J. Physiol. (1984), **346**, *pp.* 111–125
With 9 text-figures
Printed in Great Britain

SELECTIVE ABSENCE OF CALCIUM SPIKES IN PURKINJE CELLS OF *STAGGERER* MUTANT MICE IN CEREBELLAR SLICES MAINTAINED *IN VITRO*

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(*Received 14 December 1982*)

SUMMARY

1. The bioelectrical properties of Purkinje cells (intrasomatic recordings) were studied in sagittal cerebellar slices of both adult *staggerer* and control mice.

2. Mean input resistances of Purkinje cells were 25 ± 4 MΩ and 48 ± 7 MΩ in normal and *staggerer* mice respectively. In both groups, time-dependent inward rectifications were apparent in the hyperpolarizing voltage-responses.

3. In normal mice, tetrodotoxin (TTX)-sensitive simple spikes and slower-rising multiphasic spikes, abolished when Ca was replaced by Cd in the bath, spontaneously occurred in Purkinje cells. These Na- and Ca-dependent spikes were also elicited by depolarizing current pulses.

4. In the mutant, Ca spikes were never observed, even in strongly depolarized cells. On the contrary, TTX-sensitive simple spikes occurred spontaneously or were elicited by depolarizing current pulses.

5. When Ca was replaced by Ba in the bath, the Ca spikes evoked in normal Purkinje cells by direct stimulation were first enhanced and then replaced by prolonged action potentials (1–6 s in duration) which were TTX-resistant and Cd-sensitive. These (Ba) action potentials were also triggered by climbing fibre activation of the cells.

6. In *staggerer* mice, Ca spikes were never elicited by direct stimulation in Ba-containing medium, although in a few cells prolonged action potentials were occasionally elicited by depolarizing current pulses. However, this latter type of response was never evoked by climbing fibre activation of Purkinje cells.

7. In the mutant, extracellular application of tetraethylammonium (TEA) generated prolonged action potentials, the plateaux of depolarization of which were less positive than those elicited by Ba in control mice. These plateaux were abolished by TTX and left unaffected by the substitution of Ca by Cd in the bath, suggesting that they were due to a non-inactivating Na conductance.

8. On the whole, the present study strongly suggests that voltage-dependent Ca channels are missing in most *staggerer* Purkinje cells or at least that their characteristics and/or distribution are such that they cannot be activated. Na channels appear unaffected.

INTRODUCTION

Several types of ionic conductances have now been identified in invertebrate and vertebrate neurones (see Adams, 1982), in addition to the fast Na and K currents initially described by Hodgkin & Huxley (1952) in the squid axon. In particular, in vertebrates, some classes of neurones not only exhibit Na-dependent spikes but also can fire Ca spikes, the latter probably being generated in the dendrites (Barrett & Barrett, 1976; Schwartzkroin & Slawsky, 1977; Wong, Prince & Basbaum, 1979; Fain, Gerschenfeld & Quandt, 1980; Llinas & Sugimori, 1980*a*, *b*; Llinas & Yarom, 1980). Since in unicellular animals and in invertebrates, point mutations can selectively modify a given class of ionic channels – as for instance K or Na conductances in *Drosophila* and Ca spikes in *Paramecium* (Kung, Chang, Satow, Van Houten & Hansma, 1975; Jan, Jan & Dennis, 1977; Wu & Ganetzky, 1980; Salkoff & Wyman, 1981) – one wonders whether the genome controls bioelectrical properties of nerve cells in the same way in vertebrates and especially in mammals.

Cerebellar Purkinje cells in rodents might be a suitable model for such studies. Firstly, they exhibit at least six ionic conductances, and in particular, they can fire simple (Na) spikes at a somatic level, and slower-rising (Ca) spikes in their dendrites (Llinas & Sugimori, 1980*a*, *b*). Secondly, in the mouse, several point mutations affect the cerebellum (Sidman, Green & Appel, 1965) and some of them seem to have a direct impact on Purkinje cells (see Sotelo, 1980). Among the latter, the recessive *staggerer* (*sg*) mutation is of special interest since in homozygous (*sg/sg*) animals, Purkinje cell dendrites never form spiny branchlets and synaptic contacts with parallel fibres, despite the presence of a substantial number of these axons during synaptogenesis (Sidman, 1972; Sotelo & Changeux, 1974; Landis & Sidman, 1978). This therefore suggests that Purkinje cell dendrites are very defective in this mutant. Furthermore, in the adult, Purkinje cell membranes express immature components on their surface, which also stresses a possible direct effect of the mutation on the membrane of these neurones (Trenkner, 1979).

Therefore, we have investigated the bioelectrical properties of Purkinje cells in adult *staggerer* mice, compared with those in normal mice, with special reference to Na and Ca spikes. This was achieved by using cerebellar slices maintained *in vitro*, since this preparation allows very stable recordings as well as bath application of various drugs. This work has previously been presented in abstract form (Crepel, Dupont & Gardette, 1983).

METHODS

Hybrids of C57Bl and DBA 2J mice, bearing the *staggerer* mutation, were intercrossed to obtain homozygous mutants (*sg/sg*) from heterozygous animals. The mutant and control mice of the same hybrid strain were used for electrophysiological studies 5–14 months after birth. In both groups of animals, sagittal cerebellar slices 400 μm thick were obtained as previously described (Crepel & Dhanjal, 1982; Crepel, Dhanjal & Sears, 1982). They were transferred in less than 3 min to a recording chamber fed with a Krebs solution containing (mM): NaCl (124), KCl (5), KH_2PO_4 (1·15), $MgSO_4 . 7H_2O$ (1·15), $CaCl_2$ (2·5), $NaHCO_3$ (25), glucose (10). The solution was gassed with a mixture of O_2 (95%) and CO_2 (5%). The characteristics of the chamber, as well as the stimulating and recording procedures, were the same as in our previous *in vitro* studies (Crepel, Dhanjal & Garthwaite, 1981; Crepel & Dhanjal, 1982; Crepel *et al.* 1982, 1983).

