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STELLATE CELL INHIBITION OF PURKINJE CELLS IN THE TURTLE CEREBELLUM *IN VITRO*

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SUMMARY

1. The stellate cell-mediated inhibition of Purkinje cells was studied by intracellular recordings in an *in vitro* slice preparation of the turtle cerebellar cortex. A graded inhibitory postsynaptic potential (IPSP) was recorded in Purkinje cells upon stimulation of the parallel fibre–stellate cell pathway.

2. The IPSP was abolished by bicuculline, and had a reversal potential around −75 mV, consistent with a $GABA_A$ receptor-operated Cl^- conductance dominating the response investigated here.

3. Paired recordings from synaptically coupled stellate cells and Purkinje cells demonstrated that the inhibitory input from a single stellate cell is sufficient to reduce the firing in a Purkinje cell.

4. The extracellular-evoked IPSP interacted with the active postsynaptic membrane properties in the Purkinje cell. Interaction with both the Na^+ plateau and the I_A prolonged the responses to an IPSP, making the net effect of the inhibitory response dependent on the membrane potential in each postsynaptic neurone.

5. A precisely timed IPSP was particularly efficient in reducing dendritic Ca^{2+} influx.

6. The voltage-dependent Ca^{2+} component of a climbing fibre response (CFR) as well as of a parallel fibre (PF) input was reduced by the IPSP.

7. It is suggested that Ca^{2+} spike-mediated reduction in Purkinje cell excitability may be prevented by the stellate cell IPSP-mediated reduction in Ca^{2+} influx.

INTRODUCTION

In the preceding paper (Midtgaard, 1992*b*) the electrophysiological properties of stellate cells were examined. In the present paper the inhibitory effect of stellate cells on Purkinje cells is analysed by means of intracellular recordings, supplementing the analysis of the parallel fibre–Purkinje cell pathway and the analysis of synaptic integration of Purkinje cells (Chan, Hounsgaard & Midtgaard, 1989; Hounsgaard & Midtgaard, 1989). It is shown that part of the inhibitory response is determined by the time- and voltage-dependent local membrane properties of the Purkinje cell.

In mammals Purkinje cells receive inhibitory inputs from basket and stellate cells (Eccles, Ito & Szentagothai, 1967; Ito, 1984). Turtles were chosen for these

experiments because they, as other lower vertebrates, lack cells giving rise to the anatomically typical basket structure (Larsell, 1932; Llinás & Hillman, 1969). However, stellate cells are present in lower vertebrates, including turtles (Larsell, 1932; Llinás & Hillman, 1969), and the ones located deep in the molecular layer are considered to be the forerunners of the basket cells found in higher vertebrates (Eccles *et al.* 1967). In addition, turtles lack Purkinje cell axonal collaterals to the molecular layer. Parallel fibres originating from the granule cells are believed to be excitatory only. Hence the IPSPs observed can be attributed to the activity of the stellate cells. The stellate cell synapses are located at all levels of the Purkinje cell membrane from the soma to the spiny dendrites (Larsell, 1932; Hillman, 1969; Palay & Chan-Palay, 1974), corresponding to the localization of Na^+ spikes and Na^+ plateau, and the Ca^{2+} spikes, respectively (Llinás & Sugimori, 1980; Hounsgaard & Midtgaard, 1988).

Part of this work has appeared in abstract form (Midtgaard, 1992*a*).

METHODS

The preparation and the experimental techniques in general have been described in the previous paper (Midtgaard, 1992*b*). Pairwise recordings of stellate cells and Purkinje cells were made with the recording electrodes within 100–150 μm of each other in the rostro-caudal direction, corresponding to the extension of the axon of stellate cells visualized by horseradish peroxidase (HRP) or biocytin injections. First an intracellular recording from an interneurone was obtained, and then a second electrode was used to make an intracellular recording from a postsynaptic Purkinje cell. Due to the close proximity of the electrodes, and the search for synaptically connected cells, the mechanical stability of the recordings was not always optimal. More than twenty pairs were tested for connectivity, which was present in two pairs. During these experiments, parallel fibre stimulation in the molecular layer was achieved as described previously (Midtgaard, 1992*b*). This paper is based on recordings from more than forty Purkinje cells, and eleven interneurones.

RESULTS

Composite nature of the IPSP

When the molecular layer was stimulated by a stimulating electrode placed 100–200 μm 'off-beam' for the intracellular Purkinje cell electrode, a smoothly graded response was recorded with increasing stimulus strength (Fig. 1 *A* and *B*). The time course of the IPSP is dependent on the prestimulus membrane potential, but the IPSP duration is maximally some hundred milliseconds.

With the voltage resolution employed here (1–2 mV), no unitary IPSPs could usually be discerned, unlike the case in mammals (Eccles, Llinás & Sasaki, 1966) and elasmobranchs (Nicholson & Llinás, 1969). In addition to the data from pairs of synaptically connected stellate cells and Purkinje cells, this suggests that the contribution to the IPSP from individual turtle stellate cells may be modest, and that the IPSP investigated here is the result of the synchronous synaptic activation of a number of stellate cells converging on the same Purkinje cell.

