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Organic and inorganic calcium antagonists inhibit veratridine-induced epileptiform activity in CA3 neurons of the guinea pig

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Summary Veratridine is believed to cause epileptiform discharges via its effects on sodium channels. We addressed the question whether calcium currents, known to contribute to the generation of paroxysmal depolarization shifts (PDS) in most models of epilepsies, also contribute to veratridine-induced epileptiform activity. Therefore, we recorded from CA3 neurons ($n = 50$) of veratridine-treated hippocampal slices and analyzed the effects of two calcium antagonists. Veratridine (0.5—1.0 μM) elicited spontaneous epileptiform bursts, paroxysmal depolarization shifts (PDS) lasting 100—300 ms, and depolarizations (LD) lasting up to several minutes. Most often PDS directly preceded LD which resulted in typical composite depolarizations termed veratridine-induced complexes (VC). VC persisted even in the presence of CNQX and APV (25 μmol/l, both), or in nominally calcium-free saline, revealing the non-synaptic nature of these potentials. Cobalt (1—2 mM) abolished VC within minutes, but allowed LD type-like potentials to be elicited by depolarizing current pulses. Verapamil (50 μM) also diminished or abolished amplitudes of VC. All inhibitory effects of cobalt and verapamil were at least partly reversible. Due to the effects of both calcium antagonists we conclude that veratridine-induced epileptiform activity depends not only on sodium, but also on calcium currents.

Introduction

Veratridine induces slow long-lasting depolarizing potentials (LD) in hippocampal slices (Tian et al., 1995). These LD follow stimulated or spontaneous bursts, or paroxysmal depolarization shifts (PDS). Since all types of veratridine-induced seizure-like activities are inhibited by sodium channel blockers such as tetrodotoxin or cocaine, and because they persist during blocking synaptic transmission, e.g., by kynurenic acid (Tian et al., 1995) or low calcium (Inenaga et al., 1993), they are believed to be primarily due to altered sodium channel functions. Indeed, veratridine shifts the activation of sodium currents to more negative membrane potentials (Garber and Miller, 1987; Leibowitz et al., 1986) and leads to a delayed inactivation of activated sodium channels, which most likely explains long-lasting

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sodium inward currents and increases of intracellular sodium concentration upon exposure to veratridine (Ashton et al., 1990; Bouron and Reuter, 1996; Lopez et al., 1995). The role of sodium currents in epileptogenesis is also strengthened by the finding that in E1 mice both, the number of neuronal sodium channels and the frequency of spontaneous seizures are increased (Sashihara et al., 1992). Also the tottering mouse, another epileptic animal model, exhibits increased conductance of sodium channels (Willow et al., 1986). In human brain, a mutation in the β_1-subunit of the voltage-gated sodium channel is associated with febrile seizures and generalized epilepsy (Wallace et al., 1998).

On the other hand, there is compelling evidence that excessive transmembraneous inward calcium currents contribute to the generation of the PDS in most epilepsy models (Speckmann and Walden, 1986; Witte et al., 1987; Speckmann et al., 1992; Bingmann and Speckmann, 1989; Straub et al., 1997). Moreover, calcium currents starting with the onset of each single PDS increased and decreased intracellular and extracellular calcium concentration, respectively (Lücke et al., 1990; Moraidis et al., 1991; Wiemann et al., 1996). Application of inorganic or organic calcium antagonists such as verapamil, flunarizine or nifedipine reduced these transmembraneous calcium currents and decreased amplitude and duration of the PDS until epileptiform activity was abolished (Bingmann and Speckmann, 1989; Moraidis et al., 1991; Speckmann and Walden, 1986; Straub et al., 1997; Witte et al., 1987).

Although there are hints for an enlargement of calcium currents by veratridine (Adam-Vizi and Ligeti, 1986; Ashton et al., 1990; Lopez et al., 1995; Moreno-Sanchez and Hansford, 1990), the question regarding just how much calcium currents contribute to veratridine-induced epileptic discharges has not yet been investigated. Due to the profound role calcium currents play for most other epileptic model systems, we hypothesized that inorganic and organic calcium antagonists can modulate veratridine-induced discharges. Results show that cobalt and verapamil, in fact, abolish this form of non-synaptic epileptiform activity.

