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Short Communications and Technical Notes

Inhibition of Cerebellar Purkinje Cells by Climbing Fiber Input*

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Summary. The inhibitory effects of the climbing fiber input on cerebellar Purkinje cells was studied in cats.

1. The excitatory and the inhibitory effect of the CF input was observed independent from each other.

2. At C-T intervals longer than 20 msec the EPSP of the Purkinje cell produced by CF activation was reduced by a preceding inferior olive stimulation.

3. At the period of greatest depression of a CF response the N_3 wave of a mossy fiber input was not reduced by a conditioning CF input.

It is suggested that basket cells are the interneurons mainly involved in the inhibition of Purkinje cells.

Key words: Climbing Fiber — Purkinje Cell — Cerebellum.

It is known, that after a climbing fiber (CF) response [7] there is a pause in the Purkinje cell (PC) firing [2, 13, 14]. Various hypotheses on the mechanisms underlying this phenomenon have been advanced in previous papers [3, 13, 14, 16, 17]. While some authors [13, 14] concluded that the inhibitory effect is due to an excessive depolarization of the PC membrane others support the hypothesis of a postsynaptic inhibitory and/or disfacilitatory mechanism [3, 16]. Latham *et al.* [16] did not find a significant inhibition of PCs during the post-CF pause, therefore, they concluded that disfacilitation underlies the silent period produced by the CF input.

The aim of the present work is to obtain additional information on the role of basket and Golgi cells in the pause which follows the CF activation of a PC.

The experiments were performed on sixty cats anaesthetized by thiobarbituric acid. A moderate depth of anaesthesia was maintained by supplementary doses of 5 mg/kg as required. The animals were paralyzed

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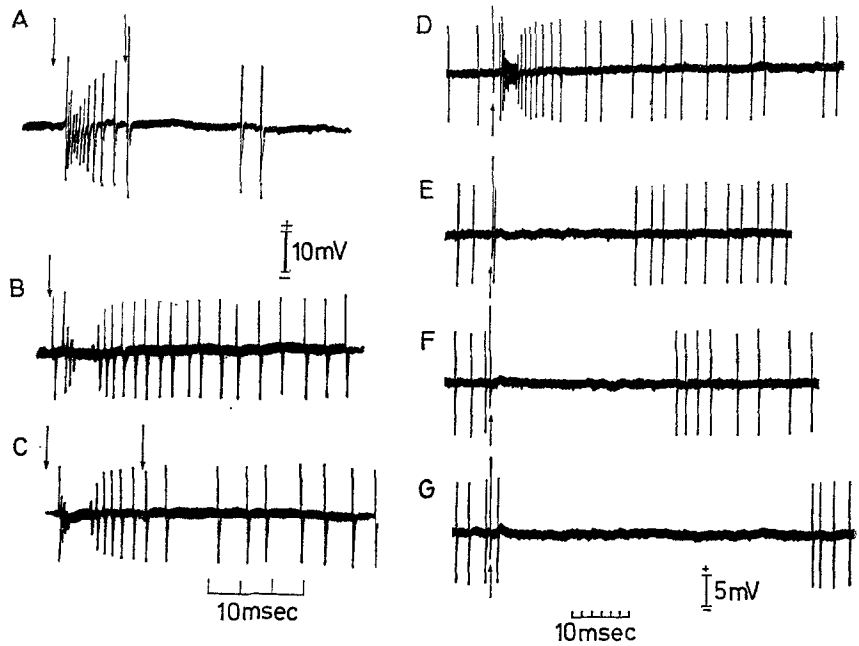

