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Research Note

Spontaneous and evoked release of endogenous Zn^{2+} in the hippocampal mossy fiber zone of the rat in situ

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Summary. In rats anaesthetized with urethane, push pull cannulae were stereotaxically introduced in the hippocampus (bilaterally) and Zn^{2+} assayed in the perfusate by atomic absorption spectrophotometry. When the cannula was located in the immediate vicinity of the mossy fiber zone, both spontaneous and K^+ evoked release of Zn^{2+} were observed, this release was associated with a reduction in the histologically demonstrable Zn^{2+} as assessed by means of the Timm's stain. Neither spontaneous nor evoked release of Zn^{2+} was observed when the cannula was located in the medial part of CA1, the fimbria or the thalamus. These observations suggest that Zn^{2+} is released in the mossy fiber zone.

Key words: Zn^{2+} – Push pull – Mossy fibers – Hippocampus

Introduction

A partial or complete destruction of the ammon's horn constitutes the most frequent pathological sequela observed in epileptic patients (Spielmeyer 1927; Margerison and Corsellis 1966). In experimental conditions, the granular neurones of the fascia dentata and their mossy fibers, which project to the apical dendrites of the pyramidal cells of CA3 (Amaral and Dent 1981) play a crucial role in this seizure related vulnerability of the ammon's horn (see references in Ben-Ari 1985). Thus, the CA3 pyramidal neurons are readily destroyed by either repetitive electrical stimulation of the perforant pathway (Sloviter 1983b) or intracerebral administration of kainic acid (Ben-Ari et al. 1980; Nadler et al. 1980) and the latter effect is only produced when the mossy fibers are mature (references in Ben-Ari 1985) and operational (Nadler and Cuthberson 1983). Furthermore, the destruction of the vulnerable CA3 zone after parenteral KA is not due to a local hypoxia (Pinard et al. 1984). It is not clear why the mossy fibers confer such a particular vulnerability to their post-synaptic targets. It has been known for some time that zinc ions are contained in high concentrations in the mossy fibers (e.g. Haug 1973; Frederikson 1984) notably in synaptic vesicles (Haug 1967) and that following parenteral administration, radioactive zinc accumulates in this zone (Von Euler 1962). We now report that there is both spontaneous and evoked release of endogenous Zn^{2+} from the mossy fibers in the anaesthetized rat.

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Methods

10 adult male wistar rats were anaesthetized with urethane and placed in a stereotaxic frame. Two push pull cannulae were stereotaxically introduced in the mid septo-temporal position of the ammon's horn usually just above the mossy fiber zone. The external diameter of the cannulae was 0.6 mm and the flow rate of perfusion was of 10 µl/min (see Charriere et al. 1983 for details about the push pull device). The composition of the artificial cerebro-spinal fluid was (mM): 126.5 NaCl; 2.4 KCl; 1.1 $CaCl_2$; 0.83 $MgCl_2$; 0.5 KH_2PO_4; 0.5 Na_2SO_4; 27.5 $NaHCO_3$; 5.9 Glucose (pH adjusted to 7.4). The photomicrographs in Fig. 2A and B) illustrate the traces made by two cannulae in a typical experiment. We used deionized water and "suprapur" grades (Merck) to minimize trace concentrations of Zn^{2+} and all collecting items (catheter and plastic vials) were left overnight in nitric acid (1%) to remove zinc. The concentration of Zn^{2+} in the samples was determined with an atomic absorption spectrophotometer (Varian AA 1275 with a graphite chamber GIA-95). Following introduction of the push pull cannulae, the perfusing system was left for over 1 h to stabilize, then samples (10 µl per min) were collected for 6 min to determine the spontaneous release of Zn^{2+}. A brief pulse of K^+ (2 min, 30 mM) was then administered and 1 min samples collected for 10–30 min. At the end of the experiment a K^+ pulse (10 min, 30 mM) was applied unilaterally and the animal was immediately perfused intracardially with sulphide followed by paraformaldehyde and brain sections treated with a modified Timm procedure (Sloviter 1983a).