RESULTS

Purkinje cells were penetrated at the somatic level under direct visualization of the cortical layers. Stable intracellular recordings were obtained from forty Purkinje cells in controls and forty-five Purkinje cells in *staggerer* mice. The mean resting potentials were $-56 \pm 1{\cdot}9$ mV and $-62 \pm 2{\cdot}3$ mV respectively. Identification of the cells was further confirmed by their typical antidromic activation and climbing fibre responses (Eccles, Ito & Szentagothai, 1967), elicited by bipolar electrical stimulation of the underlying white matter (Fig. 1). In both groups, antidromic spike potentials lasted about 1 ms, and they often presented an inflexion on their rising phase, suggesting that they consisted of an 'initial segment' (i.s.) component and a 'somatodendritic' (s.d.) component (Fig. 1; see also Fig. 3). In *staggerer* mice, as

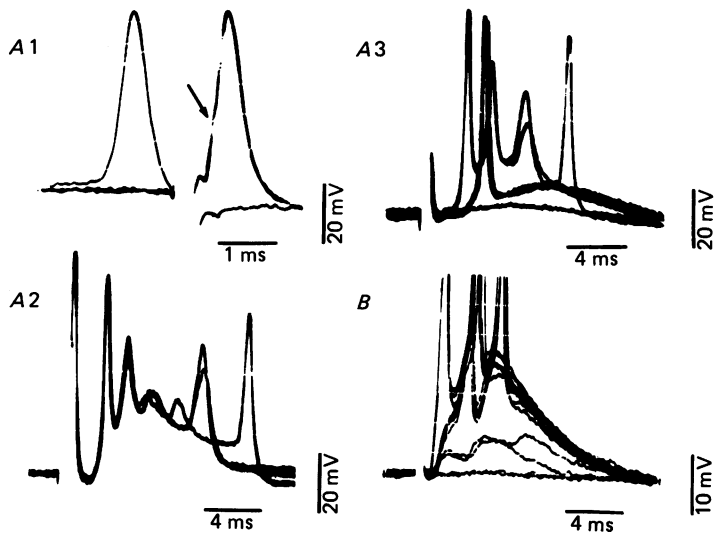

Fig. 1. Identification of Purkinje cells in *staggerer* mice. Cells were identified by their intrasomatic responses to electrical stimulation of the white matter. Superimposed sweeps in all records. In *A*1 an antidromic spike, with an i.s.–s.d. inflexion on its rising phase (arrow), was elicited at each stimulation (except in one sweep, when there was a collision with a previously occurring simple spike). *A*2 and *A*3, climbing fibre responses of the same neurone as in *A*1. In *A*2 the intensity of the stimulus was adjusted to give the maximum response, whereas it was progressively decreased in *A*3, to reveal the stepwise variation of the climbing fibre response. *B*, as *A*2 and *A*3, in another Purkinje cell. Positive is up in these and all succeeding intracellular records.

expected from *in vivo* experiments, the climbing fibre responses were graded by steps (three to six) with the intensity of the stimulus (Fig. 1 *A*2, *A*3, *B*), thus confirming the multiple innervation of Purkinje cells by climbing fibres in these mutants (Crepel, Delhaye-Bouchaud, Guastavino & Sampaio, 1980; Mariani & Changeaux, 1980).

Input resistance of Purkinje cells

Input resistances were determined by measuring the sizes of the electrotonic potentials induced by small depolarizing current pulses (0·2–1·4 nA) passed across the

cell membrane through the recording micro-electrode. In control and mutant mice, as in guinea-pig (Llinas & Sugimori, 1980*a*), marked 'sags' were apparent in most of the recorded voltage-responses (Fig. 2*A*, *C*, *D*), thus suggesting the presence of 'active components' in these hyperpolarizations. For this reason, transmembrane voltage changes were arbitrarily measured at the plateau of polarization, near the end of the responses. Under these conditions, mean input resistances were 25 ± 4 MΩ and 48 ± 7 MΩ in control and *staggerer* mice respectively. In both groups, bursts of simple spikes were often elicited immediately after the hyperpolarizing responses (Fig. 2*B* and *D*).

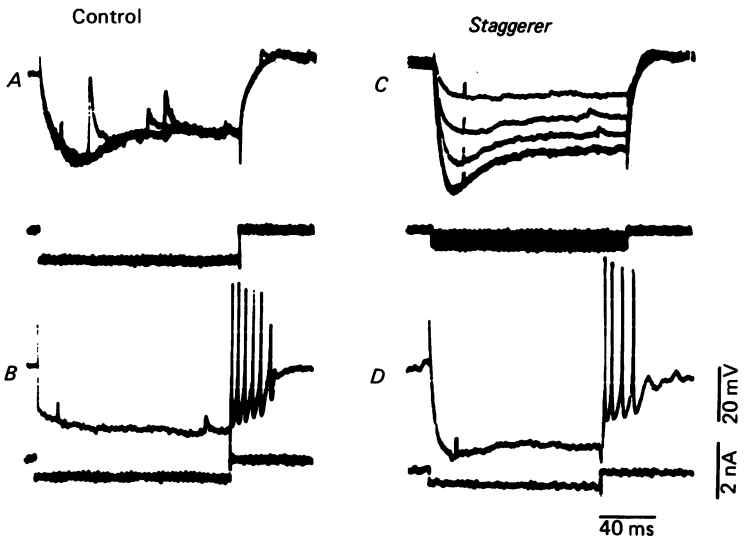

Fig. 2. Input resistances of Purkinje cells in control and *staggerer* mice. *A* to *D* illustrate membrane potentials (upper traces) produced by hyperpolarizing current steps (lower traces) in four different Purkinje cells. Note the presence of 'sags' in the voltage-responses in *A* and *C*, and the bursts of simple spikes elicited after the hyperpolarizing responses in *B* and *D*.

