Actions of pentylenetetrazol (PTZ) on CA3 neurons in hippocampal slices of guinea pigs*

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Summary. The actions of the convulsant drug pentylenetetrazol (PTZ) were studied on CA3 neurons of hippocampal slices (300–400 μm thick). In 9 out of 109 neurons, epileptic reactions were elicited during a single application of PTZ. After repeated applications of PTZ, 53 neurons showed periodic paroxysmal activity. They developed according to the following sequence: (i) Paroxysmal hyperpolarizations, (ii) burst activity, and (iii) typical paroxysmal depolarization shifts (PDS). The rate of occurrence was about 8/min. Paroxysmal hyper- and depolarizations appeared synchronously in pairs of neurons. The developmental sequence occurred in reverse during washing. After the onset of paroxysmal activity, bursts and PDS persisted if PTZ concentration in tissue ranged between about 2 and 10 mmol/l. When this range was exceeded in either direction, epileptic activity was abolished at a calcium concentration of 2.75 mmol/l. Decreasing and increasing the calcium concentration shifted the epileptogenic concentration range to lower and higher levels, respectively. It is concluded that repetition of PTZ application alters membrane properties of neurons and thus leads to paroxysmal events triggered by synaptic processes.

Key words: Hippocampal slices – CA3 neurons – Pentylenetetrazol – Paroxysmal hyperpolarizations – Paroxysmal depolarizations

Introduction

In experimental epileptology pentylenetetrazol (PTZ) is often used to provoke seizure discharges. A great number of such experiments have been carried out on mammalian neurons in vivo (cf. Prince 1972; Stone 1972). However, for an analysis of the elementary mechanisms underlying the convulsive actions of PTZ in more detail, neurophysiological techniques applicable in such experimental conditions are rather limited. Therefore, many experiments have been carried out using in vitro preparations. Thus, the overwhelming majority of investigations have been performed in snail neurons (Faber and Klee 1972; Chalazonitis and Arvanitaki 1973; Speckmann and Caspers 1973; Wilson and Escueta 1974; Dureux and Gola 1975; Williamson and Crill 1976; Pacheco et al. 1981; Doerner et al. 1982; Sugaya et al. 1982). The question is, however, whether the results obtained are representative also for mammals. Therefore, the intention was to study PTZ effects on mammalian neurons preserving the technical advantages of in vitro preparations (cf. McDonald and Barker 1977). Consequently CA3 neurons of hippocampal slices were selected for further investigations, as these cells are known to have a high seizure susceptibility with various drugs (Schwartzkroin and Prince 1978, 1980; Alger and Nicoll 1980; Hablitz and Andersen 1982; Misgeld et al. 1982; Bingmann et al. 1983). There are, however, only a little information about the actions of PTZ on hippocampal neurons (cf. Louvel and Heinemann 1981; Lux and Heinemann 1983; Zeise et al. 1983; Piredda et al. 1985). Hence the studies presented here aim to analyze more in detail effects exerted by PTZ on CA3 neurons.

Parts of the results have already been published in abstract form elsewhere (Bingmann and Speckmann 1983).

Methods

The experiments were carried out on guinea pigs weighing 300–400 g. Under ether anaesthesia the brain was removed and
the hippocampus was dissected within ca. 2 min. Transverse slices of the hippocampus (300–400 μm thick) were cut parallel to the alvear fibres by means of a hand held razor blade which was guided by a slit in a perspex block. After preparation, the slices were submerged in 28°C warm saline which was equilibrated with 95% O₂ and 5% CO₂. The saline solution contained (mmol/l) NaCl 124, KCl 5, CaCl₂ 0.75, MgSO₄ 1.3, KH₂PO₄ 1.25, NaHCO₃ 26, glucose 11. After a preincubation of ca. 2 h slices were transferred into a perspex chamber (1.5 × 4 cm) and attached to the bottom which consisted of optically plane glass. The chamber was mounted on an inverted microscope (Zeiss Invertoskop D). This arrangement allowed a detailed inspection of the excised tissue. The slices were superfused laminarily by an approximately 3 mm thick layer of 32°C warm saline. The flow rate was about 2–3 ml/min. Thus, the bath fluid was exchanged within 1–2 min. The composition of the saline was the same as that used during preincubation except for the Ca ++ concentration which was usually elevated to 2.75 mmol/l (cf. Yamamoto 1972).

Intracellular recordings were achieved by means of micropipettes with tip diameters of less than 0.5 μm which were filled either with 2 mol/l potassium methylsulphate or with 3 mol/l potassium chloride. The resistance of the electrodes ranged between 60 and 120 MΩ. Under microscopic control the tips of the microelectrodes were placed within the stratum pyramidale and were moved by means of a step motor driven hydraulic microdrive. For intracellular current injections via the recording microelectrode a passive bridge was used. The membrane resistance was determined by injection of hyperpolarizing currents (0.1–0.2 nA, 500 ms, repetition rate 0.1 Hz).

In order to facilitate displays and superimpositions of spontaneous phenomena a special technique was developed (Bingmann and Sasse 1985). The bioelectric events under investigation were used as trigger signals and simultaneously delayed in a chain of charge coupled devices. After preselected times of 25 to 100 ms the delayed signals were fed into a storage oscilloscope and thus appeared automatically in a predetermined section on the screen. For off-line analysis signals were stored by an FM tape recorder.

The distribution rate of PTZ within the slices was studied using PTZ sensitive double barreled microelectrodes according to the technique of Walden et al. (1984).

Results

In 109 CA3 neurons it was tested whether PTZ elicits the characteristic epileptic neuronal reaction, the so-called paroxysmal depolarizations (PD) (cf. Prince 1972). The experiments showed on the one hand that a single application of PTZ in a concentration of 2–15 mmol/l was able to induce bursts or paroxysmal depolarization shifts (PDS) in only 9 out of the above mentioned neurons. In the remaining cells, ictal activity was missing during the first exposition. This was true even when the neurons were exposed continuously to this drug for 1 to 2 h (n = 9). Changes of PTZ concentrations in tissue are described below. On the other hand with repetitive applications PD appeared in 53 neurons out of 91. The development of such an epileptic neuronal reaction is shown in Fig. 1. In this experiment, the preparation was exposed alternatively to a PTZ solution (15 mmol/l) for 10 min and washed with control solution for ca. 20 min. With this procedure a significant accumulation of PTZ could be avoided (see below and Fig. 9). During the 1st-4th applications no PDS occurred (Fig. 1A, B), while they did appear during the 5th exposition (Fig. 1C). The first appearance of PDS was most often preceded by typical neuronal reactions to PTZ which consisted in slow changes in resting membrane potential (RMP), membrane resistance and excitability, and in alterations of action potentials (AP). Therefore, the subsequent description is subdivided into several parts. The first deals with the responses to PTZ before the onset of PD while the second deals with those occurring afterwards. The epileptogenic range of PTZ concentrations and the interactions between PTZ and the extracellular Ca ++ concentration are taken into account in parts 3 and 4, respectively.

1. Neuronal responses to PTZ before the onset of paroxysmal epileptic activity

CA3 neurons selected for further investigations exhibited an RMP of −55 to −65 mV in control solution. When the intracellular recording proved to be stable for at least 10 min, PTZ (2–20 mmol/l) was added to the bath.

Especially during the first application of the drug, a slow transient hyperpolarization often occurred (Fig. 2A), which was associated with a decline of the