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Electrical inhibition of Purkinje cells in the cerebellum of the rat

(field effect/parallel fibers/basket cells/neuronal synchronization)

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ABSTRACT **Monosynaptic activation of cerebellar Purkinje cells by a volley of parallel-fiber impulses is followed by a powerful disynaptic chemical inhibition mediated by molecular layer interneurons, including basket cells. Active zones established by basket preterminal axons on the body surface of the Purkinje cell account for this inhibition. However, morphological studies indicate that branches of the presynaptic fibers further descend along the initial segment of the Purkinje axon. Terminals from several basket cells converge and encapsulate each initial segment with a peculiar architectural structure that is reminiscent of that characterizing the axon cap of the teleost Mauthner cell. Because no function has yet been attributed to this pinceau, we have reanalyzed the successive Purkinje cell responses to activation of their presynaptic elements. Electrophysiological data provided by field-potential and single-unit measurements indicate that the classical phases of excitation and inhibition after a parallel-fiber volley are preceded by a brief inhibition of the Purkinje cells. Transmembrane hyperpolarizing potentials that exhibit the characteristics expected of electrically mediated potentials underlie this early phase of inhibition; their properties are consistent with the hypothesis that they are generated by currents through terminals of nearby basket cells. Therefore, these hyperpolarizations, which are similar in their mechanism of generation to those described in the Mauthner cell system, represent a known case of electrical inhibition in the mammalian central nervous system.**

Field effects represent a distinct mechanism of neuronal interaction that, unlike chemical or electronic transmission, does not require specialized junctions of the classical synaptic type. The term field effect may be defined as a situation in which the extracellular currents generated by one cell alter the excitability of other neurons as they are channeled across their membranes. So far, these effects have been reported in but a few instances (1); there are two known examples of electrically mediated inhibition in the vertebrate central nervous system. The initial segment of the Mauthner cell (M cell) of teleosts is directly hyperpolarized by an inward current originating from adjacent fibers. One important feature of this inhibition is that active impulse conduction fails in those terminals, which thereby serve as passive current sources (2). In turn, impulses in the M cell generate a "passive hyperpolarizing potential" (PHP) in the neurons of origin of these fibers (3). It has been shown (2, 4) that this mutual inhibition depends on the fact that all the involved neurons lie in part within the axon cap, which is a peculiar region surrounding the initial segment of the M cell and has been extensively studied morphologically (reviewed in ref. 5). The higher extracellular resistance between this region and the surrounding tissue is critical for the magnitudes of both the intracellular channeling of current in the polysynaptic cell and the resultant transmembrane potentials.

For the study reported in this paper, we used both extracellular and intracellular recording techniques to show that, in a mammal (i.e., in rat), a field effect inhibition is exerted in the cerebellum by presynaptic elements, presumably basket cells, on the initial segment of Purkinje cells.

MATERIALS AND METHODS

Young adult albino rats, 150–180 gm in body weight, were anesthetized with sodium pentobarbital (35 mg/kg) injected intraperitoneally, paralyzed with gallamine triethiodide (3.0 mg/kg), and artificially respired. The cerebellar vermis was exposed, and its pulsations were minimized by opening the cisterna magna and covering the recording area with a 4% agar gel in a 10% sucrose solution.

Our basic experimental technique (Fig. 1*A*) was similar to the now-classical one used in cat (6). Purkinje and basket cells were excited orthodromically by activation of their common beam of presynaptic parallel fibers by using a flat-tip bipolar concentric electrode; a pair of silver wires insulated except at their tips was inserted in the cerebellar white matter near the juxtafastigial nucleus for antidromic activation of Purkinje cells, for evoking their climbing fiber responses, or for both. Extracellular and intracellular recordings were made at the uppermost superficial layer of lobules V–VIII by using glass microelectrodes filled with 2 M NaCl (2–10 MΩ) or 3 M KCl (20–40 MΩ). Data were stored on tape for analysis with a Nicolet 1074 computer.