Spontaneous activity of Purkinje cells

In normal mice, most Purkinje cells were spontaneously active in slices maintained in standard Krebs solution. At normal resting potential, this activity consisted of a regular firing of simple spikes, 1 ms in duration and 50–70 mV in amplitude, with firing frequencies ranging between 20 and 50/s (Fig. 3*A*1). When steady depolarizing currents (0·2–0·5 nA) were passed through the recording micro-electrode, or when cells were depolarized by deterioration, slower-rising, multiphasic spikes 30–50 mV in amplitude were also elicited. They gave rise, together with the simple spikes, to a regular bursting activity of the cells (Fig. 3*A*2). These slow spikes were still visible in more depolarized cells, whereas simple spikes were completely inactivated (Fig. 3*A*3). The characteristics of the simple spikes and of the slower-rising multiphasic action potentials were therefore identical to those of Na and Ca spikes previously recorded *in vitro* from Purkinje cells in the guinea-pig (Llinas & Sugimori, 1980*a*, *b*). As expected from this analogy, the simple spikes were selectively abolished

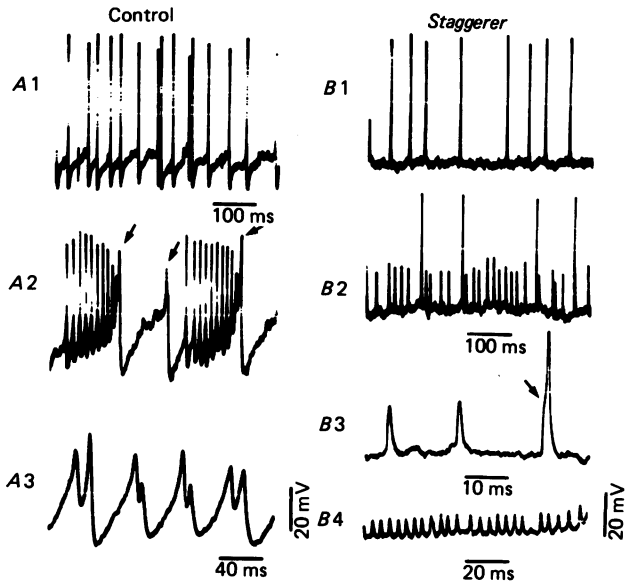

Fig. 3. Spontaneous activity of Purkinje cells in control and *staggerer* mice. *A*, control mouse. At normal resting potential of −58 mV (*A*1) the spontaneous activity of this cell consisted of a regular firing of simple spikes. In *A*2 a steady depolarizing current of 0·3 nA was applied through the micro-electrode and led to a bursting activity of simple and slower-rising spikes (arrows). *A*3, same cell as in *A*1 and *A*2 but with a depolarizing current of 0·6 nA. Note the complete inactivation of the simple spikes and the prominent bursting of Ca spikes. *B*, *staggerer* mouse. *B*1, spontaneous firing of simple spikes at normal resting potential (−61 mV). *B*2 and *B*3, activity of the same cell as in *B*1 at a membrane potential of −47 mV. Note that the 's.d.' components of the spikes were missing in most cases, or were delayed when they were present (arrow in *B*3). In *B*4 the cell was further depolarized up to −35 mV, leading to a marked inactivation of the 'i.s.' spikes. Full explanations in the text.

when tetrodotoxin (TTX) was added in the bath at a concentration of 2×10^{-6} to 5×10^{-6} M, whereas Ca spikes were no longer present in Ca-free Krebs solution containing 0·5–1 mM-Cd, a cation known to block Ca conductance (see Adams, 1982) (not illustrated).

In staggerer mice, slow Ca spikes were never observed, even when the cells were strongly depolarized by deterioration or by steady currents of up to 5–7 nA (not illustrated). On the contrary, spontaneous simple spikes were routinely recorded in most neurones, and their duration and amplitude were similar to those of the Na spikes seen in controls (Fig. 3*B*1). In the case of the cell illustrated in Fig. 3*B*, no hyperpolarizing after-potentials followed these simple spikes. They were clearly visible in other cells (see Fig. 7*A*1). As in controls, spontaneous simple spikes, as well as those elicited by electrical stimulation, were abolished when TTX was added to the bath at concentrations ranging between $0{\cdot}2 \times 10^{-6}$ and $0{\cdot}8 \times 10^{-6}$ M (Fig. 4), thus confirming that they were due to fast Na currents. In slightly depolarized neurones, the s.d. component of the spike was often delayed or missing (Fig. 3*B*2, *B*3), whereas in more strongly depolarized cells the i.s. spike was also severely reduced in amplitude or even completely inactivated (Fig. 3*B*4).

