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SHORT COMMUNICATION

Serotonin but not zonisamide inhibits theophylline-induced epileptiform activity in guinea pig hippocampal CA3 neurons☆

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Summary To test the putative serotonin (5-HT)-like effect of zonisamide (ZNS) we employed xanthine-induced epileptiform activity in the hippocampus slice preparation from guinea pigs. In this model Na^+- and T-type Ca^{2+} channel blockers are hardly effective while 5-HT should be inhibitory. Bath application of 5-HT hyperpolarized neurons and abolished theophylline-induced epileptiform activity. In contrast, ZNS failed to alter epileptiform bursting. We conclude that 5-HT augmenting effects of ZNS are missing or are not sufficient to inhibit epileptiform activity in hippocampal slice preparations.

Introduction

Zonisamide (ZNS) is a novel antiepileptic drug (AED) which is effective in animal models and under clinical conditions. The broad-spectrum of possible applications includes the control of partial and generalised seizures especially in patients refractory to other AED (Faught, 2004; Leppik, 2004). Moreover, ZNS has been shown to improve motor function in Parkinson disease (Murata et al., 2007), and to act neuroprotective (Owen et al., 1997; Yoshida et al., 2005). It may also be successfully used as a migraine prophylaxis or to treat neuropathic pain (Pappagallo, 2003). According to this large spectrum of beneficial effects ZNS appears to have multiple target sites within nervous tissue (Leppik, 2004). With respect to ion channels there is evidence for a blockage of voltage-sensitive sodium channels (Schauf, 1987; Rock et al., 1989) and for a reduction of voltage-sensitive T-type calcium currents (Suzuki et al., 1992; Kito et al., 1996).

In this study we focused on the putative effect of ZNS to diminish neuronal excitability by increasing serotonin (5-HT) levels in the hippocampus. 5-HT hyperpolarizes hippocampal neurons via 5-HT_{1A} receptor binding, thus suppressing several types of epileptiform discharges (Salgado and Alkadhi, 1995). However, literature concerning the influence of ZNS on the serotonergic system is conflicting. While Okada et al. (1999) showed a biphasic effect of ZNS on the serotonergic system in a microdialysis study in vivo, and a 5-HT-induced neuronal hyperpolarization followed by an enhancement of seizure threshold, Murata (2004) reported no effect of ZNS

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on 5-HT or its main metabolite 5-hydroxy-indole acetic acid. To address the presumed 5-HT augmenting effect ZNS and to further investigate its pharmacological mechanisms, we compared effects of ZNS and 5-HT on theophylline-treated hippocampal slices. In this system epileptiform activity is elicited by xanthine derivatives which antagonize adenosine receptors and modulate intracellular Ca^{2+} transients via ryanodine receptors (Moraidis and Bingmann, 1994; Margineanu and Klitgaard, 2004). These xanthine-induced epileptiform discharges are sensitive to L-type calcium channel blockers and blockage of ryanoide receptor opening. In contrast, blocking of T-type calcium channels or Na+ channels, both of which appear to be part of ZNSs mode of action, have no significant effect on xanthine-induced epileptiform activity (Margineanu and Klitgaard, 2004).

Based on this distinct profile we hypothesized that ZNS has an inhibitory effect on theophylline-induced activity provided that it amplifies 5-HT effects, whereas no effect was expected if ZNS effects were mainly restricted to Na^+ channels or T-type calcium channels.

Methods

General statements

All experiments described in this study conformed to the specifications of the Animal Research Committee and international guidelines on the ethical use of animals. Effort was made to minimize the number of animals and to avoid suffering.

Tissue preparation

Brains were excised from isoflurane anaesthetized adult guinea pigs (400—600 g) and transverse hippocampal slices (500 ± 100 μm) were cut with a guided razor blade. Slices were pre-incubated in a CO_2/HCO_3^--buffered solution containing (in mM), NaCl (124), KCl (3), $CaCl_2$ (0.75), $MgSO_4$ (1.3), KH_2PO_4 (1.25), $NaHCO_3$ (26) and glucose (10) at 28 °C; pH was adjusted to 7.35—7.40 by gassing with 5% CO_2, 95% O_2. After 1—2 h, slices were transferred from the pre-incubation bath to the recording chamber (volume 4 ml, perfusion rate 6 ml/min) mounted on the stage of an inverted microscope (Zeiss ID 03) for electrophysiological recordings.

Solutions and chemicals

Epileptiform activity was induced with 2 mM theophylline which was added to the superfusate to reach a stable state of hyperexcitation characterized by enhanced bioelectric activity, epileptiform bursts and afterhyperpolarisation (Moraidis and Bingmann, 1994). Alternatively, 4-aminopyridine (4-AP) was used as an epileptogenic drug (Rutecki et al., 1987). Zonisamide and all other chemicals were purchased from Sigma and were dissolved in the solutions immediately before the experiment.

