co. The reversal potential (E_{rev}) of I_h shifted from −48 mV in the control solution (*A*1) to near −60 mV in the Na-free medium. *B*, same experiment as in *A*1 in another cell. In this case E_{rev} was about −50 mV. Ten sweeps were averaged for each trace in *A*1, 2 and *B*. *C*, plots of the ratio $I(V_H \rightarrow V)/I(V \rightarrow V_H)$ (see Fig. 9 and explanations in the text) in the same Purkinje cell as in Fig. 9. The holding potential V_H was −65 mV. The curve with filled circles and continuous line was obtained in the control (Co plus TTX) solution whereas the curve with open circles and dotted line was obtained after replacement of Na by choline chloride in the bath. The straight lines were obtained by the least square criterion. Their intercept with the abscissa gives the approximate value of E_{rev} in the two media. Note that E_{rev} was shifted in the negative direction in the Na-free solution.

setting the membrane potential at various levels following a constant hyperpolarizing jump from $V_H = -60$ mV. In the control (TTX plus Co) medium ($n = 4$), the I_h current tail relaxations reversed between -45 and -52 mV, depending on cells (Fig. 10*A*1 and *B*), thus in agreement with results obtained by the indirect method. Unfortunately, in the Na-free medium, marked changes occurred in the behaviour of the cells when they were depolarized. In current-clamp experiments, these changes consisted of the occurrence of prolonged plateaux of depolarization, 50–60 mV in amplitude and up to several seconds in duration (not illustrated), thus resembling the prolonged action potentials recorded under Ba in Purkinje cells (Llinãs & Sugimori, 1980*a*, *b*; Crepel *et al.* 1984). In voltage-clamp experiments these depolarizations also occurred and they invariably unclamped the neurones. This in turn led to oscillations of the clamp which damaged the cells. Since similar results were obtained in Na-free solution where choline was replaced by Tris-HCl, they do not seem to be solely due to a blockade of outward K currents by choline which would unmask residual regenerative Ca currents unblocked by Co, as already described in the case of crustacean muscle fibres (Fatt & Katz, 1953). Whatever the cause of these depolarizing events was, it rendered the analysis of the effect of external Na on the reversal potential of I_h by the post pulse method rather difficult, so that only two cells could be successfully studied in the Na-free medium. In these two experiments, the reversal potential of I_h was shifted from -50 and -45 mV in the control medium to -60 and -54 mV in the Na-free solution (Fig. 10*A*1 and *A*2), i.e. here again in agreement with results obtained by the indirect method.

It seems very likely, therefore, that Na is one of the charge carriers of I_h, although its contribution is certainly small, given the lack of effect of Na substitution by choline on the 'on' relaxation and given the rather small shift of the reversal potential of I_h observed in these conditions.

Effect of Cl ions. In *Aplysia* neurones, Chesnoy-Marchais (1983) has recently described a Cl conductance activated by hyperpolarization of the cells. A possible contribution of such a current in the rectifying properties of Purkinje cells was therefore examined. In seven voltage-clamped cells, there was no change in the magnitude and the kinetics of I_h when the Cl concentration in the bath was reduced to 30 mM by substituting 186 mM-mannitol to 75% of the NaCl of the Ringer (Fig. 11). In three other experiments performed in a normal bathing medium, there was also no change in the inward rectification of the cells after injecting Cl ions into the neurones during 6 min, by a steady negative current of 2·5 nA (not illustrated). Finally, in current- as in voltage-clamp experiments, there was no systematic change in the amplitude and kinetics of the inward rectification over time, despite the probable leakage of Cl ions from the KCl filled micro-electrodes. From these data, an important contribution of a voltage-dependent Cl conductance to I_h appears unlikely.