Materials and methods

Experiments were carried out on hippocampal slices (400—500 μm thick) prepared from ether anaesthetized guinea pigs (350—500 g). After cutting the slices by a guided razor blade, they were preincubated for at least 2 h in a 28 °C saline which contained (in mM): NaCl (124), KCl (3), $CaCl_2$ (0.75), $MgSO_4$ (1.3), KH_2PO_4 (1.25), $NaHCO_3$ (26), and glucose (11). Thereafter, slices were transferred to a perspex chamber (1.5 cm × 4 cm) and positioned onto the bottom of optically plane glass through which the slices could be examined by means of an inverted microscope (Zeiss, Invertoskop D). The chamber was perfused with a ca. 2 mm layer of 32 °C warm saline at a rate of 4—6 ml/min. The composition of this saline was identical to the aforementioned pre-incubation saline except the calcium concentration which amounted to 1.75 or nominally 0 mM. Epileptiform activity was elicited by adding veratridine (Sigma) dissolved in 1 M HCl to the perfusate (final concentration: 0.15—1.5 μM). Glutamatergic synaptic transmission was suppressed by adding 25 μM 2-amino-5 phosphonovalerate (APV) plus 25 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to the bath saline. Cobalt(II)-chloride-hexahydrate (Merck, Germany) and verapamil (Knoll GmbH, Germany) were used as calcium antagonists. The pH of all solutions was 7.4 ± 0.02.

Intracellular recordings from CA3 neurons were obtained with glass micropipettes filled with potassium methylsulphate (2 M). Electrodes had a tip diameter of less than 0.5 μm (100—200 MΩ) and were positioned under microscopic control within the stratum pyramidale of CA3. A passive bridge circuit was used for current injections via the recording microelectrode. Hyperpolarizing pulses of 100 ms duration and (typically) 0.1 nA amplitude were injected intracellularly to monitor changes of the membrane resistance. Analogue bioelectrical signals were recorded digitally (sampling rate: 12 kHz) and analyzed using the program DAPAS (Widman and Bingmann, 1996).

Results

Veratridine-induced epileptiform activity

A total of 50 spontaneously active CA3 neurons with membrane potentials (MP) ranging from −50 to −65 mV and action potentials (AP) exceeding 50 mV in amplitude were subjected to veratridine treatment. Exposure to 0.5—1 μM veratridine progressively depolarized most neurons. The mean (±S.D.) depolarization measured in CA3 neurons ($n = 9$) exposed to veratridine for more than 60 min prior to further treatment amounted to 8.7 ± 6.0 and 13.3 ± 9.1 mV after 30 and 60 min, respectively. In five neurons we observed slow oscillations of the membrane potential with periods lasting up to minutes while amplitudes often exceeded 10 mV. These changes in MP were not accompanied by predictable changes of the membrane resistance which — in this phase — typically ranged between 30 and 40 MΩ. Paralleling these long-lasting membrane potential shifts, a typical sequence of further bioelectric events appeared, altogether lasting from several minutes to about 1 h (Fig. 1). The following consecutive stages could be distinguished:

PSP stage

The onset of veratridine effects was characterized by excitatory and inhibitory postsynaptic potentials (PSP), which transiently increased in amplitude and frequency of occurrence.

Burst stage

Within minutes, veratridine led to grouped activity characterized by short bursts (Fig. 1A). These bursts lasted about 100 ms and persisted while CNQX and APV were applied (Fig. 2). We conclude that these bursts were not synchronized among neighboring cells, as field potentials recorded extracellularly were small compared to those elicited by other epileptogenic substances such as caffeine, penicillin or pentylenetetrazol. Furthermore, small MP fluctuations lasting about 100 ms (encircled regions in Fig. 2C) indicated bioelectric activity asynchronous to the recorded neuron.

PDS stage

Isolated paroxysmal depolarization shifts (PDS) were generated which progressively replaced the former arrhythmic activity (Fig. 1A). Later on PDS appeared periodically at 5—15 s intervals.