When required, special techniques were used: (*i*) to discriminate between field effect and chemically mediated inhibition, γ-amino butyric acid, the presumed inhibitory transmitter of basket cells (7) was antagonized by picrotoxin (2–4 mg/kg) injected intravenously; (*ii*) conduction along parallel fibers was locally blocked by xylocaine injected by pressure through a broken tip microelectrode; or (*iii*) inadvertent complications of mossy and climbing fiber stimulation were avoided in chronically deafferented cerebella obtained by sectioning the inferior and medial peduncles 1–5 days before recordings by using the procedure developed previously (8).

RESULTS

Short Latency Inhibition of Antidromic Spikes of Purkinje Cells. As reported (9), the chemically mediated inhibition of Purkinje cells that follows their activation by a parallel fiber volley and has been attributed to the action of cortical interneurons (10) can be preceded by a brief inhibition phase. This earlier event is thus the first consequence at the level of the Purkinje cell to be detected after a surface stimulus (Fig. 1 B_1-B_4). Single-unit analysis shows that it can block or delay their antidromic spikes. However, this inhibition is not easily demonstrated in all investigated cells; even when it is present, repeated trials are necessary to establish its statistical signifi-

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Abbreviations: EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; M cell, Mauthner cell; PHP, passive hyperpolarizing potential.

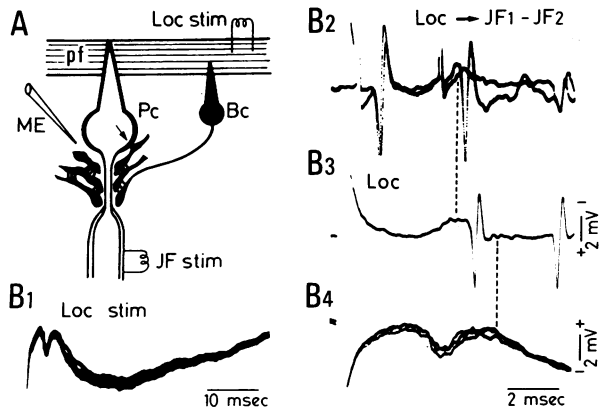

FIG. 1. Short-latency inhibitory action of a parallel-fiber volley on antidromic spikes of Purkinje cells. (*A*) Experimental setup, showing a Purkinje cell (Pc), one of its associated basket cells (Bc), a beam of parallel fibers (pf) presynaptic to both neurons, surface electrodes (Loc stim) for orthodromic stimulation, and juxtafastigial electrodes (JF stim) for antidromic activation of the cells. The microelectrode (ME) is close to the brush-like structure formed around the initial segment of the Purkinje cell by terminal axons of several basket cells, one of which establishes an inhibitory synapse (arrow) on the soma of the Purkinje cells. (B_1–B_4) Comparison of extracellular and intracellular recordings from a Purkinje cell. (B_1 and B_4) Intracellular IPSP evoked by a local stimulation and recorded at two different speeds (several superimposed sweeps). (B_2 and B_3) Extracellular recordings from the same cell before its impalement. In B_2, the second of two antidromic spikes evoked by paired juxtafastigial stimulations (JF_1–JF_2) was blocked when timed to occur during the maximum negativity of the field potential produced by a local stimulation (two superimposed sweeps, one with and one without the local stimulus) and in B_3, a local stimulation alone orthodromically fired the cell. The inhibitory action shown in B_2 occurred before both activation of this neuron (first vertical dashed line) and the onset of its intracellular recorded IPSP (second vertical dashed line).