Pharmacology and ionic nature of the IPSP

The reversal potential of the IPSP was between -72 and -78 mV (Fig. 1 *C*; $n = 6$), a value compatible with the possible involvement of a $GABA_A$ receptor Cl^- channel (see Discussion). As in other species (Bisti, Iosif, Marchesi & Strata, 1971; see

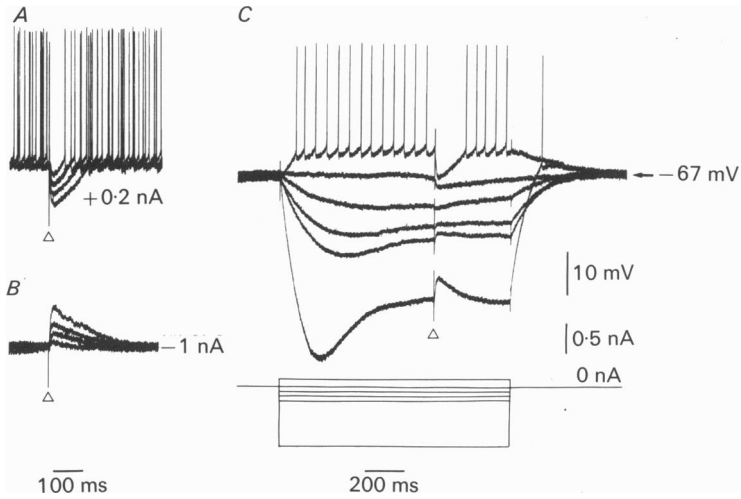

Fig. 1. Purkinje cell, proximal dendrite. Graded inhibitory response to 'off-beam' molecular layer stimulation with four different stimulus strengths at +0·2 nA holding current (*A*), and at −1·0 nA holding current (*B*), causing reversal of the PSP. *C*, determination of reversal potential, same stimulus strength at all sweeps; note that the membrane potential physiologically traverses a range from just below IPSP reversal potential, and that the synaptically induced membrane potential displacement interacts with voltage- and time-dependent membrane conductances both at sub- and supra-threshold levels. Triangle in *A*, *B* and *C* denotes molecular layer stimulation: 50 μs, up to 50 V. Arrow in *C* indicates resting membrane potential, −67 mV.

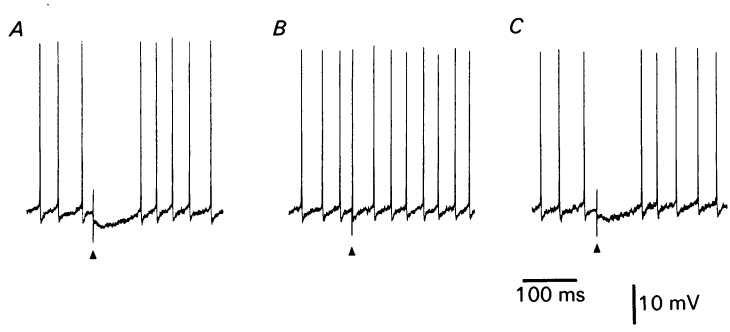

Fig. 2. Effect of bicuculline on the IPSP. Purkinje cell recording. Soma–proximal dendrite. Molecular layer stimulated (triangle) 'off-beam' to produce an IPSP. A small EPSP may contribute to the initial part of the response. *A*, control. *B*, 25 min in 4 μM bicuculline. *C*, 30 min after wash-out of bicuculline. Holding current in all sweeps, +0·2 nA.

also Ito, 1984), the IPSP recorded in turtle Purkinje cells was sensitive to bicuculline (Fig. 2) and picrotoxin (not shown), suggesting that the IPSP is primarily mediated by $GABA_A$ receptors.

In addition to the effect on the evoked IPSP these antagonists often increased the excitability of Purkinje cells, compatible with the observation that most stellate cells are spontaneously active in the slice (Midtgaard, 1992*b*).

Stellate cell–Purkinje cell pathway

At the stimulus strength and duration normally used for extracellular stimulation (up to 50 V, 100 μs), the inhibitory response was probably mediated indirectly through excitatory synaptic activation of the stellate cells. This conclusion was

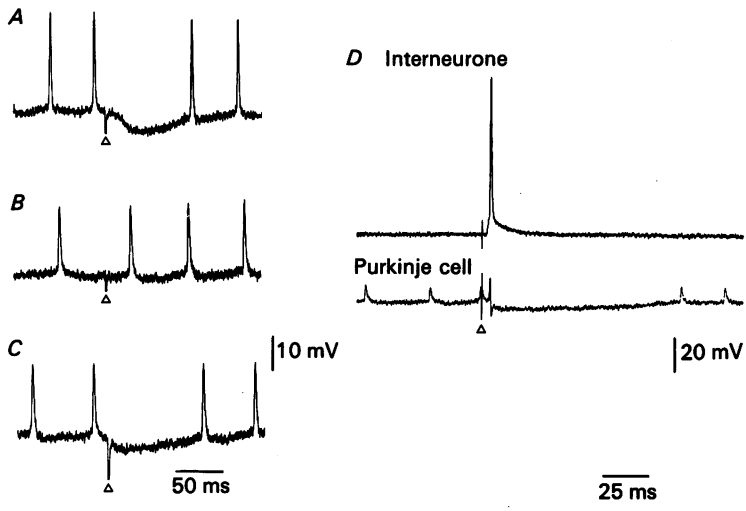

Fig. 3. Parallel fibre–stellate cell pathway of IPSP. Purkinje cell, proximal dendrite. *A*, IPSP in normal medium. *B*, same stimulus strength (40 V, 100 μs) in 10^{-5} M CNQX. *C*, stimulus strength increased 3 times; an IPSP now follows the stimulation with a much shorter latency than in *A*. Response was abolished by picrotoxin, 10^{-5} M (not shown). The reduction in Na^+ spike amplitude between *A* and *B* is due to an uncompensated increase in electrode time constant. *D*, simultaneous recording from interneurone (upper sweep) 'on-beam' and Purkinje cell dendrite (lower sweep) 'off-beam' for the extracellular stimulation electrode. Note that the duration of the IPSP greatly outlasts the duration of synaptic activity in the interneurone. Triangle denotes molecular layer stimulation.