Ionic basis of anodal breaks

In most cells recorded in standard Ringer in the current-clamp mode, hyperpolarizing responses induced by negative current pulses were followed by clear-cut rebounds of depolarization up to 15 mV in amplitude and up to 250 ms in duration. These rebounds of depolarization were markedly enhanced when Ca was replaced by

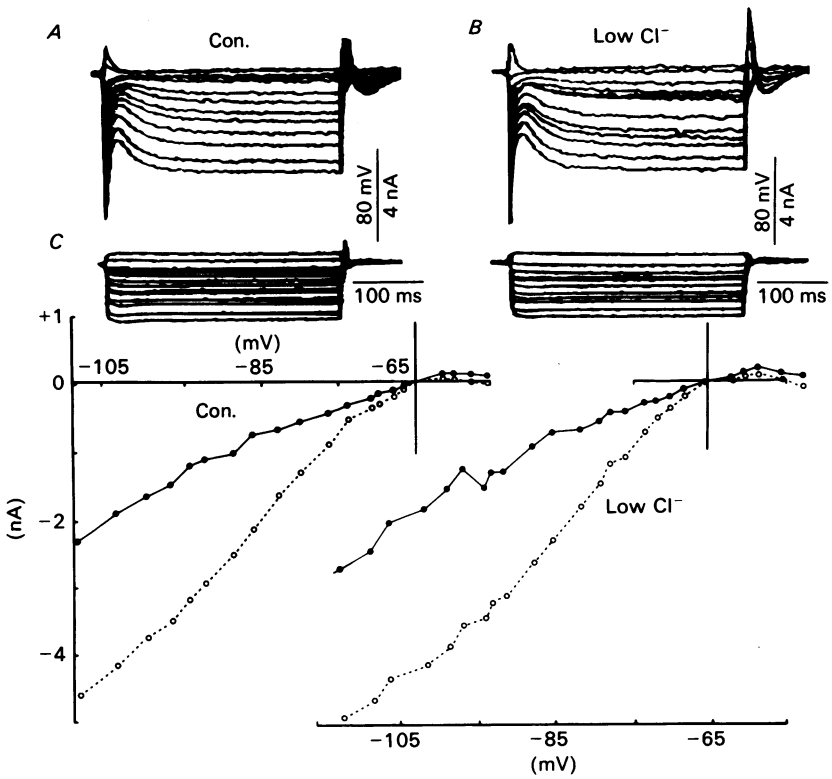

Fig. 11. Effects of external Cl ion on the inward relaxation of a Purkinje cell under voltage clamp. *A*, 'on' and 'off' relaxations and corresponding voltage commands in control (Con.) solution. Holding potential: −65 mV. *B*, same as in *A* in low-Cl bathing medium. Note that both the amplitude and the kinetics of the 'on' relaxation were left unaffected by lowering the Cl concentration in the bath. *C*, instantaneous and steady-state *I–V* curves in standard Ringer (Con.) and in the low-Cl solution.

Ba in the bath (Fig. 12*A* 1, 2). In contrast they were only slightly attenuated or even not affected depending on cells ($n = 6$), by the replacement of Ca by 1 mM-Cd in the superfusing medium (Fig. 12*B* 1, 2). Similarly, they were only slightly attenuated (Fig. 12*C* 1, 2) when 5×10^{-6} M-TTX was added to the standard Ringer (four experiments). However, they were markedly attenuated or even completely abolished in a Ca-free solution containing 1 mM-Cd plus 5×10^{-6} M-TTX (Fig. 12*C* 3). These anodal breaks appear therefore to be due, at least in part, to both Na and low threshold Ca currents de-inactivated by previous hyperpolarization of the cells (see Discussion) although a contribution of tail currents of I_h to these anodal breaks appears also probable and might explain why they were not always completely abolished under TTX and Cd. The fact that Ca-channel blockers or TTX, alone had only a slight depressant effect on these anodal breaks is certainly due to the fact that, in these regions of membrane potential, current–voltage relationships are highly non-linear (see earlier). Finally, in these current-clamp experiments, the inward rectification of Purkinje cells was clearly increased under Ba (Fig. 12*A*), in contrast