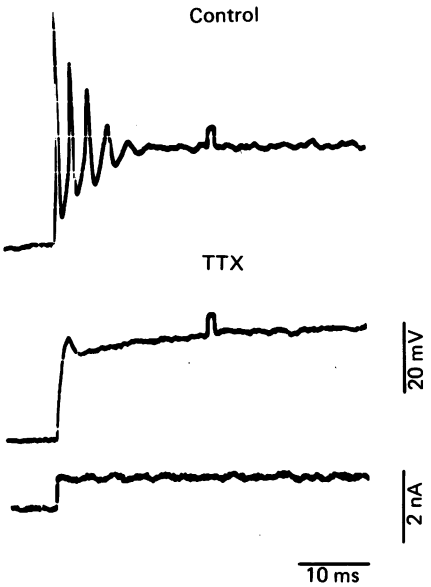

Fig. 4. Effect of TTX on simple spikes in *staggerer* mice. The burst of simple spikes elicited in a Purkinje cell (top trace) by a depolarizing current step of 1 nA (bottom trace), in normal Krebs solution, was abolished when $0{\cdot}5 \times 10^{-6}$ M-TTX was added to the bath (middle trace).

Responses induced by direct electrical stimulation in standard Krebs solution and after substitution of extracellular Ca by Ba

In normal mice and in standard Krebs solution, small depolarizing current pulses (less than 0·3 nA) elicited a marked depolarization of the cells, which in turn generated a repetitive firing of Na spikes (Fig. 5*A*1). With higher stimulus intensities (up to 1 nA) the burst of Na spikes terminated before the end of the pulse, either abruptly (Fig. 5*A*2) or progressively (not illustrated). This revealed the presence of a plateau of depolarization on which slower-rising, multiphasic action potentials were or were not superimposed (Fig. 5*A*2 and *A*3). In all cells tested, at the end of the current pulse, prolonged after-depolarizations were observed which were graded in amplitude with the stimulus intensity (Fig. 5*A*1, *A*2 and *A*3).

Following entire substitution in the bath of Ca by 2·5 mM-Ba (i.e. by an ion known to block voltage-dependent K conductances of nerve cells, to move more easily than Ca through Ca channels and not to activate the Ca-dependent K conductance: refs. in Llinas & Sugimori, 1980*a*, and in Schwindt & Crill, 1980) both the presumed Ca spikes and the after-depolarizations were first enhanced (Fig. 5*A*4) and then often replaced by prolonged action potentials 1–6 s in duration and 50–60 mV in amplitude (Fig. 5*A*5 and *B*). These prolonged action potentials were very similar to the Ba-dependent action potentials recorded from Purkinje cells in the guinea-pig under the same conditions (Llinas & Sugimori, 1980*a*). Accordingly, the prolonged Ba action potentials were not affected by TTX (not illustrated) but they disappeared when Cd (1 mM) was added to the Ba-containing medium (Fig. 6*A*–*C*). However, in medium containing Ba plus Cd, their disappearance revealed underlying plateaux of de-

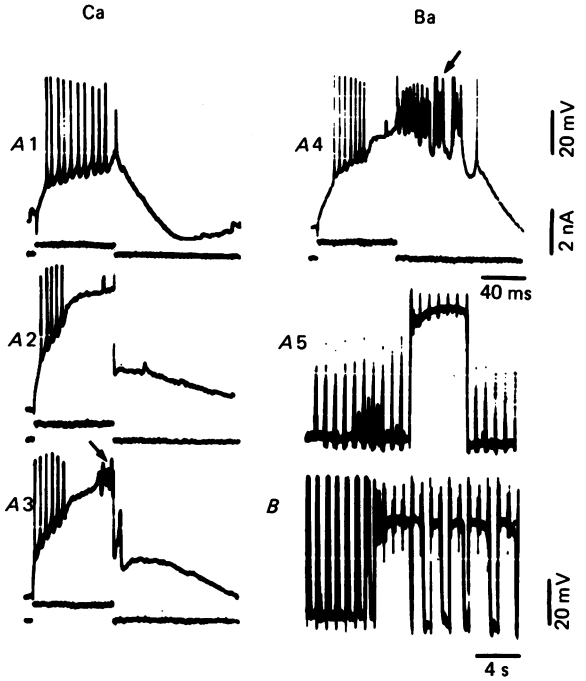

Fig. 5. Responses of normal Purkinje cells to direct electrical stimulations. *A*, responses of a Purkinje cell to stimulating currents of two different intensities (lower traces) in normal Krebs solution (*A*1, *A*2, *A*3) and after substitution of Ca by Ba in the bath (*A*4, *A*5). Note in *A*4 the enhancement in Ba-containing medium of the Ca spikes (arrow) and of the post-depolarizations elicited by the same stimulating current as in *A*3. Note also the presence of a prolonged action potential in *A*5, which was triggered by the same stimulating current pulse as in *A*3 and *A*4 (full explanations in the text). *B*, prolonged action potentials elicited in another Purkinje cell by short depolarizing current pulses (0·3 nA, 200 ms) applied at a frequency of 1·2/s in the presence of Ba. The first seven stimulating current pulses only gave rise to bursts of Ca spikes and post-depolarizations.

polarization of lower amplitude (25–35 mV) (Fig. 6*E* and *D*). These latter responses were abolished, together with the fast Na spikes, when TTX was added to the bath (Fig. 6*E* and *F*). Therefore, these experiments indicate that in normal mice two classes of ionic conductances are activated in Ba-containing medium: namely a non-inactivating Na conductance, and a non-inactivating Ca (Ba) conductance giving rise to larger (50–60 mV) depolarizations. These results therefore fully corroborate previous results of Llinas & Sugimori (1980*a*).