based on the following observations. The IPSP developed after some latency, and was abolished by CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; Fig. 3*B*), an antagonist of non-NMDA glutamate receptors (Honore, Davies, Drejer, Fletcher, Jacobsen, Lodge & Nielsen, 1988). The sensitivity to CNQX is in agreement with preliminary results from intracellular recordings in stellate cells (Midtgaard, 1992*b*) that the parallel fibre EPSP has a substantial CNQX-sensitive component. With increased stimulus strength a graded IPSP was again evoked, but now with much reduced latency (Fig. 3*C*). This IPSP was probably due to direct activation of the stellate cells.

Another observation in line with this interpretation was made from simultaneous recordings from interneurones and Purkinje cells. When an interneurone was recorded from 'on-beam' (Fig. 3*D*, upper trace), an EPSP and eventually a spike was elicited here before the onset of the IPSP in the 'off-beam' Purkinje cell (Fig. 3*D*, lower trace). Note that the duration of the IPSP in the Purkinje cell outlasts the synaptically evoked activity in the interneurone. Turtle stellate cells *in vitro* generally fire only once per parallel fibre stimulus (Chan & Nicholson, 1986;

Midtgaard, 1992*b*). The responses are in agreement with the differences in time constants and excitability between interneurones and Purkinje cells (Midtgaard, 1992*b*). In conclusion, the IPSP studied here reflects the parallel fibre–stellate cell synaptic properties as well as the translational properties of the interneurones.

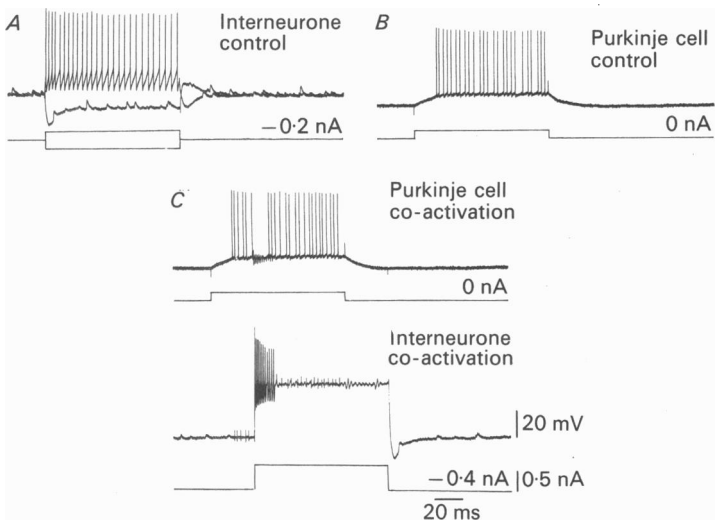

Fig. 4. Spiking activity in interneurone (*A* and *C*) inhibits the firing in Purkinje cell soma (*B* and *C*). *A* and *B*, control sweeps. *C*, simultaneous activation; upper sweep Purkinje cell, lower sweep interneurone. Note especially the immediate onset of the inhibition following initiation of spikes in the stellate cell. Small 'spikes' in the Purkinje cell during stellate cell firing and vice versa, are due to capacitive coupling artifacts between the recording electrodes. The effect of firing in the interneurone was only discernible just around threshold in the Purkinje cell, where small changes in membrane potential will affect firing.

The effect of individual stellate cells on their postsynaptic targets was assessed by paired intracellular recordings from synaptically connected stellate and Purkinje cells (Fig. 4). Here the Na^+ spike firing in the Purkinje cell (Fig. 4*A*), is inhibited by the spiking in the interneurone (Fig. 4*C*).

The effect on Purkinje cells was very weak, and could easily be overcome by a slight increase in the depolarization of the Purkinje cell. This supports the suggestion that the more robust IPSP observed with extracellular stimulation is the result of a synchronous activation of several stellate cells.

Interaction between synaptic current and intrinsic membrane properties

In addition to the currents directly activated by transmitters, the local membrane properties of the Purkinje cell, at all levels from the soma to the spiny dendrites, contributed to the effect of the postsynaptic inhibitory response.