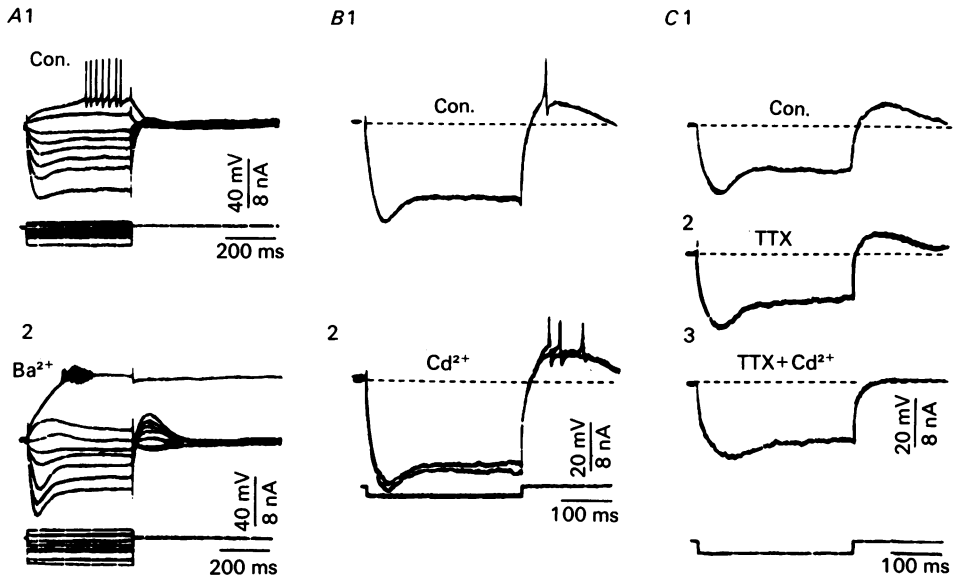

Fig. 12. Rebounds of depolarization following hyperpolarizing commands in Purkinje cells. Current-clamp experiments. *A*1, control responses of a Purkinje cell to various current pulses in standard solution. *A*2, *idem* as in *A*1 after replacement of Ca by Ba in the bath. Note that both the inward rectification induced by hyperpolarizing currents and rebounds of depolarization were enhanced by Ba. *B*1, response of another Purkinje cell to a hyperpolarizing current pulse of 1·7 nA. *B*2, response of the same cell as in *B*1 to the same current pulse in a Ca-free medium containing 1 mM-Cd. *C*1, *idem* as in *B*1 in another cell. *C*2, response of the same cell as in *C*1 to the same current pulse in a standard Ringer containing 5×10^{-6} M-TTX. *C*3, *idem* as in *C*1, *C*2 in a Ca-free medium containing 5×10^{-6} M-TTX + 1 mM-Cd.

with results obtained under voltage clamp. One interpretation of this difference is that, in current clamp the activation of I_h by hyperpolarization of the cells leads to a depolarization appearing as a sag in the voltage records. This depolarization occurring below the resting potential might be sufficient to activate the low threshold Ca current, thus giving rise to an increase of the sags, especially under Ba which passes more easily than Ca through Ca channels (ref. in Hagiwara, 1983).

DISCUSSION

Two separate sections will be considered in the following discussion, namely the nature and properties of the inward rectification and the nature of anodal breaks following hyperpolarizing commands.

Nature and properties of the inward rectification

As for all the other inward rectifiers studied so far (Gay & Stanfield, 1977; Attwell & Wilson, 1980; Di Francesco, 1981*a*, *b*, 1982; Halliwell & Adams, 1982; Constanti & Galvan, 1983; Hagiwara, 1983; Mayer & Westbrook, 1983; Bader & Bertrand, 1984), the inward rectification of Purkinje cells below resting potential was readily

blocked by Cs. However, it was clearly different from the classical anomalous rectifier of marine fish eggs and striated muscles (Hagiwara *et al.* 1976; Gay & Stanfield, 1977) since it was not blocked by Ba. In this respect, the inward rectification of Purkinje cells is very similar to I_Q, I_h and I_h–I_f–I_{k2} (Brown & Di Francesco, 1980; Di Francesco & Ojeda, 1980; Halliwell & Adams, 1982, Mayer & Westbrook, 1983).