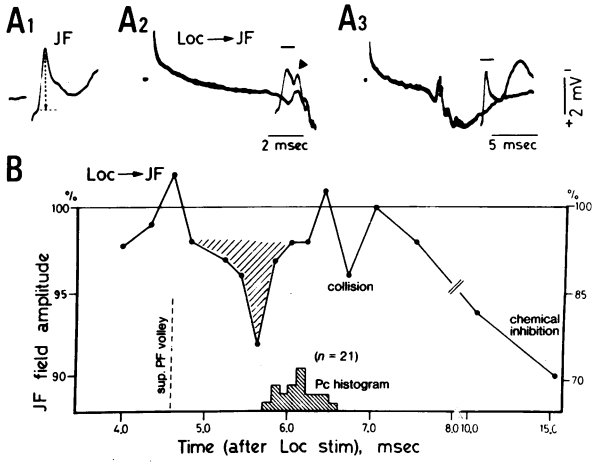

FIG. 2. Evidence for two phases in the inhibition of the antidromic spike potential of Purkinje cells by a parallel-fiber volley. (A_1–A_3) Field potentials evoked by a juxtafastigial (JF) stimulation only (A_1) (the amplitude of this control record was measured as shown by the vertical dotted line) and when conditioned by a preceding local (Loc) stimulation (A_2 and A_3) two superimposed sweeps, with and without the JF stimulation. As the JF stimulation (A_2) was adjusted to occur just before the time of activation of the Purkinje cells (arrow), the antidromic field potential was both reduced in amplitude (control value indicated by a horizontal bar in this and next recordings) and fractionated into several components (triangle) and, as the interval between stimulations was increased (A_3), the antidromic field potential decreased during the late chemical inhibition. (*B*) Relationship between the amplitude of the test antidromic field potential (ordinates) and time (abscissae) as the interval between the conditioning Loc stimulation and the JF stimulation was progressively increased. The latency histogram is from one Purkinje cell recorded along the same track as that used for the field potentials. Just before the firing of this cell, the antidromic field potential was reduced to about 92% of its initial value (left-hand ordinate); this early phase of inhibition lasted for about 1 msec (hatched lines). Firing of cells was followed by (*i*) an early depression of the antidromic field potential due to collision with the JF-evoked antidromic spikes and (*ii*) a subsequent long-lasting phase of the classical chemical inhibition (right-hand ordinate) that reduced the antidromic spike to 70% of its control value. The vertical dashed line indicates the time of occurrence of the positive peak of the superficially recorded (Sup) loc-evoked parallel-fiber volley. (For abbreviations not defined here, see legend to Fig. 1.)

cance. In fact, we found that the decreased excitability is more easily shown if timed to occur during the relative refractory period of the Purkinje cell (i.e., after either a spontaneous discharge or a conditioning antidromic stimulation). A series of experiments was therefore conducted by using the response evoked by the second of adequately timed juxtafastigial stimulations as a test response (Fig. $1B_2$). Under those conditions and taking as a control the number of failures attributable solely to refractoriness, the number of blocked spikes can be increased during this early phase of inhibition by as much as 29–70% (mean = 46; n = 12 neurons).

Field-Potential Analysis Shows That the Early Inhibition of Purkinje Cells Is Different from a Chemically Mediated One. The action of a parallel-fiber volley on the antidromic spike potentials was studied by progressively increasing the time delay between the stimuli by steps of no more than 0.25 msec, at least in the period preceding synaptic activation of the Purkinje cells; this increment is notably briefer than those used in cat (8) and is necessary to detect locally evoked influences that would be of short duration. In test-control curves obtained in this manner (Fig. 2), the earliest component of inhibition usually began 0.3–0.8 msec after the time of arrival of the surface presynaptic volley, which is signaled by the positive peak of the triphasic locally evoked superficial field potential (see Fig. $4A_1$). This latency is too short for the two synaptic delays required by chemical inhibition mediated through cortical interneurons. The early phase of inhibition lasted 0.8–1.5 msec—i.e., until activation of the Purkinje cell—and, in most cases, decreased the antidromic field potential by no more than 8–12% (although 25–30% reductions were occasionally observed). In any case, this depression appeared to be less effective than the subsequent chemically transmitted inhibition; unlike the latter, it was not abolished by picrotoxin. Nevertheless, common interneurons might generate the two inhibitions because (*i*) their latencies increased and they finally disappeared simultaneously during a progressive conduction block produced in parallel fibers by xylocaine and (*ii*) both persisted in chronically deafferented preparations.