IPSP effect on Na^+ excitability

As shown in Figs 1, 2 and 3, an IPSP effectively abolishes firing for up to a few hundred milliseconds. For the same presynaptic stimuli the duration of this inhibition was dependent on Purkinje cell excitability and varied greatly. Turtle

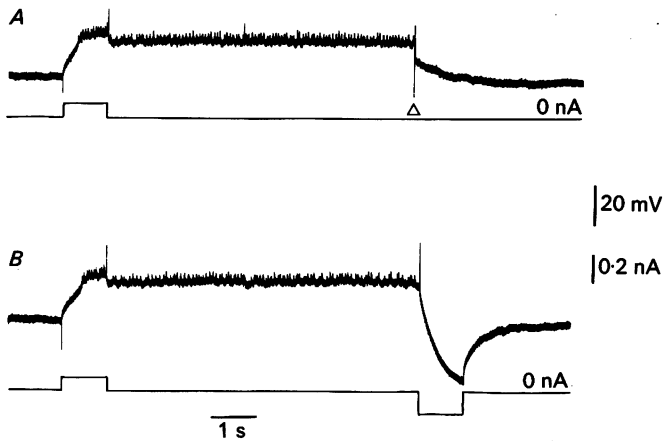

Fig. 5. Purkinje cell, distal dendrite (note the very small Na^+ spikes). *A*, triangle denotes 'off-beam' molecular layer stimulation evoking an IPSP. The current pulse through the electrode shifts the cell from a stable resting potential to a stable potential above Na^+ spike threshold. The IPSP abolishes Na^+ plateau and firing, and shifts the cell into the stable resting state, which greatly outlasts the duration of the IPSP. *B*, the bi-stable behaviour controlled by intracellular injection of current mimics the effect of the IPSP.

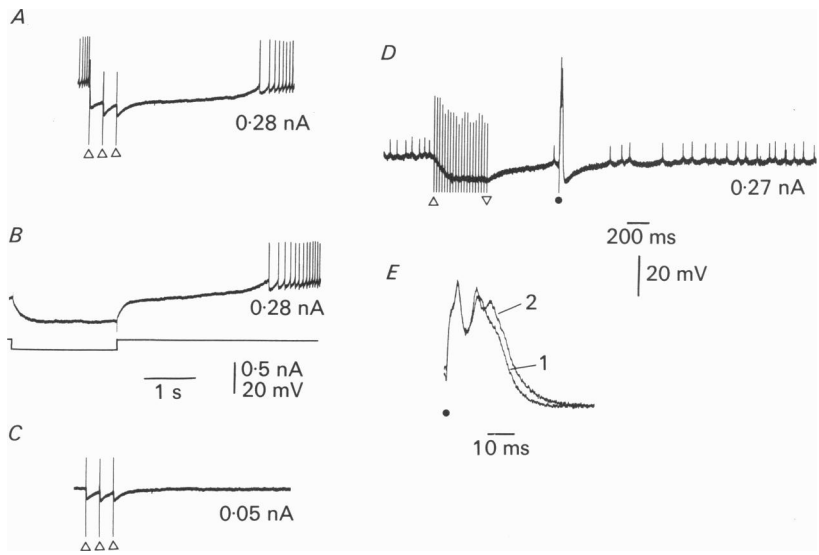

Fig. 6. *A–C*, Purkinje cell proximal dendrite; *D–E*, Purkinje cell at a more distal dendrite. *A*, three IPSPs hyperpolarize the cell and are followed by a slow return to prestimulus levels. *B*, the long-lasting effect of the IPSPs is mimicked by a hyperpolarizing current pulse. *C*, no slow return following stimulation is seen at more hyperpolarized membrane potential. *D*, train of IPSPs hyperpolarizes the cell followed by a climbing fibre response during the slow return of membrane potential to prestimuli levels. *E*, superposition of two climbing fibre configurations: (1) with preceding IPSPs (from *D*), and (2) without preceding IPSPs. Triangle denotes molecular layer stimulation, upward triangle in *D* onset, and downward cessation of stimulation. Dots denotes climbing fibre stimulation by an extracellular stimulus electrode placed in the fibre layer just ventral to the Purkinje cell layer.

Purkinje cells can display a Na^+-dependent bi-stability due to the somatically located Na^+ plateau underlying Na^+ spike firing (Hounsgaard & Midtgaard, 1988). In this way the cell can maintain steady Na^+ spike firing for very prolonged periods of time, or can be in a stable resting state well below the threshold for the Na^+ plateau and spikes. An IPSP evoked by extracellular molecular layer stimulation is effective in terminating the plateau and thus the Na^+ spike firing (Fig. 5). Thus, by interacting with the postsynaptic determinants of Na^+ excitability, a brief activation of the interneurones may be translated into a much longer lasting reduction in the axonal output of the Purkinje cell.

IPSP deinactivation of transient outward current

A transient A-like conductance is present in Purkinje cell spiny dendrites (Hounsgaard & Midtgaard, 1988). Figure 6*A* shows the ability of inhibitory synaptic input to hyperpolarize the cell enough to deinactivate this A-like conductance, as evidenced by the slow return to prestimulus membrane potentials.

This effect was mimicked by a hyperpolarizing current pulse through the recording electrode (Fig. 6*B*). At a less depolarized membrane potential the IPSPs are followed by a much faster return to the prestimulus membrane potential (Fig. 6*C*). This can be explained by a lack of I_A activation at this more negative membrane potential. It has previously been demonstrated that this outward current determines the Ca^{2+} influx during the activation of a climbing fibre input (Chan *et al.* 1989). Figure 6*D* and *E* shows the delayed effect on the climbing fibre response by an inhibitory input, which is consistent with activation of I_A following the IPSP-induced hyperpolarization. The temporal summation of several IPSPs was generally required to produce a hyperpolarization followed by a clear-cut activation of I_A. This suggests that the temporal aspects of stellate cell firing determined, in addition to the amplitude of the response, the after-effects of the postsynaptic response.