In staggerer mice, and in standard superfusing fluid, Ca spikes and after-depolarizations were never elicited in Purkinje cells by depolarizing current pulses (Fig. 7*A*), even when stimulus intensities were as high as 3–5 nA (i.e. the maximum currents which could be passed through the recording micro-electrodes in these experiments). On the contrary, these direct stimulations invariably induced the same type of repetitive firing of Na spikes as in controls (Fig. 7*A*).

When Ca was replaced by Ba in the bath, a steady depolarization ranging between 10 and 30 mV in amplitude progressively developed in the nineteen cells that were tested. This depolarization led in turn to a faster inactivation of the Na spikes within

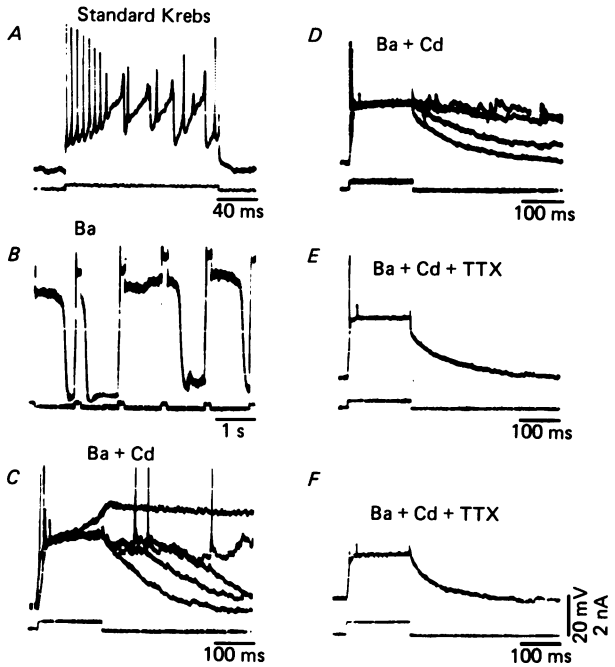

Fig. 6. Effect of divalent cations and TTX on responses of normal Purkinje cells. All responses in this Figure (upper traces) were elicited in the same neurone by direct electrical stimulation (lower traces) under the following conditions: standard Krebs (*A*), after replacement of Ca by Ba in the bath (*B*), after addition of Cd (1 mM) to the medium containing Ba (*C*, *D*), and after addition of TTX to the bath (*E*, *F*). Note that the prolonged action potentials elicited under Ba were abolished by Cd, except in one sweep in *C*, taken during the transition from the medium containing Ba to the Ba + Cd perfusing solution. This revealed the presence of underlying plateaux of depolarization of various durations which, in turn, were abolished by TTX, together with the initial fast (Na) spikes of the responses (full explanation in the text).

the bursts elicited by short current pulses (compare Fig. 7*A*3 and *A*4). In seventeen out of these nineteen cells no Ca spikes, after-depolarizations or prolonged action potentials were ever elicited under these conditions. In the two other cells no Ca spikes or after-depolarizations were observed in the presence of Ba. However, prolonged action potentials (50–60 mV in amplitude) were elicited on rare occasions by depolarizing current pulses of 0·4–0·8 nA. They were characterized by a rather long duration of their plateau phase and/or their repolarization (Fig. 7*A*5 and *B*).

Effect of extracellular replacement of Ca by Ba on climbing fibre responses of Purkinje cells

The above experiments failed to reveal the presence of any Ca spike in *staggerer* Purkinje cells following depolarization initiated in their somata. However, Ca channels are normally located in the dendrites (Llinas & Sugimori, 1980*a*, *b*). Therefore, another set of experiments was performed to investigate the possibility of activating Ca channels in control and in mutant mice, in the presence of Ba, by synaptic activation of the Purkinje cell dendrites via the climbing fibres.