Intracellular recording and measurement of neuronal activity

Intracellular recordings were obtained from hippocampal CA3 neurons with sharp glass mircoelectrodes filled with 2 M potassium methylsulphate (150—180 MΩ) as described (Bingmann and Speckmann, 1986). Electrodes were connected to an amplifier (BA-1S, npi-advanced electronics, Tamm, Germany) using the bridge mode. Input resistance of neurons was calculated from the voltage deflection upon hyperpolarizing current pulses (0.1 nA, 200 ms). Analogue bioelectric signals were converted and recorded digitally using a DAPAS system (Widman and Bingmann, 1996) operating with 12 kHz sampling rate and 10-bit resolution. After attaining a stable intracellular recording, theophylline (2 mM) or 4-AP (50 μM) was applied to constantly increase neuronal excitability. ZNS (final concentration 50 or 100 μM) was added about 20 min later and applied for 20—55 min unless otherwise stated.

Results

Effects of ZNS and 5-HT on theophylline-induced epileptiform activity

Intracellular recordings were obtained from 10 different neurons (10 slices from 7 animals) located 60—230 μm underneath the tissue surface. In all neurons theophylline (2 mM) led to epileptiform bursts which were followed by a pronounced after-hyperpolarization. Theophylline-induced bursting appeared after 10—15 min and became highly regular after 20—30 min. Using a sequential treatment of the same neurons ($n = 7$), we compared the effect of 5-HT (50 μM) and ZNS (50 μM). Membrane potential hyperpolarized upon bath application of 5-HT (50 μM) and epileptiform bursting ceased within 4—6 min after the onset of 5-HT treatment. Hyperpolarization measured at this point in time amounted to 6.9 ± 3.8 mV. The inhibitory effect of 5-HT was reversible upon washout (Figure 1B).

However, none of the neurons susceptible to 5-HT ($n = 7$) responded to 50 μM ZNS applied for 20 min (Figure 1A). Mean rate of APs riding on epileptiform bursts (counted in 10 s intervals) was 21.3 ± 9.6 and 19.9 ± 8.2 ($n = 7$, $P = 0.303$, t-test for paired-samples) before and after ZNS treatment, respectively. Also 100 μM ZNS ($n = 3$) applied for 40—50 min failed to abolish epileptiform bursting or to change the shape of single bursts (Figure 1B). Results were not influenced by the sequence of applications. As is shown in Figure 1C, the same lot of ZNS reversibly reduced frequency of APs on epileptiform bursts elicited by 4-AP (Figure 1C).

Discussion

In the present study 5-HT consistently hyperpolarized theophylline-treated hippocampal CA3 neurons and abolished theophylline-induced epileptiform activity. The hyperpolarizing effect of 5-HT on hippocampal neurons has been demonstrated before and it has been shown that bicuculline or kainite-induced epileptiform activity of hippocampal neurons was reduced by 5-HT. Most likely this happened via 5-HT_{1A} receptors which are present in all subfields of the hippocampus (Salgado and Alkadhi, 1995; Birzniece et al., 2001). Though the effect of 5-HT has not yet been characterized for CA3 neurons of the guinea pig hippocampus, its similarity to rat hippocampus is likely since in all of our experiments neurons treated with 50 μM 5-HT hyperpolarized and stopped burst firing. Surprisingly, the putative 5-HT modulator ZNS, when applied to the same neurons, had no effect in our model. With respect to the integrity of the lot of ZNS used in the present study, we would like to emphasize that there was a strong anti-epileptiform effect of ZNS in the 4-aminopyridine model of guinea pig hippocampal slices (Figure 1C). These experiments serve as positive con-