Direct short-term effect of IPSPs on Ca^{2+} spikes

A more precisely timed action of the IPSP on Ca^{2+} excitability was also observed (Fig. 7). Several features were noticeable. The effect of a single IPSP on Ca^{2+} spike configuration was modest outside a narrow time window (Fig. 7*A–D*).

Only a negligible effect on the Na^+ excitability was observed (Fig. 7*E*). Nevertheless, a strong effect on the Ca^{2+} spikes was present. Note also that in this case only a change in configuration of the Ca^{2+} spike occurred, not a total abolition. This suggests that a local Ca^{2+} response was still present, and/or that other spiny branchlets still fired a full-blow Ca^{2+} spike; this implies that a differential Ca^{2+} influx over the dendrites may be provided by the IPSP (cf. Llinás & Nicholson, 1971). The effect of the IPSPs on Ca^{2+} influx was strongly dependent on the holding current passed through the recording electrode. At slightly less depolarized levels than shown in Fig. 7, the Ca^{2+} response was totally abolished during the IPSP. The effect of an inhibitory input on climbing fibre (CF)-induced voltage-dependent Ca^{2+} influx is illustrated in Fig. 7*F–I*. Panels *F–H* illustrate the effect of the relative timing of the CFR and the IPSP at a positive holding current. The effect on the various components of the CFR depends on the stimulus interval. Note that the reduction of the CF-induced Ca^{2+} influx is largest for the late voltage-dependent component of

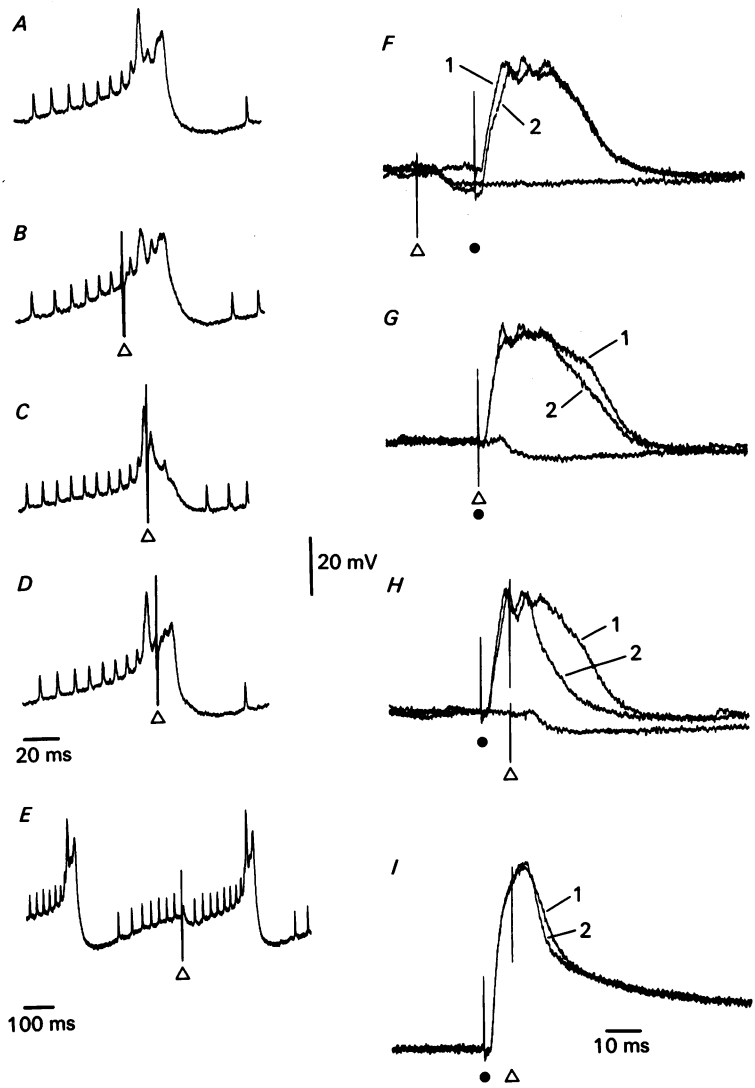

Fig. 7. Short-term effect of an IPSP on Ca^{2+} excitability. *A–D*, Purkinje cell recording, all sweeps from distal dendrites. Effect of IPSP on Ca^{2+} spikes (+1·0 nA holding current). *A*, control sweep without IPSP. An IPSP evoked just before Ca^{2+} spike (*B*), in the middle of the Ca^{2+} spike complex (*C*) and more towards the end of the Ca^{2+} spike (*D*). Note that the pause in Na^+ spike firing following the Ca^{2+} spike (*A*) depends on the IPSP-induced reduction of the Ca^{2+} spike: the greater the reduction in Ca^{2+} spike amplitude, the sooner the Na^+ spiking recommences after the Ca^{2+} spike (*B–D*). *E*, small direct inhibitory effect of IPSP on Na^+ spike firing at this holding current. *F–I*, another Purkinje cell, distal dendrite. *F–H*, +0·36 nA holding current; *I* at rest (no holding current). Effect of a single IPSP on climbing fibre-induced Ca^{2+} influx. Figures composed of three superimposed sweeps: IPSP stimulation alone, climbing fibre stimulation alone (1), and climbing fibre stimulation combined with IPSP stimulation (2). Triangle denotes IPSP activation, dot denotes climbing fibre activation. The relative timing between the IPSP and the climbing fibre stimulation is varied in *F–H*. *F*, IPSP stimulation prior to climbing fibre stimulation; *G*, simultaneous activation; *H*, IPSP activated after climbing fibre. In *F* the rate of rise and the amplitude of the CFR is slightly reduced, but the duration is unchanged. *G* shows

the CFR (Fig. 7*H*), occurring just after the CF EPSP itself (compare *H* with *I*). The effect of the IPSP on the CF EPSP *per se* is moderate at rest (*I*), demonstrating the interaction of the IPSP with voltage-dependent postsynaptic properties.

