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# CONTINUOUS INCREASE OF EPILEPTOGENIC EFFECTS FOLLOWING APPLICATION OF PROTEOLYTIC ENZYMES (BUCCAL GANGLIA OF *HELIX POMATIA*)*

U. ALTRUP,** A. ÜRE and A. JOSCHKO

Institute of Experimental Epileptology, University of Münster,
Hüfferstr. 68, H-48149 Münster, Germany

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Epileptic activity of neurons consists of paroxysmal depolarization shifts (PDS) which can be induced presumably in any nervous system by application of an epileptogenic drug. The spontaneous appearance of epileptic activity, however, is based on a largely unknown process which increases susceptibility to epileptic activity (seizure susceptibility in man). It is presently shown that the treatment of ganglia with proteolytic enzymes (Pronase) decreases the effective concentration of epileptogenic drugs, i.e. increases seizure susceptibility. Since proteolytic enzymes are known to primarily affect glial cells a contribution of glia to seizure susceptibility is discussed.

*Keywords:* Buccal ganglia – *Helix pomatia* – epilepsy – epileptogenicity – protease – pacemaker potential

Epilepsy shows itself in epileptic seizures which are a transient loss of function within a circumscribed part of the central nervous system. On the neuronal level, epileptic seizures result from paroxysmal depolarization shifts (PDS) which consist of a steep depolarization with action potentials superimposed, a plateau depolarization of 30 to 40 mV of amplitude, and a steep repolarization (cf. Fig. 1B) [5]. PDS appear suddenly, repeatedly, for a short period of time, and synchronized within many neurons. As to the nature of PDS, they have been shown to represent enlarged and extended pacemaker potentials. Synchronization of neurons during a seizure probably results from non-synaptic exocytosis during the PDS [1, 4]. Whereas PDS are understood at least in part, it is largely unknown what happens in a circumscribed part of the brain which enable PDS. This epileptogenicity is hardly understood presently. The application of a drug like pentylenetetrazol is well known to introduce epileptogenicity since during application of pentylenetetrazol PDS appeare in almost every nervous system. With washing of the drug, PDS disappeare immediately. It is presently shown that after the application of proteolytic enzymes, epileptogenicity

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** Corresponding author; e-mail: altrup@uni-muenster.de

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increased continuously during several hours. Thus, a treatment with proteolytic enzymes may provide a model to study the endogenous emergence of epileptogenicity in a relatively simple nervous system.

The buccal ganglia of *Helix pomatia* were isolated from the animal and kept in an experimental chamber continuously perfused with a control solution. Test substances were added to the control solutions. Neurons B3 were recorded with intracellular microelectrodes since they reliably generated PDS when treated with pentylenetetrazol (40 mM) or etomidate (0.6 mM) [3]. Proteolytic enzymes (Pronase E, Merck, Darmstadt, Germany) were applied in 0.1 to 1.0 mM concentration. Duration of treatment lasted from 10 min (mostly 1.0%) to 3 h (mostly 0.1%).

With increasing concentration of pentylenetetrazol or etomidate, pacemaker potentials of neuron B3 (Fig. 1A1) first increased in duration and amplitude (Fig.