Fig. 1A—G. Responses evoked on PCs by IO stimulation. A shows the effect of two stimuli applied to IO. The first stimulus evokes a CF response, the second stops the single spike discharge. In B and C another PC shows a CF response to IO stimulation (first arrow) and in C a slowing of the discharge frequency by a second weaker stimulus applied to IO. In D—G another PC is recorded. In D, IO stimulation evokes a CF response with a following long lasting single spike discharge. In E—G with the same parameters of stimulation the discharge of the cell is stopped for more than 100 msec

with gallamine triethiodide. The culmen of the anterior lobe of the cerebellum was exposed. Stimulating electrodes were placed in the inferior olive (IO) (1). The typical responses to IO stimulation at the surface of the cerebellum [5, 12, 15] indicated the precise localization of the stimulating electrode within the 10. The position of the stimulating electrode was marked by electrolytic lesion. In all experiments the stimulating electrode was placed near the ventral raphe at the olivary level in order to prevent spread of current to the lateral reticular nucleus and to stimulate predominantly the olivo-cerebellar fibers leaving the IO. Glass microelectrodes filled with 4 M NaCl (DC resistance 4—8 MOhm) were used for recording field potentials and with 1 M Kcitrate (DC resistance 6—15 MOhm) for intracellular recording. Average field potentials were compiled (n = 16) with the aid of a Fabri Tek 1062 computer.

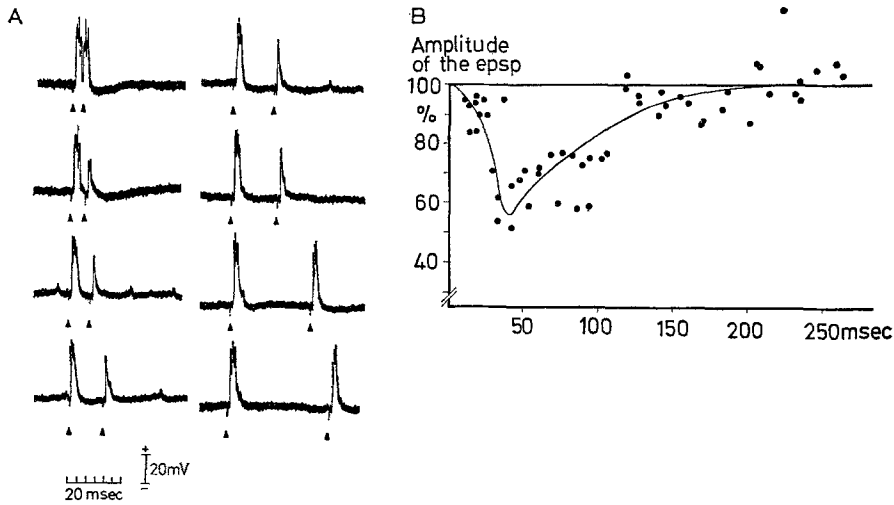

Fig. 2A and B. Intracellular records of a PC showing the time course of the interaction between two CF-EPSPs evoked by IO stimulation. A shows the changes in amplitude of a CF evoked EPSP at different intervals after a preceding CF response. In B the amplitude of the second EPSP in percentage of the control record was plotted against the intervals in milliseconds

Extracellular Recording

In some cells stimulation of the IO nucleus produced an inhibition of the spontaneous activity of the PC without an excitatory effect on this cell. Fig. 1 A shows the typical CF response of a PC following IO stimulation. A second stimulus applied to the IO, however, stops the activity of the cell without an excitatory effect. The same effect, but less intense, was observed in another cell (Fig. 1 B and C). In B the cell discharges with a typical burst to IO stimulation. In C a second stimulus, which was weaker than the first slowed down the frequency of the discharge without an excitatory response. In Fig. 1 D—G another cell discharged sometimes with the excitatory CF response (D) composed of an initial spike and several partial spikes with a frequency of about 500 per second. Sometimes (E—G) the IO stimulation was followed by interruption of spontaneous spike activity.

Intracellular Recording

By double stimulation of the IO at C—T intervals shorter than 15—20 msec, a second excitatory postsynaptic potential of normal size could usually be evoked by the second impulse. Fig 2 A illustrates the reduction in size of a second ESPP within intervals longer than 20 msec.

