Facilitation of synaptic transmission by diazepam in a perfused frog cerebellum[1]

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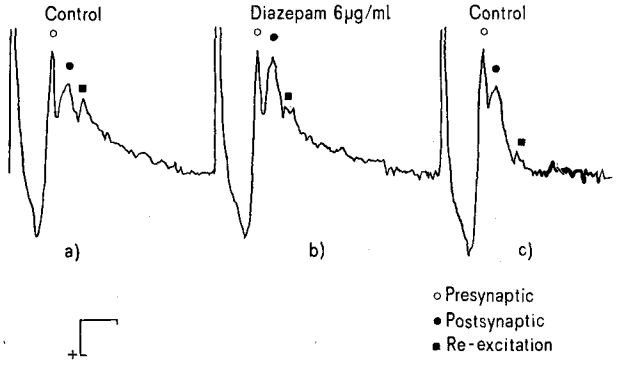
Summary. The action of diazepam on the parallel fibres-Purkinje cell synapse was studied in a perfused frog cerebellum. Diazepam facilitates the excitatory input to Purkinje cells and hence increases the inhibition produced by Purkinje cells activity.

Diazepam is one of the most widely used tranquilizers and anti-epileptic drugs. The action of diazepam on the central nervous system has been examined by several authors and it is generally accepted that the electrical activity in the brain is depressed by the drug. Diazepam is effective in the treatment of photo-myoclonic epilepsy, and this was attributed to the reduction of the visually evoked response produced by the drug[3]. Convulsions produced by soman, a potent anticholinesterase, could be terminated by diazepam. The EEG was markedly depressed after successful treatment with the drug[4]. In most systems which have seen examined, the interpretation of the data is difficult because complicated pathways and many synapses are involved. We report here on the action of diazepam on the well-defined synapse between the parallel fibres and Purkinje cells in the frog cerebellum. We found that diazepam facilitates the excitatory input to Purkinje cells, and hence increase the inhibition produced by Purkinje cells activity. The facilitation of an inhibitory mechanism ('change of sign') may thus play a role in the action of diazepam on the central nervous system.

The physiology and anatomy of neuronal circuits in the brain are better understood in the cerebellum than in any other higher center. Excitatory input to Purkinje cells is transmitted along the climbing and parallel fibres. The Purkinje cell axons are the only output of the cerebellar cortex, and the axons form inhibitory synapses with various subcortical structures[5]. Comparative studies show that the 'cerebellar circuitry' in the frog is simple but very similar to that of higher animals[6]. It has recently been shown that the frog cerebellum is well maintained in vitro and the preparation may be used in the investigation of central synaptic mechanisms[7,8].

The method for perfusing the frog cerebellum with Ringer solution has already been described[8]. This preparation has the advantage of keeping the isolated cerebellum in a well-defined environment. The parallel fibres were stimulated at a rate of 2 min by stainless steel electrodes coated with Teflon. The surface field potential was recorded by a glass micropipette filled with 3 M KCl and the response was averaged in a CAT (TMC) computer. When the cerebellum was perfused with normal Ringer (figure, a), the shape of the surface field potentials evoked by stimulation of the parallel fibres was very similar to those described in vivo[9]. The field potential comprized 2 distinct components: A presynaptic component which arises from the action potential of the parallel fibres and a postsynaptic component which arises from the resulting depolarization of Purkinje cells. The ratio between the postsynaptic and presynaptic components may thus represent, qualitatively, the efficiency of the transmission in the synapse. Following the perfusion of diazepam (5–20 µg/ml) for 5 min (figure, b), the amplitude of the postsynaptic wave was increased, whereas the presynaptic action potential was not markedly changed. The effect lasted for about 10–45 min after withdrawal of diazepam.



Diazepam effect on the parallel fibres-Purkinje cell synapse in a perfused frog cerebellum. Each record is a computer average of 10 responses. *a* Following the stimulus artefact, the surface field potential exhibits a presynaptic component which arises from the action potential of the parallel fibres (circle) and a postsynaptic component which arises from the chemically induced depolarization of the Purkinje cells (filled circle). *b* Following diazepam administration, the postsynaptic component is increased, whereas the presynaptic component is not changed. *c* After prolonged washing (45 min), the effect of the drug is abolished. Calibration: vertical bar, 1 mV; horizontal bar, 5 msec.

The ratio between the post- and presynaptic waves was measured in 16 experiments, and it always increased following diazepam application. The mean increase in the 'efficiency' of synaptic transmission was 33% ± 12% (range 11–64%). This was never found in control experiments in which the response was constant for up to 45 min, and the postsynaptic wave always deteriorated more rapidly. In some experiments, additional waves which represent Purkinje cell re-excitation were observed[9]. These waves deteriorated over a long period of time (figure, c).

The increase in the ratio between the post- and presynaptic waves shows that for a given number of activated parallel fibres, the transmission in the parallel fibres-Purkinje cell synapse is facilitated by diazepam. Whether the drug increases transmitter release from nerve terminals, or increases the sensitivity of postsynaptic receptors, is not yet known. The facilitation of the excitatory input to Purkinje cells may account for the increase in the firing rate of Purkinje cells following i.v. administration of the drug[10]. Since Purkinje cell axons comprize the entire output of the cerebellar cortex, and since Purkinje cells are inhibitory in their projections to subcortical structures[5], the facilitation of the parallel fibres-Purkinje cell synapse may play an important role in the anti-convulsant action of the diazepam.

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