Spontaneous and stimulus-triggered epileptic discharges: delayed antiepileptic effect with triggering

Abstract

The aim of the present study was to test whether the organic calcium channel blocker verapamil acts not only on spontaneously occurring epileptiform field potentials (EFP) but also on EFP triggered by single electrical stimuli in the low-Mg$^{2+}$ epilepsy model. The experiments were carried out on hippocampal slices of guinea pigs. EFP were elicited by omission of Mg$^{2+}$ from the perfusate and recorded from stratum pyramidal and stratum radiatum in the CA1 subfield. Single electrical stimuli were applied to the Schaffer collateral pathway. Verapamil was added to the bath solution in concentrations of 40 and 60 µmol verapamil/l at normal (4 mmol/l) and elevated (8 mmol/l) K$^+$ levels. After omission of Mg$^{2+}$ from the perfusate, spontaneously occurring EFP appeared in all trials. These spontaneously occurring EFP were suppressed dose-dependently upon addition of verapamil to the perfusate. At elevated K$^+$ levels, the latencies to suppression were significantly reduced and the dose dependency was abolished for the two doses of verapamil used. Triggered EFP reappeared upon stimulation after spontaneously occurring EFP had been suppressed, except for trials with 60 µmol/l bath solution with elevated K$^+$ levels. The stimulus-evoked EFP were abolished with continuing perfusion of verapamil except for trials with 40 µmol/l at normal extracellular K$^+$ concentrations. This effect was again dose dependent and enhanced by elevating the K$^+$ level. In all experiments, stimulus-evoked EFP reappeared upon wash-out of verapamil. A primary action of verapamil on pacemaker functions in epileptogenic tissue is assumed.

Key words

Verapamil · Calcium channel blockers
Epilepsy · Hippocampus · Guinea pig

Introduction

Calcium and calcium-dependent ion fluxes are supposed to play a major role in the generation of epileptic activity (for an overview see Speckmann et al. 1986; Speckmann and Walden 1993). Consequently, organic calcium channel blockers have been demonstrated to suppress epileptic activity in various experimental models both in vivo and in vitro. Among these calcium channel blockers, the diphenylalkylamine derivative verapamil has been studied extensively and found to exert a prominent antiepileptic effect (Walden et al. 1986; Bingmann et al. 1988; Bingmann and Speckmann 1989; Boulton et al. 1989; Aicardi and Schwartzkroin 1990; Straub et al. 1990a,b, 1992a,b; Moraidis et al. 1991; Pohl et al. 1992).

Epileptic activity is represented by paroxysmal depolarization shifts (PDS) on the level of single neurons. The appearance of PDS is thought to be based on two mechanisms. The first consists in the ability to generate rhythmic epileptic discharges spontaneously. The second mechanism comprises triggered activity in response to the spontaneously occurring discharges. This model is in accordance with concepts commonly being used in presurgical evaluation of epileptic patients suffering from seizures with focal onset, who are potential candidates for surgery (Wieser and Elger 1987). In these concepts, different epileptogenic zones are being distinguished. One among these is the primary epileptogenic zone with pacemaker structures (Wyler and Ward 1980; Lüders and Awad 1991; Wieser and Siegel 1992, 1993). Another is the secondary epileptogenic zone, which is less relevant for the actual appearance of epileptic discharges and whose activity is dependent on the primary epileptogenic zone (Lüders and Awad 1991; Wieser and Siegel 1992, 1993). The present investigations aim to differentiate whether the antiepileptic effect of organic calcium channel blockers is primarily being exerted (1) on pacemaker mechanisms or (2) on the general ability to generate epileptic discharges.
have already been published in abstract form (Köhling et al. 1993).

As an experimental preparation, the hippocampal slice was chosen because of its defined anatomy and the possibility of activating distinct pathways by electrical stimulation. With low-Mg²⁺-induced epileptic activity, the CA3 subfield of the hippocampus corresponds to the primary epileptogenic zone with pacemaker function, and the CA1 subfield corresponds to the secondary epileptogenic zone being driven by CA3. This notion is supported by observations of Tancredi et al. (1988, 1990) which showed that severing CA1 from CA3 leads to a breakdown of epileptic activity in CA1 but not in CA3. Furthermore, in a number of experiments it has been shown that the CA3 subfield generally is the leading structure in epileptic hippocampus (Mody et al. 1987; Pohl et al. 1992; cf. Colom and Saggau 1993). This is in line with unpublished data demonstrating that, after separating the two regions, CA1 changes its activity from discharges synchronous to CA3 to discharges with a characteristically different rhythmicity.

Epileptic activity elicited by omission of Mg²⁺ from a bath solution has been studied extensively in hippocampal slices (Anderson et al. 1986; Walther et al. 1986; Swartzwelder et al. 1987; Tancredi et al. 1990). This model has two major advantages: firstly, no additional epileptogenic agent is needed; secondly, the organic calcium channel blocker verapamil has already been demonstrated to suppress spontaneously occurring epileptiform field potentials (EFP) in low-Mg²⁺-induced epilepsy (Boulton et al. 1989; Pohl et al. 1992).

**Materials and methods**

The experiments were carried out on 23 hippocampal slices from guinea pigs weighing 300–400 g. The brain was removed under deep methohexitol anesthesia. The hippocampi were dissected and cut into slices of 400–500 μm thickness. The slices were preincubated at 28°C for 60 min in artificial cerebrospinal fluid (CSF). The CSF contained (in millimoles per liter): NaCl 124, KCl 4, CaCl₂ 1.0, NaH₂PO₄ 1.24, MgSO₄ 1.3, NaHCO₃ 26, and glucose 10. The CSF was continuously exposed to 5% CO₂ and thus the pH stabilized at 7.35–7.4. After preincubation, the slices were transferred to a submersion recording chamber and perfused with CSF with CaCl₂ elevated to 2.0 mmol/l at 33°C. Field potentials were recorded with glass micropipettes (approx. 1 MΩ) from stratum radiatum and stratum pyramidale in CA1. The Schaffer collateral pathway was stimulated by a bipolar platinum electrode lateral to the Schaffer collaterals. Interstimulus intervals, 10 s. Period 1 served to detect slices showing spontaneously occurring EFPs under control conditions. In the present study there were none. Single electrical stimuli evoked typical population spikes and a positive field excitatory postsynaptic potential (fEPSP) in CA1 stratum pyramidale and stratum radiatum, respectively (Fig. 1A, a).

Induction of spontaneously occurring epileptiform field potentials (period 2)

Epileptic activity occurred in all slices with omission of Mg²⁺ from the perfusate (period 2, Fig. 1B). EFP appeared synchronously at stratum pyramidale and stratum radiatum within 18 ± 4 min at normal (n = 10) and within 6 ± 1 min at elevated K⁺ levels (n = 13), the two groups thus differing statistically (Fig. 4). EFP appeared in a regular manner (Fig. 1B). Within 20–30 min, the repetition rate of EFP rose to a steady state level. Spontaneously occurring EFP appeared as longlasting monophasic and polyphasic deflections of up to 500 ms duration with superimposed sharp oscillations (Figs. 2, 3). The area integrals of spontaneous EFP varied from 85 to 1000 μVs.

**Results**

Stimulus-evoked nonepileptic field potentials (period 1)

Period 1 served to detect slices showing spontaneously occurring EFPs under control conditions. In the present study there were none. Single electrical stimuli evoked typical population spikes and a positive field excitatory postsynaptic potential (fEPSP) in CA1 stratum pyramidale and stratum radiatum, respectively (Fig. 1A, a).