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Short communication

ucb L059, a novel anticonvulsant, reduces bicuculline-induced hyperexcitability in rat hippocampal CA3 in vivo

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Abstract

Local diffusion of bicuculline from a bicuculline-containing recording micropipette increased the orthodromic field population spike (PS_2), elicited upon commissural stimulation in the hippocampal CA3 region of anaesthetized rats. This increase in PS_2 was consistently reduced by 5.4 mg/kg of the novel anticonvulsant drug ucb L059 ((*S*)-α-ethyl-2-oxo-pyrrolidine acetamide), when injected i.v. 10 min prior to lowering in place the bicuculline-containing micropipette. ucb L059 had no effect on PS_2 in the absence of bicuculline. Paired-pulse stimulation produced marked inhibition of the second PS_2 at low interstimulus interval, an effect which was significantly reduced by bicuculline. Although ucb L059 reduced the effect of bicuculline on both PS_2s elicited by paired stimulations, the drug did not alter the reduction by bicuculline of paired-pulse inhibition at low interstimulus interval. These results suggest that ucb L059 prevents increases in CA3 neuronal excitability by bicuculline through a non γ-aminobutyric acid-ergic mechanism.

Keywords: ucb L059; Bicuculline; Hippocampal CA3, rat; Input-output curve; Paired-pulse inhibition

1. Introduction

Hippocampal area CA3 has an extensive local circuitry, involving both recurrent excitation and inhibition, which makes this area particularly prone to the generation of epileptiform bursts (Wong and Traub, 1983; Scharfman, 1994). This area is therefore of particular interest when evaluating the effects of potential antiepileptic drugs. Commissural stimulation elicits in hippocampal CA3 a field potential consisting of two population spikes (PS): a shorter latency antidromic PS_1 and a longer latency orthodromic PS_2 (Andersen, 1975). Increasing the intensity of the stimulating current (I_{stim}) recruits more neurons via non-synaptic activation and produces a saturating increase in the PS_1 amplitude, whereas the orthodromic PS_2 decreases when I_{stim} is raised above 0.5 mA. This biphasic shape of the stimulus-PS_2 response curve suggests that increasing stimulus strength progressively activates inhibitory circuits in CA3 (Margineanu and Wülfert, 1995).

ucb L059 ((*S*)-α-ethyl-2-oxo-pyrrolidine acetamide) is a new potential antiepileptic drug, with a high therapeutic index, which has been shown to exert anticonvulsant effects in various models of generalized seizures in rats and mice (Gower et al., 1992; Löscher and Hönack, 1993). A binding site for this drug has been identified in the central nervous system (Gillard et al., 1994), but its mode of action is still unclear. The purpose of the present investigation was to explore whether ucb L059 exerts its anticonvulsant effects via an interaction with γ-aminobutyric acid-ergic (GABA-ergic) mechanisms. Here we report data showing that ucb L059 prevented the increase in excitability of rat hippocampal CA3 pyramidal neurons in vivo, produced by local application of bicuculline. Furthermore, the drug had no effect per se on excitability and did not influence the paired-pulse inhibition occurring at short interstimulus intervals, which is thought to be mediated via $GABA_A$ receptors (Joy and Albertson, 1992). These results suggest that ucb L059 reduces bicuculline-induced increases of neuronal excitability in CA3 via non GABAergic mechanisms.

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2. Materials and methods

2.1. Animals and surgery

Adult male Sprague-Dawley rats were used, which were kept on a 12 h light:12 h dark cycle, with free access to water and to a standard cube diet. Animals, of 300–350 g weight, were anaesthetized with 1.6 g/kg i.p. urethane, and a catheter was introduced in the left jugular vein, for subsequent i.v. injections. Procaine was injected at the incision site and at the stereotaxic pressure points.