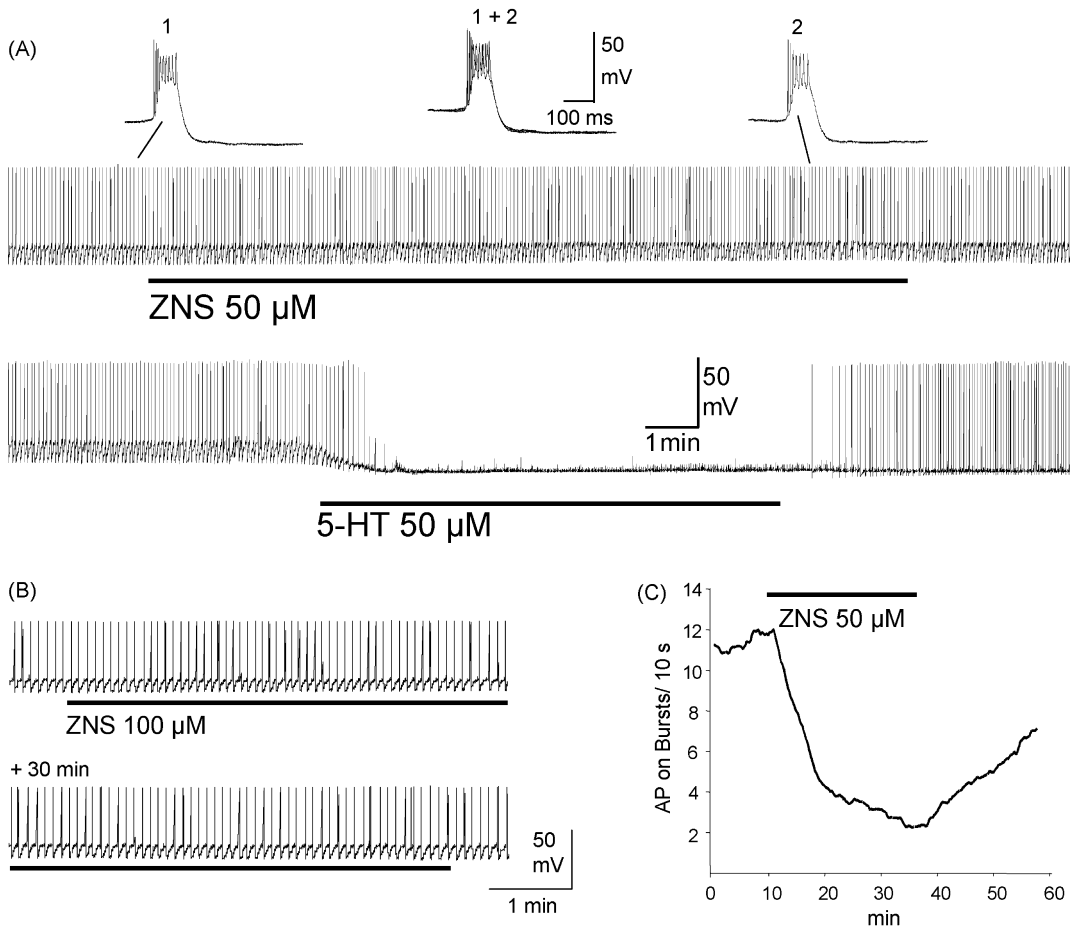

Figure 1 Effect of ZNS and 5-HT on theophylline-induced epileptiform bursting of hippocampal CA3 neurons. (A) Upper recording: ZNS failed to alter the frequency of epileptiform bursts. Single bursts (1 and 2) are shown above at an expanded time scale. Their similarity before and after treatment is shown by superposition (1 + 2). Lower recording: 50 µM 5-HT hyperpolarized the same neuron; epileptiform activity stopped until 5-HT was washed out. (B) Also 100 µm ZNS failed to change epileptiform bursting. (C) ZNS reduced epileptiform burst activity upon 50 µM 4-AP. The example is representative for three other neurons and demonstrates the efficacy of the lot of ZNS used in our study.

trols sufficient to exclude that ZNS was simply inactive. Also the concentration of ZNS (50—100 µM) as well as application periods should have been sufficient as they match the conditions of other studies (Okada et al., 1999). Therefore, our experiments do not support the notion that ZNS acts as an antiepileptic drug via increased levels of 5-HT. As Okada et al. (1999) found elevated 5-HT levels upon ZNS in an in vivo microdialysis study, our deviating results may be explained by the use of brain slices. We cannot exclude that 5-HT levels were moderately increased by ZNS. It may also be possible that 5-HT — in case that it was increased within the superficial tissue layer — was washed out by the superfusate. In any case, however, changes in the concentration of 5-HT were too small to influence burst firing.

The missing effect of ZNS on xanthine-induced epileptiform activity may help to indirectly characterize ZNSs mode of action. For example, possible effects of ZNS on $GABA_A$ receptor currents, adenosine receptors or L-type calcium channels, all of which are known to impair xanthine-induced epilepsy (Moraidis and Bingmann, 1994; Margineanu and Klitgaard, 2004), do not seem to play a major role in the antiepileptic action of ZNS on hippocampal CA3 neurons (Rock et al., 1989; Suzuki et al., 1992; Murata, 2004). On the other hand, voltage dependent Na^+ or T-type calcium channels are among the possible targets of ZNS (Rock et al., 1989; Suzuki et al., 1992) but xanthine-induced hyperactivity is hardly influenced by carbamazepin, whose antiepileptic activity primarily reflects sodium channel blockage (Moshé, 2000), or T-type calcium channel inhibition (Margineanu and Klitgaard, 2004). This leaves the possibility that ZNS preferentially acts via Na^+ channels and T-type calcium channels which has no effect in the xanthine-treated hippocampus model.

Recent findings also suggests an interaction of ZNS with intracellular calcium stores. Based on the work of Yoshida et al. (2005) we speculate that ZNS and xanthines may interact at the level of ryanodine receptors. We assume that excitation of these receptors, e.g. by theophilline or caffeine, releases Ca^{2+} from intracellular stores leading, e.g. to an insensitivity for effects of ZNS. Further studies on genetically engineered ryanodine receptors should be conducted to shed more light on the interaction of ZNS and xanthines.

In conclusion, despite its numerous modes of action clinically relevant concentrations of ZNS failed to inhibit

theophylline-induced epileptiform activity. As 5-HT was effective in the same model ZNS has no apparent 5-HT like effects in xanthine-treated CA3 neurons of guinea pigs.

Conflict of interest

Authors state that there exist no financial or other conflicts of interest (e.g. ownership, equity position, stock options, consulting fees, patent rights and corporate affiliations) related to the submitted manuscript.

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