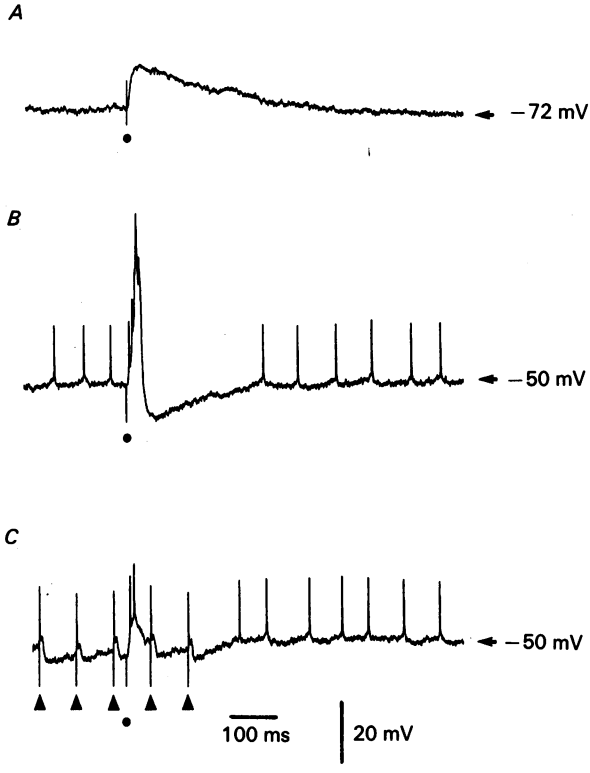

Fig. 8. IPSPs abolish PF-induced Ca^{2+} response. Purkinje cell, proximal dendrite. Triangle denotes IPSP activation, dot denotes PF EPSP. *A*, PF EPSP at rest. *B*, response to same stimulation at a more depolarized membrane potential (+0·2 nA holding current). The PF EPSP evokes a voltage-dependent Ca^{2+} spike. *C*, +0·2 nA holding current. Train of IPSPs hyperpolarize the cell, and reduce the PF-evoked voltage-dependent Ca^{2+} response.

The EPSP evoked by 'on-beam' stimulation of the parallel fibres (see Eccles *et al.* 1967) can elicit a voltage-dependent, graded, regenerative response (Llinás & Nicholson, 1971), which is due to activation of Ca^{2+} conductances localized to the spiny dendrites (Chan *et al.* 1989). The IPSP abolished parallel fibre-induced Ca^{2+} influx (Fig. 8), extending the observations made by Llinás, Nicholson, Freeman & Hillman (1968), and demonstrating that the IPSP can prevent PF-induced voltage-dependent Ca^{2+} influx in a manner similar to the effects on the voltage-dependent Ca^{2+} part of the CFR.

a somewhat reduced CFR duration, while *H* shows a large reduction in CFR duration. *F–H* demonstrate the large effects of the IPSP on the active voltage-dependent components of the climbing fibre response and *I* the small effects on the climbing fibre EPSP at rest.

DISCUSSION

This paper describes the interaction between inhibitory synaptic input from stellate cells and the cell-specific postsynaptic membrane properties in Purkinje cells.

The anatomy and pharmacology will be briefly discussed first. Stellate cell axons do not make the characteristic basket-like structure around the Purkinje cell soma that the basket cells of birds and mammals do. However, stellate cell axons contact Purkinje cells at all levels from the soma to the spiny dendrites in amphibians, reptiles, birds and mammals (Llinás & Hillman, 1969; Hillman, 1969; Palay & Chan-Palay, 1974), providing the basis for interaction of the inhibitory input with the local membrane properties of Purkinje cells.

Anatomical differences apart, both stellate cells and basket cells appear to be mainly GABAergic, with small amounts of taurine present as shown by immunocytochemical studies (Magnusson, Madl, Clements, Wu, Larson & Beitz, 1988; Ottersen, 1988; Ottersen, Madsen, Storm-Mathisen, Somogyi, Scopsi & Larsson, 1988; Batini, 1990). Mediation of the stellate cell inhibition by $GABA_A$ receptors is suggested by the blocking effect of bicuculline (Fig. 2) on the IPSP which also agrees with studies on mammalian Purkinje cells showing the presence of $GABA_A$ receptors *in vivo* and *in vitro* (Bisti *et al.* 1971; Kaneda, Wakamori & Akaike, 1989; see also Ito, 1984). However, the present experiments do not allow one to differentiate between taurine and GABA in the inhibitory response of Purkinje cells as the effects of both may be antagonized by bicuculline and picrotoxin (Fredericksson, Neuss, Morzorati & McBride, 1978; Horikoshi, Asanuma, Yanagisawa, Anzai & Goto, 1988). The relative locations of the inhibitory synapses with respect to the recording electrode is not known in this study, and this, together with the cable-like nature of the Purkinje cell imposes some limitations on the determination of the value of the reversal potential. The values found (−72 to −78 mV), however, are close to the value (−72 mV) attributed to a GABA-activated Cl^- current in hippocampal neurones (Andersen, Dingledine, Gjerstad, Langmoen & Mosfeldt Laursen, 1980). GABA is known to activate a Cl^- current in isolated mammalian Purkinje cells (Kaneda *et al.* 1989). Taken together, the results suggest that the IPSP studied here may be mediated by $GABA_A$ receptor-operated Cl^- channels.