Passive Hyperpolarizing Potentials Underlie the Early Inhibition of Purkinje Cells. By using intracellular recordings, we found that, in about half of the investigated Purkinje cells, local stimulation produced a PHP that preceded initiation of postsynaptic potentials (Fig. 3); however, this hyperpolarization could be observed only when the recording electrode was near the lower border of the Purkinje cell layer, as indicated by the configuration of the externally recorded corresponding field potential (Fig. $3A_4$). This depth indicated that the intracellular recording site was in the cell soma, close to the initial segment. In cells exhibiting membrane potentials of 40–60 mV and antidromic spikes or climbing fiber responses of no less than 25 mV, the amplitude of the PHPs was 0.4–3.0 mV (mean = 1.14; SD = 0.33), and their duration was 0.83–2.30 msec (mean = 1.36; SD = 0.37). There was a delay between the peak of the early hyperpolarization and the onset of the second phase of

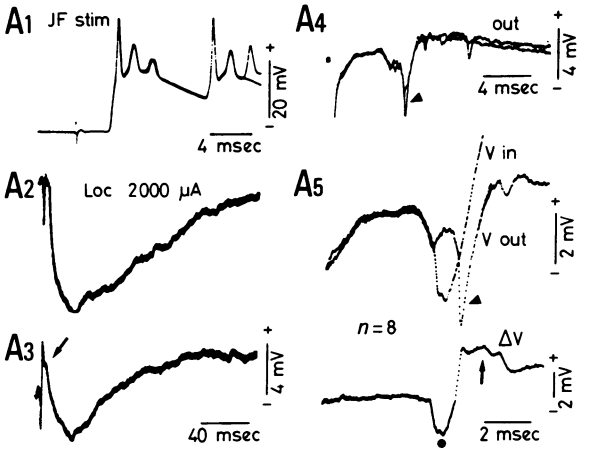

FIG. 3. Intracellular recording of PHP in a Purkinje cell. (A_1) Climbing fiber response produced by a JF stimulation (A_2 and A_3). Potentials evoked in this cell by Loc stim at a constant strength that was lower than threshold for initiation of a distinct EPSP. The second hyperpolarizing phase, the IPSP, began to invert into a depolarizing response (arrow in A_3) because of Cl^- leakage from the recording microelectrode. (A_4) Extracellular recording outside this cell; the Loc stimulation induced both a field potential and a spike (arrow head), the latter indicating firing of a nearby Purkinje cell. (A_5, upper traces) Expanded computer-averaged sweeps corresponding to the early phase of the Loc-evoked responses shown in A_3 and A_4. The intracellular (V in) and extracellular (V out) potentials have been superimposed (n = 8); (lower trace) transmembrane potential change obtained from the computer-calculated difference (ΔV) between the two tracings, showing that a net hyperpolarization of about 2.8 mV (dot) was the first event produced in this cell by the Loc stimulation. Although the decaying phase of this PHP was apparently curtailed by the extracellularly recorded spike and a subsequent potential corresponding to the onset of the depolarizing IPSP (arrow), its duration was nevertheless not shorter than 1 msec. (For abbreviations not defined here, see legend to Fig. 1.)

hyperpolarization due to the inhibitory postsynaptic potential (IPSP; Fig. 4B_2). This interval was difficult to determine exactly because of possible contamination by the locally evoked excitatory postsynaptic potential (EPSP) that occurred at this time, although a distinct EPSP that crosses the baseline was generally recorded in these cells at a higher stimulus strength than that required for inhibition. The PHP time to peak was 0.61–1.31 msec (mean = 0.85; SD = 0.21).

That PHPs are electrically mediated is indicated by their short latency from the onset of the deeply recorded (Fig. 4A_2) presynaptic volley (mean = 0.75 msec; SD = 0.30; n =13), which leaves time for only one chemical synaptic delay between parallel fibers and one set of cortical interneurons. Furthermore, and as expected of field effects (3, 4), PHPs are unaffected by changes in membrane potential and are insensitive to Cl^- injections, which, by contrast, convert the later IPSPs into depolarizing responses. The IPSPs were longer in duration (mean = 57 msec; SD = 17) and larger (mean = 6.5 mV; SD = 4.2) than the PHPs, reflecting that chemically transmitted inhibition is more effective than that generated by field effects.