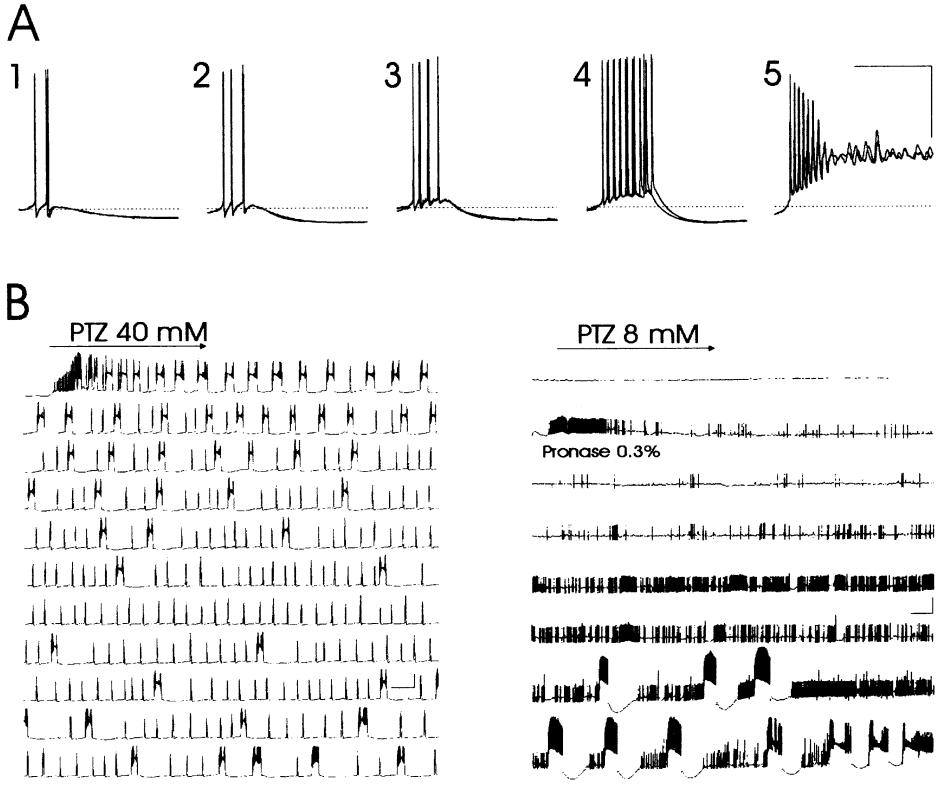


*Fig. 1.* Development of epileptic activity (PDS) from pacemaker potentials (A) and effects of long-term application of pentylenetetrazol (B, PTZ 40 mM) as well as of PTZ (8 mM) when the ganglia are treated additionally with proteases (B, PTZ 8 mM). A1: control condition, 2 to 5: pentylenetetrazol (in mM) 4, 8, 16, 40; two oscilloscope recordings are superimposed triggered from the first action potential; calibration bars: 2 s, 50 mV. B: continuous pen recordings for 24 h, arrows mark the begin of a treatment with pentylenetetrazol; duration of application of proteases (Pronase, 0.3%) is marked by a box; calibration bars: 20 min, 20 mV

1A2 to 4) and then developed into typical PDS (Fig. 1A5). When pentylenetetrazol (40 mM) was applied for ca. 24 h, PDS-activity was maximal immediately after application of the drug (Fig. 1B, PTZ 40 mM, first line). PDS-activity then declined during several hours down to zero level. After 10 h to 20 h, frequency and duration of PDS in most cases re-increased demonstrating that the decline was no "run-down". The decline could be reversed with a reduction of $[Mg^{2+}]_o$ and with application of forskolin (50 μM). The decline may be interpreted as to result from intracellular processes.

When pentylenetetrazol or etomidate were applied for 24 h in a sub-threshold concentration with respect to the generation of PDS, the enhanced pacemaker potentials (cf. Fig. 1A3) appeared regularly and without decrease of frequency or amplitude (not shown in Fig. 1). When the same experiment lasting for 24 h was done in combination with a treatment of the ganglia with proteolytic enzymes (Pronase), epileptic activity regularly increased and PDS appeared after several hours (Fig. 1B, PTZ 8 mM). The increase appeared after termination of the enzyme-treatment. The increase was also encountered when the ganglia were pre-treated with the enzymes. Latency between termination of protease treatment and appearance of PDS decreased with increasing concentration of the enzymes and with duration of their application. The increase is interpreted as an endogenous increase of epileptogenicity comparable to an increase of concentration of the epileptogenic drug pentylenetetrazol from 8 mM to 40 mM.

Concerning invertebrate neurobiology, proteolytic enzymes are often used to soften perineuronal connective tissue in order to facilitate experimental access to the ganglia. It has been shown previously that a proteolytic treatment alters physiologic properties of the ganglia [2]. It is presently shown that the treatment may accelerate epileptogenic properties which results in the appearance and accentuation of pacemaker potentials and PDS. This in turn may continuously alter network properties of the ganglia. The observations do not support the idea that the described effects only happen at high concentrations of the proteolytic enzymes.

Concerning experimental epileptology, the present observations may provide a basis for the study of epileptogenicity within a relatively simple model nervous system. So the question to be answered is what increases epilepogenicity during several hours after the application of proteolytic enzymes. It has often been thought that synaptic transmission may underlie epileptogenicity. Especially long-term potentiation and NMDA-type glutamate activities have been suggested to be involved. However, since previous studies have shown that synaptic potentials are largely depressed under epileptic conditions [1], i.e. during application of an epileptogenic drug, synaptic potentials should play minor roles only. Presently it appears possible, that a disturbed "housekeeping function" of glia might contribute since it is known that a treatment with the proteolytic enzymes Pronase predominantly affects glia [6] and leaves neurons relatively unaffected [2].

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