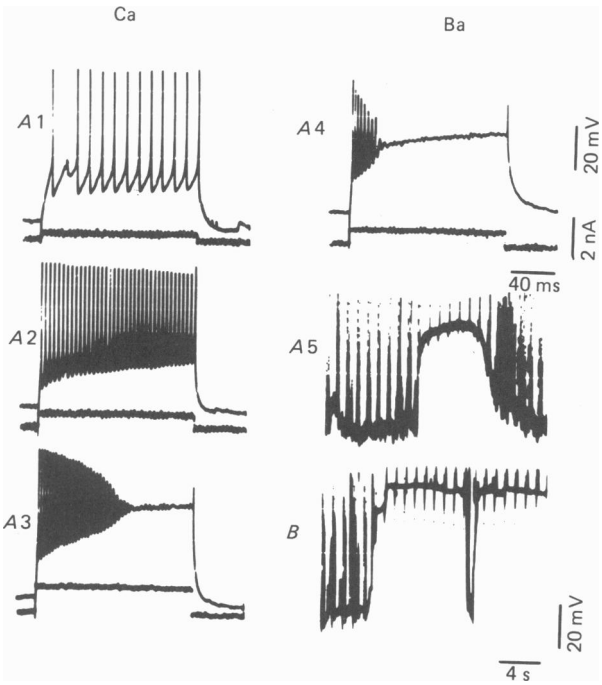

Fig. 7. Responses of *staggerer* Purkinje cells to direct electrical stimulations. *A*, responses of a Purkinje cell to stimulating currents of various intensities (lower traces) in normal Krebs solution (*A*1–*A*3) and after replacement of Ca by Ba in the bath (*A*4, *A*5). Ca spikes were never elicited in standard and Ba-containing superfusing solution. Thus, the response illustrated in *A*4 is very similar to those illustrated in *A*2 and *A*3 except for a faster inactivation of the Na spike due to a steady cell depolarization of 10 mV induced by Ba. Note also the absence of any after-depolarization in *A*1–*A*4 (compare with Fig. 5). In the cell shown in *A*5 a prolonged action potential was triggered by one of the short depolarizing current pulses (0·5 nA, 150 ms) applied at a frequency of 1·2/s. The membrane potential of the cell during the plateau phase of the prolonged action potential was about +10 mV. *B* illustrates two prolonged action potentials which were elicited in another Purkinje cell by short depolarizing current pulses, in the presence of Ba, with plateau phases culminating at about +15 mV.

In normal mice, replacement of Ca by Ba in the bath led first to a progressive lengthening of the plateau of depolarization following the initial full spike of the climbing fibre response evoked in Purkinje cells by electrical stimulation of the white matter. Fully developed plateau responses lasted up to 700 ms with an amplitude ranging between 25 and 35 mV (Fig. 8*A*1 and *A*2, traces 1, 2 and 3), i.e. that of responses due to non-inactivating Na conductance (see above). Then, the characteristics of the plateau depolarizations following the initial full spike of the responses changed in an all-or-none manner, i.e. their amplitude suddenly increased up to 50–60 mV and their duration up to 1–6 s (Fig. 8*A*2, trace 4) with an abrupt termination. This latter type of response was therefore apparently identical to the prolonged Ba action potentials elicited by direct stimulation of the cells. Following such a response, the next evoked climbing fibre response had a much shorter duration

(see for instance trace 1 of Fig. 8*A*2) and a new cycle of changes in the characteristics of the climbing fibre responses started, identical to that described above. These recurrent changes lasted for as long as Ba replaced Ca in the bath.

In staggerer mice, climbing fibre responses of Purkinje cells remained unaltered after extracellular replacement of Ca by Ba, with the exception of a reduction in their amplitude probably due to the concomitant cell depolarization caused by Ba application (see previous section). The responses never gave rise to any subsequent plateau depolarization or prolonged Ba action potentials (Fig. 8*B*1 and *B*2).

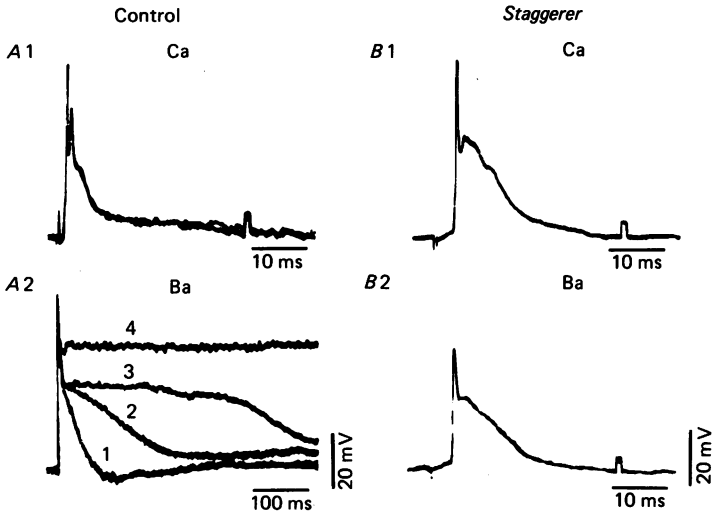

Fig. 8. Effect of replacement of extracellular Ca by Ba on climbing fibre responses in control and *staggerer* mice. *A*, climbing fibre response elicited by white matter stimulation in a normal Purkinje cell in standard Krebs solution (*A*1) and in Ba-containing medium (*A*2). The superimposed successive sweeps in *A*2 illustrate the progressive building-up of a plateau of depolarization following the response (1 to 3), and the appearance, in trace 4, of an all-or-none prolonged action potential. *B*1 and *B*2, same experiments as in *A*1 and *A*2 in a Purkinje cell from a *staggerer* mouse. Full explanations in the text.

Effect of tetraethylammonium on Purkinje cell responses in staggerer mice

This series of experiments was performed on twelve cells to ascertain that the failure of Purkinje cells to fire Ca spikes in *staggerer* mice was not due to an abnormally well-developed K conductance which might prevent the membrane potential from reaching the level required for activation of Ca channels. K currents were pharmacologically depressed by adding tetraethylammonium (TEA : 15 mM) to the superfusing standard Krebs solution. This procedure was used since it has recently been well established that extracellular application of this drug can effectively block most fast K currents in cat and frog motoneurones (Barrett & Barrett, 1976; Schwindt & Crill, 1981).