PHPs in Purkinje Cells Are Correlated with Firing in Adjacent Basket Cells. Basket cells are the only presynaptic elements close to the PHP recording site. Identification of these interneurons in the rat cerebellum (11) by using the same criteria as those used in cat (12) allowed a correlation between their firing and the PHP. That the two events occur simultaneously had been suggested by the finding that, for a given local stimulus, basket cells are activated before the corresponding adjacent Purkinje cells (Fig. 4 A_1, A_2, and C). In confirmation, the latency of the first basket cell spikes within trains of action potentials evoked by a parallel fiber volley and recorded before penetration of the Purkinje cells encountered along the same track, consistently decreases during (Fig. 4 B_1 and B_2) or at the onset (Fig. 4C) of the calculated PHP. We also found that, as the strength of the local stimulus is increased, the number of presynaptic spikes within a train, the amplitude of the PHP,

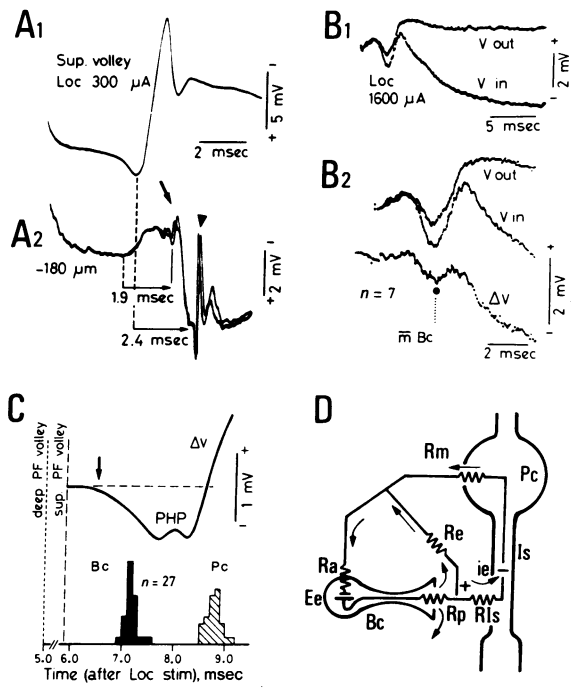

FIG. 4. Time relationships between PHPs and firing of basket cells set up by parallel-fiber volleys. Field potentials evoked by a Loc stim at the surface of the cerebellar cortex (A_1) and 180 μm below this level (A_2); for each, the peak of the earliest positive going potential change, which signals the time of arrival of the presynaptic volley, is indicated by a vertical line. In A_2, action potentials from presumed basket cells (arrow) and from Purkinje cells (triangle) were simultaneously recorded by the microelectrode; note that basket cells were activated before Purkinje cells. Computer-averaged traces (n = 7) of potentials evoked in a Purkinje cell by a Loc stimulation during another experiment. (B_1) Superimposed traces of intracellular (V in) and extracellular (V out) recordings; both a large negative early potential and an IPSP were recorded in this neuron. (B_2, upper traces) Expanded sweep of the earliest negative components visible in B_1. (Lower trace) Computer-calculated difference (ΔV) between the two traces above, showing a PHP and the subsequent onset of the IPSP. The mean time ($\bar{m}$ Bc) at which an adjacent basket cell fired its first spike before impalement of this cell is indicated by a vertical line. (C) Diagram of a calculated PHP from another cell (its onset is indicated by an arrow) and distribution of the first spike latencies of this neuron and a nearby basket cell (Bc), as recorded extracellularly along the same track. The onset of the superficial (Sup) and deep presynaptic volleys are indicated at the left by vertical dashed lines; note that, in B_2 as well as in C, the basket cells fired during the PHP. (D) Conceptual model for PHP generation in a PHP cell, illustrating the lines of current flow associated with an action potential in a basket cell, where Ee and Ra are, respectively, the driving electromotive force and the resistance of the active spike generating membrane. It is postulated that the terminal part of the axon of this cell is inactive and thus behaves as an external anode to the Purkinje cell. Part of the current, which leaves the noninvaded terminal through its passive resistance, Rp, is channeled in the Purkinje cell, possibly in part due to the high electrical resistance, Re, of the extracellular space surrounding the axon hillock and initial segment of the cell; the resistance of the initial segment of the Purkinje cell is labeled RIs; the inward current, i.e., at this region produces the PHP and returns to the active site across the soma or dendrites (or both) of the cell, the resistance of which is Rm. A remainder of the current flows through Re. (For abbreviations not defined here, see legend to Fig. 1.)

and that of the chemically mediated IPSP are increased, and their latencies are decreased in parallel, the threshold for initiating all three responses being generally the same.