The recordings of synaptically coupled stellate–Purkinje cell pairs demonstrate that a single stellate cell is capable of rapidly inhibiting the output of Purkinje cells, but the effect was very weak, suggesting that a concerted action of stellate cells may be required in order to inhibit the Purkinje cells effectively. The weak action of a single stellate cell is consistent with a relative paucity of noticeable spontaneous IPSPs in turtle Purkinje cells, even though stellate cells probably are spontaneously active *in vitro* (Midtgaard, 1992*b*).

Purkinje cells display a Na^+ plateau underlying the Na^+ spikes. Both phenomena are located in or near the somatic membrane (Hounsgaard & Midtgaard, 1988). In contrast, the intrinsic electroresponsiveness of the spiny dendrites is characterized by voltage-dependent Ca^{2+} channels, responsible for a graded Ca^{2+} response which can vary from a local response to a full Ca^{2+} spike. The intrinsic control of the amplitude of a given Ca^{2+} response is in part due to a voltage-dependent, transient, I_A-like outward current, also localized in the spiny dendrites, and already activated

at membrane potentials subthreshold for both Na^+ and Ca^{2+} spikes (Hounsgaard & Midtgaard, 1988).

The interaction between the inhibitory input and the cell-specific postsynaptic membrane properties (the Na^+ plateau and the I_A) shows that the inhibitory effect can last well beyond the duration of the activation of the stellate cells. The Na^+ plateau may well serve a dual role with respect to the response to an IPSP, rendering firing relatively immune to small, randomly occurring IPSPs, but enhancing the effect of a large IPSP sufficiently to hyperpolarize the membrane below threshold for the Na^+ plateau.

The subthreshold membrane potential in Purkinje cells traverses a range from about -55 (at Na^+ spike threshold) to about -80 mV during the Ca^{2+}-induced long-lasting after-hyperpolarization (Hounsgaard & Midtgaard, 1989). Within this membrane potential range the IPSP can change from a clear hyperpolarizing to a depolarizing (reversed) IPSP (Fig. 1*C*). Thus, the effect of the same inhibitory input may range from a simple conductance increase during presynaptic activity, with no displacement of postsynaptic membrane potential, to an elaborate temporal and spatial interaction with postsynaptic membrane potential-dependent properties, dependent on the instantaneous membrane potential in each individual postsynaptic cell.

The precise timing necessary for a direct inhibition of Ca^{2+} influx is facilitated by several distinct features in stellate cells. First, the after-effects of output activity and membrane potential displacement are within a narrow range, both in terms of the voltage range traversed and in terms of the time scale (Midtgaard, 1992*b*). This suggests that the populations of stellate cells may be rather homogeneously excitable at any one time compared to Purkinje cells which have a much more extended repertoire in terms of a larger range of membrane potential traversed over a longer time scale (Hounsgaard & Midtgaard, 1989). In addition, the response time is short in stellate cells compared to Purkinje cells (Midtgaard, 1992*b*), and thus a synchronized response to a synchronous input – e.g. the electrical stimuli employed in these experiments – is very likely to occur in the stellate cell population. The physiology of the stellate cells may then provide a basis for a temporally and spatially convergent inhibition of Ca^{2+} influx in Purkinje cell dendrites. One important electrophysiological role of Ca^{2+} influx seems to be a long-lasting reduction of Purkinje cell excitability (Hounsgaard & Midtgaard, 1989). This suggests that a very tight control on Ca^{2+} influx is essential, and this may in part be provided by stellate cells with a continuously changing convergence of inhibition. An important role for stellate cells in localized control of Ca^{2+} excitability in Purkinje cell dendrites is also suggested by the large stellate cell/Purkinje cell ratio, and the strategic localization of inhibitory synapses on the Purkinje cell spiny dendrites (Llinás & Hillman, 1969; Palay & Chan-Palay, 1974), effectively blocking the CF- and PF-evoked Ca^{2+} influx in the spiny dendrites (Figs 7*F–I* and 8). In this way short-lasting stellate cell-mediated inhibition of CF-induced Ca^{2+} influx can prevent a long-term excitability decrease of the Purkinje cells.

REFERENCES

ANDERSEN, P., DINGLEDINE, T., GJERSTAD, L., LANGMOEN, I. A. & MOSFELDT LAURSEN, A. (1980). Two different responses of hippocampal pyramidal cells to application of γ-aminobutyric acid. *Journal of Physiology* **305**, 279–296.

BATINI, C. (1990). Cerebellar localization and colocalization of GABA and calcium binding protein-D28K. *Archives Italiennes De Biologie* **128**, 127–149.