As for the latter currents, K and Na ions are also likely to be the main charge carriers of I_h, at least when this current was not contaminated by the Ca dependent component operating near rest (see below). However, if there is little doubt that K is one of the charge carriers of I_h, the participation of Na to this current deserves further comment. First, this participation is suggested by the value of the reversal potential of I_h under TTX plus Cd which, like I_Q (Halliwell & Adams, 1982), ranged between −45 and −56 mV, thus precluding that I_h might be a pure K current. The fact that this reversal potential was about 15 mV more negative than that measured for I_h in rods (Bader & Bertrand, 1984) might result from a possible contamination of tail currents by a transient outward current (Adams, 1982) in the present experiments since they were not done under tetraethylammonium. Secondly, this participation of Na to I_h is suggested by the shift in the negative direction of the reversal potential of I_h in media without Na. One might argue that these experiments were only done successfully in a very few number of cells and that the shift was small (no more than 10 mV) which might raise some doubt on their significance. However, this shift was observed by the use of two different methods, and its amplitude was very similar to that observed for I_Q in the same conditions (Halliwell & Adams, 1982). Finally, the participation of Na to I_h is also suggested by the decrease in the amplitude and the change in the kinetics of tail currents in Na-free medium. However, the participation of Na to I_h is likely to be small, given that the 'on' relaxation of the current was unaffected by replacing Na by choline in the bath and that the shift in the reversal potential of I_h was rather small in these conditions.

The blocking effect of Ca-channel blockers on the inward rectification of Purkinje cells near rest was puzzling. First, the Ca component of I_h might represent a low threshold Ca conductance (Llinãs & Yarom, 1981*a*, *b*) which would be de-inactivated by cell hyperpolarization (Llinãs & Yarom, 1981*a*, *b*; Carbone & Lux, 1984*a*, *b*; Jahnsen & Llinãs, 1984*a*, *b*; Bossu, Feltz & Thomann, 1985). Indeed, Ca-channel blockers strongly reduced I_h between −65 and −90 mV, i.e. values of membrane potential at which the low threshold Ca conductance is already operating in other cells. However, in these neurones, this conductance, once de-inactivated, has to be activated by cell depolarization, which was not the case here. Furthermore, the initial part of the rectification was also blocked by the K-channel blocker, Cs, which makes this hypothesis still more unlikely.

Secondly, the blocking action of Ca-channel blockers on the inward rectification near resting potential might be due to the suppression of a I_{K-Ca} (Meech, 1974) already present at rest, as in some parasympathetic myenteric neurones (Grafe, Mayer & Wood, 1980), although in other cells I_{K-Ca} is usually observed at less negative membrane potentials (Marty, 1981; Pallota, Magleby & Barnett, 1981; Hermann & Hartung, 1983). This interpretation would well explain why this component of the rectification was also blocked by Cs and increased when K concentration was raised in the bath (see Fig. 7). Accordingly, the sequence of events would be as follows. If

we assume that a fraction of the Ca channels responsible for the low threshold Ca conductance present in Purkinje cells (see below) are open at rest, these channels might be closed (and de-inactivated) by hyperpolarizing voltage jumps, thereby decreasing I_{K-Ca}. Because the depressant effects of the Ca-channel blockers on the inward rectification mainly occurred above E_K (see Results), i.e. in a voltage span where K^+ currents are still outward, this decrease of I_{K-Ca} would appear as an inward relaxation, as it indeed did. This interpretation is rendered even more attractive by the fact that under voltage clamp, Ba nearly had the same effect as the Ca-channel blockers on the inward rectification of Purkinje cells, i.e. it decreased the inward relaxation near rest. One knows that Ba does not activate I_{K-Ca} (Meech, 1978), and this would therefore explain why it suppressed this part of the rectification.