The major change observed in the electrophysiological properties of Purkinje cells following TEA application was the appearance of prolonged action potentials consisting of an initial fast spike followed by a plateau of depolarization the amplitude and duration of which ranged between 30 and 40 mV and between 50 and 300 ms

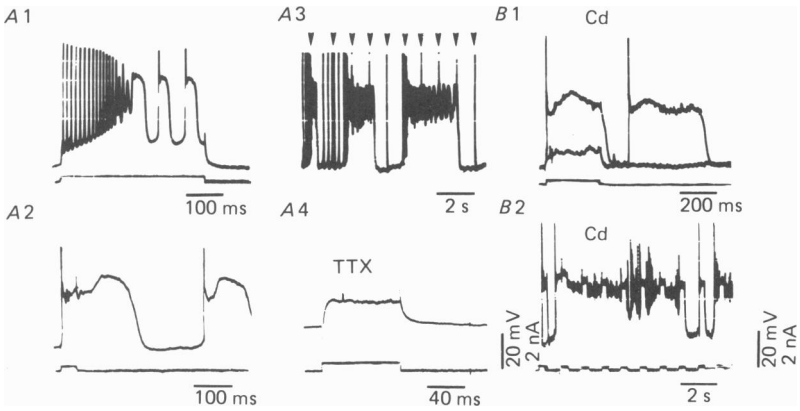

Fig. 9. Effect of extracellular application of TEA on Purkinje cell responses in *staggerer* mice. *A*1, response of a Purkinje cell to a depolarizing current step (lower trace) during the transition from the standard Krebs solution to the medium containing TEA. Note the appearance of prolonged action potentials at the end of the response. *A*2 and *A*3, responses of the same Purkinje cell as in *A*1 after complete replacement of the normal superfusing fluid by the TEA-containing solution. In *A*2 a prolonged action potential was triggered by a short depolarizing current pulse, and it was followed by a spontaneously occurring spike with similar characteristics. In *A*3 long-lasting plateau responses were triggered by climbing fibre responses (arrowheads), with marked oscillations on their plateau phases. *A*4, same cell as in *A*1–*A*3 after addition of 5×10^{-6} M-TTX to the TEA-containing medium. All regenerative responses previously elicited by direct stimulation of the cell were now abolished. *B*, prolonged action potentials and plateaux of depolarization elicited in another Purkinje cell by direct electrical stimulation (lower traces) in Ca-free Krebs solution containing 15 mM-TEA and 0·6 mM-Cd.

respectively. These prolonged action potentials were elicited by direct stimulation of the cells and also occurred spontaneously (Fig. 9*A*1 and *A*2). Much more prolonged plateau depolarizations, 30–40 mV in amplitude and 1–4 s in duration, were also elicited in these cells, either by direct electrical stimulation or by climbing fibre activation (Fig. 9*A*3). Clear-cut oscillations (up to 20 mV in amplitude) were present on the plateau phase of these latter long-lasting depolarizations (Fig. 9*A*3), suggesting that they might result from a nearly complete fusion of all-or-none prolonged action potentials such as those illustrated in Fig. 9*A*1 and *A*2.

All these regenerative responses were completely abolished when TTX was added to the bath at a concentration of 5×10^{-6} M (Fig. 9*A*4). By contrast, when Krebs solution containing TEA was replaced by a Ca-free medium containing the same concentration of TEA as before plus 0·6 mM of the Ca channel blocker Cd, these prolonged action potentials and plateau responses were still present with similar characteristics, except that the oscillations on the latter were greatly reduced in amplitude or even absent (Fig. 9*B*1, *B*2).

Similar experiments performed in seven Purkinje cells from control mice showed that, in the presence of Ca-free Krebs solution containing 15 mM-TEA plus 0·5 or 1 mM-Cd, there was first abolition of the Ca spikes elicited by direct stimulation of the cell, and then the appearance of prolonged action potentials and plateau responses, the characteristics of which were identical to those seen in *staggerer* mice

under the same conditions (not illustrated). In both groups of animals, all these regenerative responses, including the initial fast component of the prolonged action potentials, disappeared within a few minutes when Cd concentration was further raised to 1·5 mM, suggesting that at such relatively high concentrations this ion exerted non-specific effects on Purkinje cell membranes. Therefore, these experiments imply that the plateaux of depolarization and plateau phases of prolonged action potentials observed after TEA in Purkinje cells from *staggerer* mice, and in the presence of TEA plus Cd in normal mice, are mainly due to the same type of non-inactivating, TTX-sensitive Na conductance as previously described in normal Purkinje cells (see above).

DISCUSSION

In normal mice, the bioelectrical properties of Purkinje cells appear very similar to those previously described in guinea-pig and rat cerebellar Purkinje cells maintained *in vitro* (Llinas & Sugimori, 1980*a*, *b*; Crepel *et al.* 1981, 1982; Crepel & Dhanjal, 1982). In particular, in normal Krebs solution the cells can fire both fast (Na) and slow (Ca) action potentials, as previously demonstrated in the guinea-pig (Llinas & Sugimori, 1980*a*, *b*).

Several other conductances are probably also present in the Purkinje cells of normal mice. Thus, the 'sags' in voltage-responses induced by hyperpolarizing current pulses might be due to the turning off of 'M' current, or to the activation of the anomalous rectifier (see Adams, 1982). In keeping with the first hypothesis, acetylcholine depolarizes Purkinje cells and increases their membrane resistance (Crepel & Dhanjal, 1982). The prolonged after-depolarizations following application of depolarizing current pulses resemble the Ca-dependent responses induced under the same conditions in guinea-pig cerebellar Purkinje cells (Llinas & Sugimori, 1980*a*), especially because they are graded in amplitude with the stimulus intensity, and also because they are markedly enhanced by Ba. Finally, the plateau depolarizations and the plateau phases of the prolonged action potentials elicited in medium containing Ba plus Cd or in the presence of TEA plus Cd in Ca-free solution are likely to be mainly due to a non-inactivating Na conductance similar to that previously described in the guinea-pig (Llinas & Sugimori, 1980*a*), since they were abolished when TTX was added to the bath (see Results). However, voltage-clamp experiments are clearly required to elucidate the exact nature of these postulated conductances.