BISTI, S., IOSIF, G., MARCHESI, G. F. & STRATA, P. (1971). Pharmacological properties of inhibitions in the cerebellar cortex. *Experimental Brain Research* **14**, 24–37.

CHAN, C. Y., HOUNSGAARD, J. & MIDTGAARD, J. (1989). Excitatory synaptic responses in turtle cerebellar Purkinje cells. *Journal of Physiology* **409**, 143–156.

CHAN, C. Y. & NICHOLSON, C. (1986). Modulation by applied fields of Purkinje and stellate cell activity in the isolated turtle cerebellum. *Journal of Physiology* **371**, 89–114.

ECCLES, J. C., ITO, M. & SZENTAGOTHAI, J. (1967). *The Cerebellum as a Neuronal Machine*, p. 335. Springer Verlag, Berlin, New York.

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966). Intracellularly recorded responses of the cerebellar Purkinje cells. *Experimental Brain Research* **1**, 161–183.

FREDERICKSON, R. C. A., NEUSS, M., MORZORATI, S. L. & MCBRIDE, W. J. (1978). A comparison of the inhibitory effects of taurine and GABA on identified Purkinje cells and other neurons in the cerebellar cortex of the rat. *Brain Research* **145**, 117–126.

HILLMAN, D. E. (1969). Neuronal organization of the cerebellar cortex in amphibia and reptilia. In *Neurobiology of Cerebellar Evolution and Development*, ed. LLINÁS, R., pp. 279–326. American Medical Association, Chicago.

HONORE, T., DAVIES, S. N., DREJER, J., FLETCHER, E. J., JACOBSEN, P., LODGE, D. & NIELSEN, F. E. (1988). Quinoxalinediones: Potent competitive non-NMDA glutamate antagonists. *Science* **241**, 701–703.

HORIKOSHI, T., ASANUMA, A., YANAGISAWA, K., ANZAI, K. & GOTO, S. (1988). Taurine and β-alanine act both on GABA and glycine receptors in *Xenopus* oocyte injected with mouse brain messenger RNA. *Molecular Brain Research* **4**, 97–105.

HOUNSGAARD, J. & MIDTGAARD, J. (1988). Intrinsic determinants of firing pattern in turtle Purkinje cells. *Journal of Physiology* **402**, 731–749.

HOUNSGAARD, J. & MIDTGAARD, J. (1989). Synaptic control of excitability in turtle Purkinje cells. *Journal of Physiology* **409**, 157–170.

ITO, M. (1984). *The Cerebellum and Neural Control*. Raven Press, New York.

KANEDA, M., WAKAMORI, M. & AKAIKE, N. (1989). GABA-induced chloride current in rat isolated Purkinje cells. *American Journal of Physiology* **256**, C1153–1159.

LARSELL, O. (1932). The cerebellum of reptiles: chelonians and alligator. *Journal of Comparative Neurology* **56**, 299–345.

LLINÁS, R. & HILLMAN, D. E. (1969). Physiological and morphological organization of cerebellar circuits in various vertebrates. In *Neurobiology of Cerebellar Evolution and Development*, ed. LLINÁS, R., pp. 43–76. American Medical Association, Chicago.

LLINÁS, R. & NICHOLSON, C. (1971). Electrophysiological properties of dendrites and somata in alligator Purkinje cells. *Journal of Neurophysiology* **34**, 532–562.

LLINÁS, R., NICHOLSON, C., FREEMAN, J. A. & HILLMAN, D. E. (1968). Dendritic spikes and their inhibition in alligator Purkinje cells. *Science* **160**, 1132–1135.

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

MAGNUSSON, K. R., MADL, J. E., CLEMENTS, J. R., WU, J. Y., LARSON, A. A. & BEITZ, A. J. (1988). Colocalization of taurine- and cysteine sulfinic acid decarboxylase-like immunoreactivity in the rat with monoclonal antibodies against taurine. *Journal of Neuroscience* **8**, 4551–4564.

MIDTGAARD, J. (1992*a*). Interactions between postsynaptic membrane properties and IPSPs in turtle Purkinje cells *in vitro*. *Journal of Physiology* **446**, 157*P*.

MIDTGAARD, J. (1992*b*). Membrane properties and synaptic responses of Golgi cells and stellate cells in the turtle cerebellum *in vitro*. *Journal of Physiology* **457**, 329–354.

NICHOLSON, C. & LLINÁS, R. (1969). Inhibition of Purkinje cells in the cerebellum of elasmobranch fishes. *Brain Research* **12**, 477–481.

OTTERSEN, O. P. (1988). Quantitative assessment of taurine-like immunoreactivity in different cell

types and processes in rat cerebellum: an electromicroscopic study based on a postembedding immunogold labelling procedure. *Anatomy and Embryology* **178**, 407–421.

OTTERSEN, O. P., MADSEN, S., STORM-MATHISEN, J., SOMOGYI, P., SCOPSI, L. & LARSSON, L. I. (1988). Immunocytochemical evidence that taurine is colocalized with GABA in the Purkinje cell terminals, but that the stellate cell terminals predominantly contain GABA: a light- and electronmicroscopic study of the rat cerebellum. *Experimental Brain Research* **72**, 407–416.

PALAY, S. & CHAN-PALAY, V. (1974). *Cerebellar Cortex*. Springer Verlag, Berlin, New York.