To sum up, the observed effects of K^+ concentration, of Cs, of Ca-channel blockers and of Ba on the inward rectification of Purkinje cell near rest fit well with known properties of I_{K-Ca} whereas they cannot be easily explained on the basis of a participation of other currents at this rectification. This is true not only for the low threshold Ca conductance mentioned before, but also for the M current (Brown & Adams, 1980) which is not known to be affected by Ca-channel blockers and by external application of Cs (Halliwell & Adams, 1982). In keeping with a participation of I_{K-Ca} to the inward rectification of Purkinje cells near rest, one might expect that either Ca-channel blockers or Cs induce an inward shift in the holding current. This was not observed (see Results) probably because these substances also increased the membrane resistance of the cell, which by itself, had an opposite effect on the holding current. Furthermore, according to our interpretation (see above), Ca-channel blockers are likely to abolish both a steady inward Ca current and the outward I_{K-Ca} near rest, which makes the resulting shift in the holding current difficult to predict. Further investigation will be necessary to identify more accurately this I_{K-Ca} and in particular its sensitivity to tetraethylammonium and apamin since these two agents have differential effects on already well described I_{K-Ca} in other cells (Moolenar & Spector, 1979; Romey & Lazdunski, 1984; Pennefather, Lancaster, Adams & Nicoll, 1985).

Finally, a contribution of a Cl conductance to I_h such as that described in *Aplysia* neurones (Chesnoy-Marchais, 1983) appears unlikely since, in marked contrast to this current, I_h was blocked by Cs whereas its magnitude and kinetics were unchanged in low Cl solution. The lack of effect on I_h of injecting Cl ions into the cells also fits with this conclusion, although the injecting current markedly hyperpolarized the cells, which might have led to a redistribution of Cl ions across the membrane, thus leaving the internal Cl concentration at rather low levels (Chesnoy-Marchais, 1983). However, this objection cannot hold when considering the absence of effect on I_h of the probable leakage of Cl ions from the micro-electrodes (see Results).

On the whole, the present study strongly suggests that the inward rectification of Purkinje cells is due to the turning off of I_{K-Ca} near rest, and due to the turning on of I_h at more negative values of membrane potential. Both these currents contribute to buffer cell hyperpolarization and thereby are certainly partly responsible for the rather low resting potential of Purkinje cells, which in turn allows these neurones to have a sustained spontaneous firing.

Anodal breaks

The rebounds of depolarization following hyperpolarizing commands are likely to be due at least in part to Na and Ca currents which are partly inactivated at resting potential and de-inactivated by previous hyperpolarization of the cells (see Results). Furthermore, these currents seem to inactivate rather rapidly near resting potential since in voltage-clamp experiments the inward tail currents following hyperpolarizing commands which remained after complete blockade of I_h by Cs (see Fig. 3) decayed completely over a period of time which never exceeded 200–300 ms. Similarly, in current-clamp experiments, the duration of the anodal break ranged between 150 and 250 ms, and this even under Ba, i.e. when I_{K-Ca} is not activated (Meech, 1978) and when the other voltage dependent K conductances are likely to be reduced (see Schmidt & Crill, 1980). As far as Ca currents are concerned, this behaviour is typical of the low threshold Ca conductance described in other neurones (Llinãs & Yarom, 1981*a*, *b*; Bossu *et al.* 1985; Carbone & Lux, 1984*a*, *b*; Jahnsen & Llinãs, 1984*a*, *b*). It seems therefore that Purkinje cells do have such a conductance. The Na and low threshold Ca currents responsible for the anodal breaks might be the same as the sustained Na and Ca currents previously described in Purkinje cells (Llinãs & Sugimori, 1980*a*, *b*; Crepel *et al.* 1984). However, the latter currents seem to inactivate more slowly than the former and moreover, they can be elicited directly by depolarizing commands from resting potential (Llinãs & Sugimori, 1980*a*, *b*; Crepel *et al.* 1984).For these reasons, we cannot preclude that the Na and low threshold Ca currents underlying the anodal breaks are indeed different from the previously described slowly inactivating Na and Ca currents of Purkinje cells.

A possible role of these Na and Ca conductances might be to speed up the return to base line following inhibitory post-synaptic potentials induced in Purkinje cells by neighbouring interneurones. Therefore, like I_h, these currents might help to maintain the membrane potential of Purkinje cells near firing threshold and thus contribute, together with the other conductances described before, to give to Purkinje cells the main attributes of pace-maker neurones.

