Possible explanation for interictal-ictal transition: Evolution of epileptiform activity in hippocampal slice by chloride depletion

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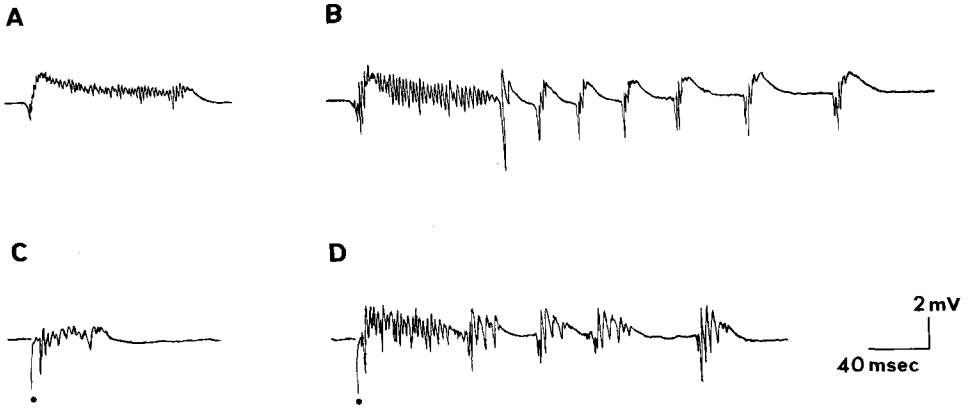
Summary. In thin hippocampal slices, paroxysmal epileptiform discharge was generated in high potassium medium. Removal of chloride from the high potassium medium caused explosive potentiation of the paroxysmal discharge and emergence of clonic relapsing discharges. Evolution of the paroxysm to regenerative seizure was attributed to the reduction of inhibitory postsynaptic potentials.

The focal electrical paroxysm, which is generally accepted as the electrographic 'interictal spike' or the 'paroxysmal depolarization shift' in intracellular recordings, is the simplest form of epileptiform activity in the epileptic focus of the mammalian brain. It is a well known phenomenon that the focal paroxysms ordinarily do not develop into self-sustained and spreading ictal discharges unless some additional manipulation is handled, and the basic mechanism of the transition from the interictal focal paroxysm into the ictal seizure discharge in the epileptogenesis is still unknown. One possible explanation might be the extracellular accumulation of potassium[1,2]. Recent investigations, however, revealed that the extracellular accumulation of potassium does not appear to be a crucial factor in the interictal-ictal transition[3,4]. Another plausible explanation is the reduction of the recurrent inhibition. Dichter and Spencer[5] observed that the inhibitory postsynaptic potential (IPSP) following the paroxysmal depolarization shift disappeared in the course of the interictal-ictal transition in the hippocampal pyramidal cell within the penicillin epileptic focus in vivo, and they suggested that the spread of the focal electrical paroxysm is restricted spatially and temporally by the powerful recurrent inhibition on the pyramidal cells in the focus. It has been established that IPSP in the hippocampal pyramidal cell was brought about by an increase in permeability of the membrane to chloride[6]. It is inferred, therefore, that if the above suggestion presented by Dichter and Spencer is valid, the focal paroxysm would tend to develop into the regenerative seizure, when chloride is removed from the extracellular space of the brain. However, it has been impossible to remove chloride from the extracellular space in in vitro experiments. The sliced preparation of the guinea-pig hippocampal formation has enabled us to study an electrical activity of mammalian cortical neurons under the modified ionic environments[3,7-10]. In the present study, therefore, the effects of chloride depletion on the paroxysmal electrical activity have been investigated in vitro by using a simplified brain slice preparation, and evolution of the paroxysm to regenerative seizure was attributed to the reduction of inhibitory postsynaptic potentials.

Materials and methods. The techniques for the preparation of the tissue, incubation, and recordings have been described in detail by Yamamoto and McIlwain[7]. The isolated hippocampal formation was prepared from adult guinea-pigs of both sexes weighing 400-600 g, and was sliced transversely to obtain a homologous histological structure, in which granule and pyramidal layers were interlocked with each other in oppositely faced 'c' shapes. Slices (350-450 µm thick) were incubated in glucose-saline medium saturated with 95% O_2 and 5% CO_2 at 37 °C. The standard medium contained (in millimolar concentrations): Na^+, 151.24; K^+, 5; Ca^{2+}, 2.6; Mg^{2+}, 1.3; Cl^-, 131.6; HCO_3^-, 26; $H_2PO_4^-$, 1.24; SO_4^{2-}, 1.3; and glucose, 10. In high potassium medium, K^+ concentration was maintained at 10 mM by adding potassium propionate. In chloride-deficient medium, NaCl was totally replaced by equivalent amounts of sodium propionate. The final concentration of chloride in chloride-deficient medium was 7.6 mM. Field potentials were recorded from the CA3 pyramidal layer with a glass pipette of 10 µm tip diameter filled with the incubating medium. For the evoked response, a single electrical pulse of 40-60 µsec duration was applied to the mossy fibres through a pair of tungsten needles insulated except for the tips.