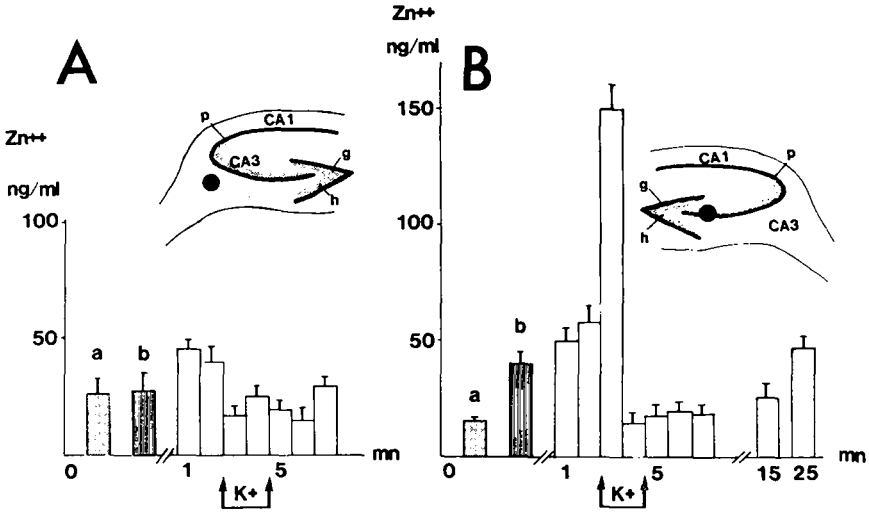

Fig. 1A and B. Spontaneous and evoked release of Zn^{2+} in the mossy fiber zone of the anaesthetized rat. Each column represents the mean Zn^{2+} concentration obtained (± s.e.m.) in successive 1 min samples (10 µl). Each sample was diluted and 3–5 determinations were made. The Zn^{2+} concentrations in the blank (artificial cerebrospinal fluid) are indicated in the dotted columns (a); the hatched columns (b) represents the mean spontaneous release observed in the perfusate (n = 6 samples). The clear columns indicate the Zn^{2+} concentrations in consecutive (1 mn) samples before, during and after the (2 min) potassium pulse. The location of the cannulae as determined by the Timm stained sections (e.g. Fig. 2) is schematically indicated for each case (dark circle). Note that spontaneous and evoked release of Zn^{2+} were observed in **B** (cannulae located in the mossy fiber zone) but not in **A** (cannulae located in the fimbria). Abbreviations: sp = pyramidal layer, g = granular layer, h = hilus

Results

The concentration of Zn^{2+} in control solutions (i.e. artificial cerebro-spinal fluid) was of 27.6 ± 3.3 (ng/ml, mean ± s.e.m.; n = 30). Spontaneous release of Zinc (20 to 50 ng/ml over this blank concentration) was consistently observed in experiments (n = 6) in which the cannulae were located in the vicinity of the mossy fiber zone of the medial part of CA3 (i.e. Fig. 1B, Fig. 2A left side). In contrast, the concentration of Zn^{2+} was not different from the control solutions when the cannulae were located in the fimbria (Fig. 1A; n = 6), the lateral thalamus (n = 3, not shown) or the CA1 field (Fig. 2, right side; n = 2).

When the cannulae were located in the mossy fiber zone (Fig. 1B, Fig. 2 left side), a brief pulse (2 min) of K^+ produced an increase in the Zn^{2+} concentration (Fig. 1B, 174 ± 40.5 mean ± s.e.m., n = 6). There was no evoked release when the cannulae were located in the other regions (Fig. 1A, Fig. 2 right side). Interestingly, the rise in Zn^{2+} concentration was of short duration (1 min) and the return to baseline levels occurred even though K^+ was still being applied (Fig. 1B); often this rise was followed by a signifiant reduction for variable periods (up to 20 min, cf. Fig. 1B). This probably reflects the important uptake of Zn^{2+} which has been described in hippocampal slice preparations (Howell et al. 1984). Also using the Timm stain, we have noted a consistent unilateral decrease in the histologically demonstrable Zn^{2+} when a K^+ pulse (10 min) was applied just before perfusion-fixation (Fig. 2B and C). A similar effect has been recently shown by Sloviter (1984) following repetitive electrical stimulation of the perforant pathway, in conditions which are also associated with neuronal damage (Sloviter 1983b).

In (n = 3) other cases, the cannulae was located in the immediate vicinity of the mossy fiber zone of the dorsal part of CA3 (CA3 a, e.g. Lorente de Nó 1934). The K^+ pulse caused a 2 orders of magnitude rise in Zn^{2+} (up to 3.000 ng/ml in one case). Experiments are currently in progress to confirm these observations with a larger group of experiments. It is interesting to note that there is an overlap of mossy fibers in the CA3-a region (Swanson et al. 1978).

Discussion

In a recent report, Assaf and Chung (1984) have reported a release of Zn^{2+} from hippocampal slices following perfusion with potassium; the concentration of zinc in the perfusate was increased from 17.4 ± 0.9 (ng/ml mean ± s.e.m., blank levels) to 30.7 ± 3.2 when the perfusion medium of the slice was replaced with a solution containing an increased concentration of K^+ (for 10 min); there was no evidence of spontaneous release. The rather small magnitude of this release is expected considering the fact that perhaps only 10% of the total hippocampal zinc is associated with the mossy fibers (Frederickson et al. 1983), the remaining "background" zinc reflect-