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REFERENCES

ADAMS, P. (1982). Voltage-dependent conductances of vertebrate neurons. *Trends in Neurosciences* **5**, 116–119.

ATTWELL, D. & WILSON, M. (1980). Behaviour of rod network in tiger salamander retina mediated by membrane properties of individual rods. *Journal of Physiology* **309**, 287–315.

BADER, C. R. & BERTRAND, D. (1984). Effects of changes in intra- and extracellular sodium on the inward (anomalous) rectification in salamander photoreceptors. *Journal of Physiology* **347**, 611–631.

BOSSU, J. L., FELTZ, A. & THOMANN, J. M. (1985). Depolarization slightly above resting potential elicits a transient Ca current in rat cranial sensory neurones. *Journal of Physiology* **358**, 72*P*.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage sensitive K^+ current in a vertebrate neurone. *Nature* **183**, 673–676.

BROWN, H. & DI FRANCESCO, D. (1980). Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *Journal of Physiology* **308**, 331–351.

CARBONE, E. & LUX, H. D. (1984*a*). A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. *Nature* **310**, 501–503.

CARBONE, E. & LUX, H. D. (1984*b*). A low voltage-activated calcium conductance in embryonic chick sensory neurons. *Biophysical Journal* **46**, 413–418.

CHESNOY-MARCHAIS, D. (1983). Characterization of a chloride conductance activated by hyperpolarization in *Aplysia* neurones. *Journal of Physiology* **342**, 277–308.

CONSTANTI, A. & GALVAN, M. (1983). Fast inward-rectifying current accounts for anomalous rectification in olfactory cortex neurones. *Journal of Physiology* **335**, 153–178.

CREPEL, F. & DELHAYE-BOUCHAUD, N. (1979). Distribution of climbing fibres on cerebellar Purkinje cells in X-irradiated rats. An electrophysiological study. *Journal of Physiology* **290**, 97–112.

CREPEL, F., DHANJAL, S. S. & GARTWAITE, J. (1981). Morphological and electrophysiological characteristics of rat cerebellar slices maintained *in vitro*. *Journal of Physiology* **316**, 127–138.

CREPEL, F., DUPONT, J. L. & GARDETTE, R. (1984). Selective absence of calcium spikes in Purkinje cells of staggerer mutant mice in cerebellar slices maintained *in vitro*. *Journal of Physiology* **346**, 111–125.

CREPEL, F. & PENIT-SORIA, J. (1984). Anomalous rectification in rat cerebellar Purkinje cells. An *in vitro* study. *Journal of Physiology* **357**, 16*P*.

DI FRANCESCO, D. (1981*a*). A new interpretation of the pace-maker current in calf Purkinje fibres. *Journal of Physiology* **314**, 351–376.

DI FRANCESCO, D. (1981*b*). A study of the ionic nature of the pace-maker current in calf Purkinje fibres. *Journal of Physiology* **314**, 377–393.

DI FRANCESCO, D. (1982). Block and activation of the pace-maker channel in calf Purkinje fibres; effects of potassium, caesium and rubidium. *Journal of Physiology* **329**, 485–507.

DI FRANCESCO, D. & OJEDA, C. (1980). Properties of the current i_f in the sino-atrial node of the rabbit compared with those of the current i_{K_2} in Purkinje fibres. *Journal of Physiology* **308**, 353–367.

FATT, P. & KATZ, B. (1953). The electrical properties of crustacean muscle fibres. *Journal of Physiology* **120**, 171–204.

GALVAN, M. & ADAMS, P. R. (1982). Control of the calcium current in rat sympathetic neurons by norepinephrine. *Brain Research* **244**, 135–144.

GAY, L. A. & STANFIELD, P. R. (1977). Cs^+ causes a voltage-dependent block of inward K currents in resting skeletal muscle fibres. *Nature* **267**, 167–170.