Results and discussion. In the standard medium, all the



Effects of depletion of chloride from high potassium medium on the field potentials recorded from CA3 pyramidal layer. *A* Spontaneous paroxysmal discharge observed in high potassium medium. *B* The recording from the same preparation as that in *A* after the depletion of cloride from the medium (i.e., in high potassium plus chloride-deficient medium). *C* The evoked potential in response to mossy fibre stimulation in high potassium medium. *D* After the depletion of chloride from the medium in the same preparation as that in *C*. In *C* and *D*, the points of mossy fibre stimulation are indicated by dots. Positivity is upward in all the traces.

slices showed absolutely no spontaneous electrical activity in field potentials. It has been shown that the paroxysmal electrical activity can be produced in hippocampal slices in vitro by increasing the potassium concentration in the medium[3,8-10]. Figure, A illustrates the paroxysmal discharge observed in high potassium medium. The rate of occurrence of the paroxysmal discharge was usually less than 0.5/sec, and the interval between 2 consecutive paroxysmal discharges was relatively regular. When chloride was removed from the high potassium medium, both amplitude and total duration of the paroxysmal discharge were explosively augmented, and, in addition, clonic relapsing discharges, each of which had relatively short duration, appeared subsequently to the original discharge (figure, B). The number of the clonic relapsing discharges ranged from 3 to 10 arbitrarily in an unpredictable manner during the observation from the same preparation. The same phenomenon as the above observations which were seen in spontaneous electrical activities could also be observed in case of the evoked response by mossy fibre stimulation (figures, C and D). In this case, the evoked response in high potassium and normal chloride medium never developed into the clonic relapsing discharges, even by an extremely strengthened stimulation. Both spontaneous and evoked clonic relapsing discharges disappeared reversibly when the concentration of chloride in the medium was restored to normal. Further development of the discharges into full-brown seizure was not observed in both spontaneous and evoked activities. This might be partly ascribed to the metabolic limitations, such as accumulation of CO_2 and depletion of O_2 or high-energy phosphate intermediates, because metabolic capacity of the sliced preparation from the mammalian brain is assumed to be poor compared with that of the intact brain. It might also be plausible that a neural circuit involved in the sliced preparation is incomplete for the generation of full-brown seizure. The possibility that the clonic relapsing discharges were due to deterioration or injury of cells might be excluded, because discharges in these cases showed completely irregular patterns and occurred irreversibly.

In a series of previous reports[3,8-10], the following findings have been confirmed by the intracellular recordings from pyramidal cells in vitro. a) IPSP is retained in high potassium medium in spite of the emergence of the paroxysmal depolarization shift. b) On the other hand, in chloride-deficient medium with normal potassium concentration, IPSP is replaced by a small depolarizing potential accompanied by spike generation, and the paroxysmal depolarization shift was not observed. Therefore, it was indicated that, although chloride depletion causes the change in excitability by facilitating the synaptic transmission, it is not essential to the initiation of the focal paroxysm. Furthermore, changes in electrical activity of the paroxysmal discharge after the removal of chloride from the medium (figure) seem to be essentially identical to that observed at the transitional stage from interictal spikes to seizure in hippocampus in vivo (see figure 13 of Dichter and Spencer[5]). Thus, the present experiments support the notion that the reduction of inhibitory processes in hippocampus may play a causative factor in the evolution of the focal paroxysm into an ictal event, whereas it does not appear to be indispensable for the generation of the focal paroxysm.

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Proteoglycans in ovine brain

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Summary. Sulphated proteoglycans were isolated using a dissociative method of extraction. Cerebrum accounted for the major portion of proteochondroitin sulphate in brain, whereas the brain stem fraction contained over half the proteoheparan sulphate. Chemical characterization studies demonstrated that heparan sulphate from cerebellum contain more N-sulphate glucosamine residues.

The existence of chondroitin and heparan sulphate in nervous tissue has been firmly established. Owing to problems in isolation little is known of the molecular organization of these compounds. However, transferases associated with the addition of neutral sugars to a protein acceptor at the potentially reducing ends of glycosaminoglycans have been demonstrated[3]. We have recently reported that chondroitin sulphate and heparan sulphate exist as proteoglycans in brain tissue using a nondisruptive method of isolation[4,5]. In this present work the distribution of proteoglycans within specific locations have been studied.

Materials and methods. Fresh adult sheep brains were freed from adipose tissue, blood vessels and other adhering tissue. Brain tissue was carefully dissected into cerebrum, cerebellum and brain stem and each fraction was defatted with chloroform-methanol (3:1, v/v). Lipid-free batches (about 10 g) were suspended in 0.1 M citrate, pH 3.1, and further extracted with 4 M guanidinium chloride[4]. On removal of guanidinium ions by dialysis against water an insoluble residue formed was collected by centrifugation and dried over acetone. This residue was then extracted with 0.4 M citrate, pH 5.0, for 12 h at room temperature and the suspension clarified by centrifugation at 35,000 × g. The supernatant (fraction B) was then chromatographed on DEAE-cellulose (figure).

Free glycosaminoglycan chains were released by papain