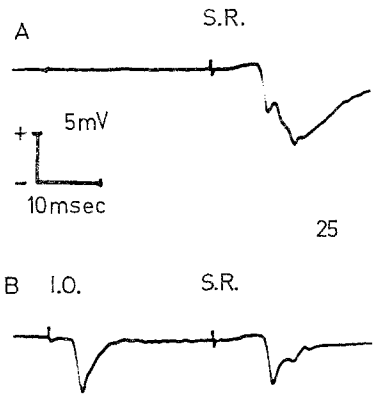

Fig. 3A and B. Averaged field potentials recorded at 250 μ depth. A shows the control potential evoked by stimulating the SRN with the N_3 and the CF component. In B the interaction effect between IO and SRN stimulation is illustrated. **(See text)**

At shorter intervals practically no reduction of the EPSP could be observed. In Fig. 2B the graph shows the reduction of the amplitude of the second EPSP at the same cell. The maximal reduction was reached at about 200 msec. By recording from other cells we mostly obtained curves which were identical in shape but differing in duration and degree of the depression.

Field Potentials

The N_3 wave of the field potential elicited by a mossy fiber (MF) input to the cerebellar cortex can be used to some extent as an indicator of the action of Golgi cells on the MF input. We recorded the field potentials produced by stimulating the superficial radial nerve (SRN) at a depth of about 250 μ within the molecular layer and conditioned this potential with that produced by stimulating the IO within the interval of the greatest depression which we have observed between two CF responses. The result of these experiments is illustrated by a typical interaction in Fig. 3. In A stimulation of the SRN produced a field potential consisting of two negative waves. Microelektrode track recordings showed, that the first negative deflection, with a latency of 8 msec, appeared at all depths of the molecular layer and was mirrored at the granular layer. It could be therefore identified as the N_3 component of the MF input [10]. The second larger negativity with a latency of 11 msec was replaced by a positive deflection at very superficial regions of the molecular layer and at the granular layer and did not follow frequencies higher than 15 cps. This is a typical behavior of the CF induced field potentials [8, 9]. In Fig. 3B the field potential produced by SRN stimula-

tion was preceded by a stimulus applied to the IO. The N_3 wave was not remarkably reduced while the CF component was strongly depressed.

The present study confirms that the CF input in addition to the strong excitatory action on PCs has also an inhibitory effect. The excitatory and the inhibitory effects sometimes occur independent from each other. Therefore, in agreement with Murphy and Sabah [17], Bloedel and Roberts [3] and with Latham and Paul (16] we assume that the inhibition of single spike firing in PCs is mediated by interneurons which are reached by CF collaterals. This interpretation is consistent with the histological finding that CF collaterals have synaptic contact with Golgi cells which are located about 5 PCs distant from the PC that is directly activated by the CF. On the other hand this interpretation is also consistent with the divergence of the basket cell—being reached by CF collaterals—on about 70 PCs.

In the post-CF period the PC again can be activated within intervals of 10—20 msec whereas in longer intervals the CF evoked EPSP is markedly reduced in amplitude. The interaction curve (Fig. 2) is very similar to the time course described by Eccles *et al.* [11] for the inhibition mediated by the interneurons of the molecular layer. The degree of the inhibitory action may be dependent on the number of the interneurons involved. Bloedel *et al.* [3] pointed out that a hyperpolarization of the PCs followed the CF evoked EPSP. On the basis of these results we assume that the decrease of the EPSP in our experiments is due to the shunt mechanism produced by inhibitory postsynaptic activity.

Another explanation for the reduction of the second EPSP in these experiments could be a depression of the IO cells by the test stimulus. In this case the depression of the EPSP would be due to a reduction of secondary spikes of IO cells. However, inhibitory phenomena in olivary cells were observed immediately after the EPSPs which were evoked by stimulating the olivary input [4] whereas in our studies we observed the depression after longer intervals, normally not before 20 msec.

In order to control the effectiveness of a CF activation on the MF input the N_3 wave produced by SRN stimulation was conditioned by IO stimulation. With C—T intervals of 25—50 msec the N_3 wave was not diminished. Since this component of the field potential provides a measure of the granule cell discharge it can be used as an index of the Golgi cell inhibition of granule cells [11].

Our results do not support the disfacilitatory hypothesis favoured by Latham and Paul [16] nor can they be explained by inactivation mechanisms. The present experiments support the view that basket cells are the neurons which likely are mainly involved in producing the inhibitory effect of the CF input.

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