GRAFE, P., MAYER, C. J. & WOOD, J. D. (1980). Synaptic modulation of calcium-dependent potassium conductance in myenteric neurones in the guinea-pig. *Journal of Physiology* **305**, 235–248.

HAGIWARA, S. (1983). Membrane potential-dependent ion channels in cell membrane. Phylogenetic and developmental approaches. In *Distinguished Lecture Series of the Society of General Physiologists*, vol. 3. New York: Raven Press.

HAGIWARA, S., MIYZAKI, S. & ROSENTHAL, N. P. (1976). Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *Journal of General Physiology* **67**, 621–638.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250**, 71–92.

HERMANN, A. & HARTUNG, K. (1983). Ca^{2+} activated K^+ conductance in molluscan neurones. *Cell Calcium* **4**, 387–405.

JAHNSEN, H. & LLINÃS, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *Journal of Physiology* **349**, 205–226.

JAHNSEN, H. & LLINÃS, R. (1984*b*). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* **349**, 227–247.

JOHNSTON, D. & BROWN, T. H. (1983). Interpretation of voltage-clamp measurements in hippocampal neurons. *Journal of Neurophysiology* **50**, 464–507.

KATZ, B. (1949). Les constantes électriques de la membrane du muscle. *Archives des Sciences Physiologiques* **3**, 285–299.

LLINÃS, R. & SUGIMORI, M. (1980*a*). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *Journal of Physiology* **305**, 171–195.

LLINÃS, R. & SUGIMORI, M. (1980*b*). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**, 197–213.

LLINÁS, R. & YAROM, Y. (1981*a*). Electrophysiology of mammalian inferior olivary neurones *in vitro*. Different types of voltage-dependent ionic conductances. *Journal of Physiology* **315**, 549–567.

LLINÁS, R. & YAROM, Y. (1981*b*). Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones *in vitro*. *Journal of Physiology* **315**, 569–584.

MARTY, A. (1981). Ca-dependent K channels with large unitary conductance in chromaffin cell membranes. *Nature* **291**, 497–500.

MAYER, M. L. & WESTBROOK, G. L. (1983). A voltage-clamp analysis of inward (anomalous) rectification in mouse spinal sensory ganglion neurones. *Journal of Physiology* **340**, 19–45.

MEECH, R. W. (1974). The sensitivity of *Helix aspersa* neurones to injected calcium ions. *Journal of Physiology* **237**, 259–277.

MEECH, R. W. (1978). Calcium-dependent potassium activation in nervous tissues. *Annual Review of Biophysics and Bioengineering* **7**, 1–18.

MOOLENAR, W. H. & SPECTOR, I. (1979). The calcium current and the activation of a slow potassium conductance in voltage-clamped mouse neuroblastoma cells. *Journal of Physiology* **292**, 307–323.

PALLOTTA, B. S., MAGLEBY, K. & BARRETT, J. N. (1981). Single channel recordings of Ca^{2+}-activated K^+ currents in rat muscle cell culture. *Nature* **293**, 471–474.

PENNEFATHER, P., LANCASTER, B., ADAMS, P. R. & NICOLL, R. A. (1985). Two distinct Ca-dependent K currents in bullfrog sympathetic ganglion cells. *Proceedings of the National Academy of Sciences of the U.S.A.* **82**, 3040–3044.

RALL, W. (1969). Time constants and electrotonic lengths of membrane cylinders and neurons. *Biophysical Journal* **9**, 1483–1508.

ROMEY, G. & LAZDUNSKI, M. (1984). The coexistence in rat muscle cells of two distinct classes of Ca^{2+}-dependent K^+ channels with different pharmacological properties and different physiological functions. *Biochemical and Biophysical Research Communications* **118**, 669–674.

SCHMIDT, P. C. & CRILL, W. (1980). Properties of a persistent inward current in normal and TEA-injected motoneurons. *Journal of Neurophysiology* **43**, 1700–1724.

WILSON, W. & GOLDNER, M. A. (1975). Voltage-clamping with a single microelectrode. *Journal of Neurobiology* **4**, 411–422.