DISCUSSION

Direct evidence was obtained in the M-cell system (13) that at least two classes of PHP-exhibiting interneurons that bring about field inhibition also mediate chemical inhibition in the vertebrate central nervous system. It was thus speculated that similar interneurons might also generate an electrical phase of inhibition provided their spikes should fail to invade the terminal process. Such processes might lie, in part, in a region of increased extracellular resistance. The data presented here indicate that cerebellar basket cells also mediate electrical and chemical inhibition.

When measured from the time of arrival of the presynaptic volley recorded at the surface, the latency of the PHP is as short as 0.2–0.4 msec. But as reported earlier (11), basket cells are apparently activated by deeper and faster conducting parallel fibers than Purkinje cells; as shown in Fig. 4 A_1 and A_2, latencies at the lower depth of the molecular layer must be calculated from the onset of the deeply recorded presynaptic volley. Under those conditions, the PHP delay is about 0.75 msec, which is the same as that necessary for activation of basket cells by the parallel-fiber volley. PHPs might be more complex than those illustrated here—for example, by having a small positive phase that precedes their dominant hyperpolarization. However, contribution of currents generated by parallel-fiber or Purkinje cell activity seems unlikely because the timing of their responses in inappropriate.

Given their comparable latencies and time course,the early phase of inhibition exerted by a parallel-fiber volley on Purkinje cells can be accounted for by the locally evoked PHPs recorded in these neurons. The real amplitude of these potentials should be, however, somewhat larger than reported here, because they are presumably generated, not at their somatic recording site, but at the level of the initial segment.

A conceptual model for the generation of PHPs in Purkinje cells, which is a modified version of that used for the M cell (2, 4, 14), is shown in Fig. 4*D*. The impulses of the basket cell produce an extracellular positivity that has an associated current flow that is inward at the initial segment of the Purkinje cells. In this case, the positivity is masked, however, by a predominantly negative field generated by active elements in the molecular layer. This current leaves the cell by way of its body or initial dendrites (or both), which are therefore depolarized. The depolarization of the dendrites, however, has a negligible value, as shown by experiments in which the tip of the microelectrode penetrated the Purkinje cell at a more superficial level than close to the initial segment. Such observations indicate that this escape pathway is of lower resistance than that used by the current entering the cell. This model of electrical inhibition, which is substantiated by our physiological data, implies that, as for the M cell, the field effect should be reciprocal.

Morphological specializations at the initial segment of both M and Purkinje cells also support a functional similarity. Projections of basket fibers that can chemically inhibit Purkinje cells (10) constitute a tightly packed meshwork of a peculiar cytoarchitecture around the initial segment of the latter, as described in detail for rat (15). Basket axons are of various diameters and, at their terminal region, most of them contain synaptic vesicles that give them the appearance of normal boutons. However, they establish but few synapses with the initial segment of the Purkinje cell (16). The fibers of this pinceau are intermingled with glial processes that cover most of the emerging axon, surrounding this entire region and having a configuration similar to that of the M-cell axon cap; this led Palay (17) to postulate that similar characteristics might be present in both structures and that they might have analogous modes of operation. This hypothesis was strengthened by observations (18) that, in some species, including rat, basket-cell axons are joined by septate-like junctions that may contribute to occlusion of the intercellular space and function as a high-resistance element channeling extracellular current flow. Comparable junctions are present in the M-cell axon cap (19).

The functional value of PHPs in the cerebellar cortex remains unclear; their role in speeding inhibition (which is weak in this case) may not be the most important. Rather, as discussed for the M cell (1), they could contribute to the synchronization of Purkinje cell discharges by resetting neuronal activity, thus maximizing the effectiveness of orthodromically evoked EPSPs during the subsequent rebound. Furthermore, firing of basket cells might be synthronized by gap junctions between them (18). Consequently, excitatory potentials, flanked by inhibitory influences, would be time locked in all Purkinje cells receiving their inhibitory inputs from the same set of basket cells.

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