In staggerer mice, the abnormally high input resistance of Purkinje cells might simply be due to the over-all reduction in size of these neurones in the mutant (Landis & Sidman, 1978). Otherwise, the presence of sags in the voltage-responses induced by square hyperpolarizing jumps, as well as the rebound excitations observed at the end of such jumps, suggest that the conductances underlying these 'active' responses (see above) remain at least qualitatively normal. Similarly, the steady depolarizations of Purkinje cells elicited by Ba, a cation known to block voltage-dependent K conductances (refs. in Schwindt & Crill, 1980), suggest the presence of such K conductances in *staggerer* mice. The same conclusion holds for fast Na currents since TTX-sensitive simple spikes, whether they occurred spontaneously or were elicited by direct stimulation, were very similar to the Na spikes recorded under the same

conditions in normal animals. Furthermore, the distribution of Na channels might also remain unaltered in the mutant: the presence of an i.s.–s.d. inflexion on antidromic action potentials and on spontaneous simple spikes, previously seen in *in vivo* experiments (Crepel & Mariani, 1975), demonstrates that the activation of the initial segment of the axon normally precedes that of the somatodendritic region of the cells. However, we cannot infer from the present data whether Na spikes are restricted to Purkinje cell somata as in controls (Llinas & Sugimori, 1980*a*, *b*), or whether they also actively invade the dendrites. Finally, as in controls, a non-inactivating (TTX-sensitive) Na conductance also seems to be present in the mutant (see Results).

The absence of any detectable response mediated via Ca channels in most *staggerer* Purkinje cells is the most salient deficit disclosed in these neurones in the present study. First, it could result from abnormally high K conductances, preventing cell depolarization from reaching the firing level of Ca spikes. The fact that Ca spikes were never elicited in the presence of Ba or TEA, two blockers of the fast K currents (refs. in Schwindt & Crill, 1980, 1981), suggests that this is not the case, although other K conductances are less affected by these agents in other vertebrate neurones (Schwindt & Crill, 1980, 1981). Secondly, since in normal animals Ca channels are mainly borne by Purkinje cell dendrites (Llinas & Sugimori, 1980*a*, *b*), the absence of Ca spikes (and of Ba spikes in Ba-containing medium) might simply result from an abnormally low electrotonic propagation into the dendrites of depolarizations applied at a somatic level. This possibility seems unlikely since Ca channels were not activated in *staggerer* mice, even when depolarizing currents were up to 5 or 10 times larger than those which routinely evoke Ca- or Ba-dependent responses in control mice. Accordingly, prolonged Ba action potentials were never elicited in *staggerer* Purkinje cells by direct activation of their dendrites through climbing fibres in medium containing Ba, whereas such responses were routinely evoked in control mice under the same conditions. However, one may argue that in *staggerer* mice climbing fibre–Purkinje cell synapses are restricted to the lowermost domain of the dendrites, whereas they extend to more distal branches in normal mice (Larramendi, 1969; Landis & Sidman, 1978). Therefore, the possibility exists that in the mutants, Ca channels are present and do have normal activation properties, but are only borne by remote dendrites, in contrast to the controls in which they are also present on more proximal branches (Llinas & Hess, 1976; Llinas & Sugimori, 1980*b*).

Finally, the lack of Ca spikes in the mutant might also result either from abnormal activation properties of Ca channels, or from a decreased density or even a complete absence of these channels. So far, it is still impossible to determine which of these hypotheses is the more plausible. Even in the two neurones which exhibited prolonged action potentials when Ca was replaced by Ba in the bath, it is not absolutely certain that the action potentials were due to the presence of Ca channels since, unfortunately, no attempt was made to determine the sensitivity of these responses to TTX and Cd. Hence the possibility remains that they might have been due to non-activating Na currents revealed by the K-current-blocker Ba, although their amplitude was 10–20 mV greater than that of the responses attributed to such Na conductances in control and mutant mice (see Results).

In any case, the present results indicate that the mutation selectively affects the

dendritic region of Purkinje cells, leaving the soma apparently unaffected with respect to electrical properties. Since Ca spikes appear later than Na spikes during normal development (Llinas & Sugimori, 1979), the absence of the former in the adult *staggerer* mice might therefore indicate, in keeping with previous observations (Trenkner, 1979), that the Purkinje cell membrane remains immature.

Finally, the question arises as to whether the absence of Ca spikes is due to a direct effect of the mutation on Purkinje cells, or whether it results from the very abnormal connectivity of these neurones in the mutant. In adult *staggerer* mice, Purkinje cells lack synapses with parallel fibres (cf. Sotelo, 1980) and receive several climbing fibres instead of only one (Crepel *et al.* 1980; Mariani & Changeux, 1980). The fact that in *reeler* mutant mice, Purkinje cells with similar abnormalities in their wiring (Mariani, Crepel, Mikoshiba, Changeux & Sotelo, 1977) do have Ca spikes (J. L. Dupont, R. Gardette & F. Crepel, unpublished) strongly supports the view that, in *staggerer* mice, the absence of any detectable Ca conductance in Purkinje cell dendrites represents a direct effect of the mutation on these neurones. Therefore, to the best of our knowledge, the present study strongly suggests, for the first time in vertebrates, the existence of a direct link between a single gene mutation and at least one class of ionic channels borne by excitable cells.

We wish to thank Professor Ph. Ascher for helpful comments during preparation of the manuscript, and W. M. Debono for skilful technical assistance. The DGRST (DN/P166) and the CNRS (ATP Génétique et Développement de la souris) are thanked for supporting this work.

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