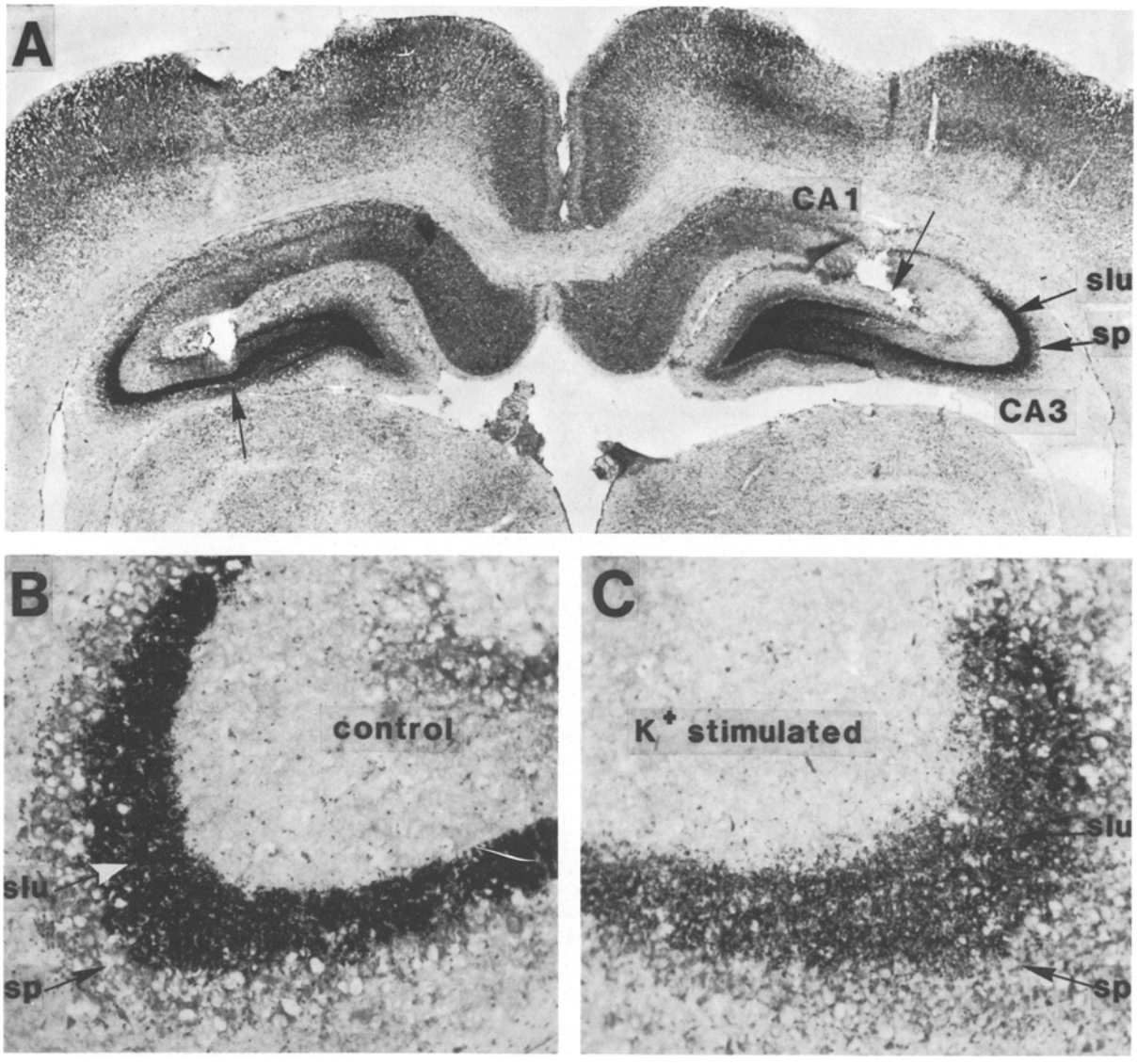

Fig. 2. A Photomicrograph illustrates the location of two cannulae (arrows) in the same experiment. Timm stained sections, note the characteristic dark zone corresponding to the stratum lucidum (slu, i.e. the mossy fiber zone) adjacent to the pyramidal layer (sp) of CA3 (e.g. Lorente de Nó 1934). Spontaneous and evoked release was observed on the left side but not the right one. **B and C** Effects of a K^+ pulse (comin) applied just before fixation to one hippocampus in another experiment. Timm stain, note the reduction in the intensity of the stimulated side

ing the multiple role of zinc in cell biology (Frederickson 1984). In the slice preparation, the whole hippocampus is stimulated and the origin of the zinc stimulated by the K^+ stimulation is not known. Our observations show that the spontaneous and evoked release and probably also the uptake are exquisitely localized. This suggests that zinc is released from the mossy fiber synaptic zone. The high concentrations of Zn^{2+} released during brief activation of the mossy fibers are likely to contribute to the notorious vulnerability of the CA3 pyramidal neurons to seizure events; perhaps by inhibiting the Na-K ATPase (Donaldson et al. 1971).

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