2.2. Experimental procedures

The response of pyramidal neurons in the CA3 region of the hippocampus (stereotaxic coordinates: antero-posterior (AP) −3.4 mm from bregma, lateral (L) 3.2 mm from the midline, dorso-ventral (DV) about −3.2 mm from brain surface) was elicited upon stimulation in the contralateral fimbria (coordinates: AP −1.7 mm from bregma, L 1 mm from the midline and DV −3.3 mm from brain surface). The extracellularly recording electrodes were glass micropipettes filled with either 0.5 M NaCl, or 8 mM bicuculline in 0.5 M NaCl (input impedance about 2 MΩ). The bipolar stimulating electrode was a twisted 0.2 mm diameter Pt-Ir wire. Monophasic, rectangular stimuli, of 0.2 ms duration, generated by a Grass S88 stimulator, were given via a PSIU6 constant current unit. The placement of stimulation and recording electrodes was histologically checked in preliminary pilot experiments. The position of the standard (NaCl-filled) recording micropipette was finely adjusted to obtain the highly characteristic response of CA3 neurons (Margineanu and Wülfert, 1995), most frequently elicited by $I_{stim} = 2$ mA. The onset of the experiment, i.e. zero time, was considered when a characteristic response was obtained. In the stimulus-response experiments reported in Fig. 1, the values of I_{stim}: 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 mA, were successively increased at 4 min intervals (4 to 40 min from onset) and the responses (averages of three successive samples, at 20 s interval) were recorded with the NaCl-filled micropipette. This complete stimulus-response curve served for strictly monitoring placement of the recording electrode and also as a pre-treatment control. After recording at $I_{stim} = 6$ mA, ucb L059 or saline was injected i.v. (at 41 min from onset of the experiment) and the NaCl-filled micropipette replaced with either a bicuculline-containing recording micropipette (in the two groups receiving bicuculline), or with another NaCl-filled micropipette (in the two control groups). Within the 0.01 mm accuracy limit of the stereotaxic instrument (Kopf 1760), the tip of the second micropipette was lowered at the same dorso-ventral depth

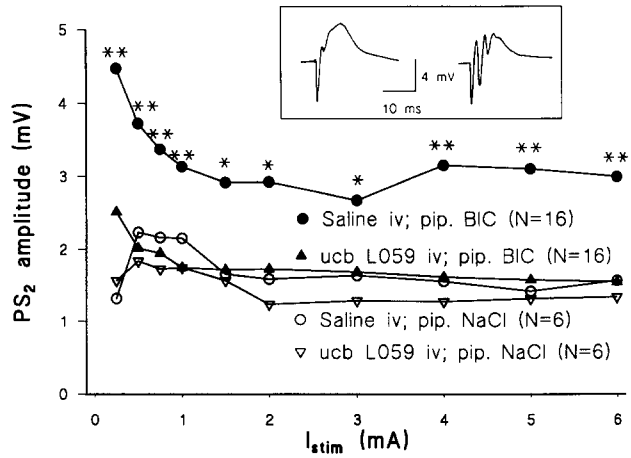

Fig. 1. Effect of intravenous administration of ucb L059 and of local administration of bicuculline on the amplitude of the orthodromic population spike (PS_2) elicited in rat hippocampal CA3 region upon commissural stimulation with increasing intensity (I_{stim}). 25 min after an i.v. injection of either 5.4 mg/kg ucb L059, or saline, the extracellular evoked responses were recorded with either micropipettes containing 8 mM bicuculline in 0.5 M NaCl (pip. BIC), or with standard 0.5 M NaCl-filled micropipettes (pip. NaCl). Symbols indicate mean values for the number of rats given in parentheses. Significant differences (two-tailed t-test) between the two groups recorded with pip. BIC are indicated as: $* P < 0.05$ and $** P < 0.005$. A pre-treatment control recording, not shown on the graph, indicated no difference between groups. The inset shows representative field potentials recorded in hippocampal CA3 region with pip. NaCl (left) and pip. BIC (right). Stimulus artifact was deleted.

as the first one, while antero-posterior and lateral coordinates were not changed. A complete stimulus-response curve was recorded again between 65 and 101 min from onset of the experiment, at least 10 min after final positioning of the second micropipette, to allow bicuculline responses to stabilize (Steward et al., 1990). In the paired-pulse experiments reported in Fig. 2, the paired stimuli were identical, with amplitudes eliciting the standard response, but separated by increasing interstimulus intervals. After obtaining the standard CA3 response as above (time 0 of the experiment), the responses to paired pulses with increasing interstimulus intervals (20, 30, 40, 50, 60, 80, 100 and 150 ms) were recorded (averages of three successive samples, at 30 s interval) with the NaCl-filled micropipette. The interstimulus interval was successively increased every 4 min (4 to 32 min from onset of the experiment). After this control recording, the i.v. injection (at 33 min from onset) and the micropipette replacement (at 45 min from onset) were done as in the stimulus-response experiments. The responses to the whole series of paired pulses with increasing interstimulus intervals were again recorded (58 to 86 min from onset) with either bicuculline-containing, or NaCl-filled micropipettes.

2.3. Data analysis

The acquisition and the on-line processing of the evoked field potentials were performed via an internally developed software which plots the averaged signal (averages of three successive samples) and returns the amplitudes and latencies of PS_1, PS_2 and, if existing, of a third PS. Mean values and stardard deviations of each parameter were obtained for groups of minimum 6 rats and statistical significance of differences was assessed using *t*-tests.

2.4. Drugs

ucb L059 (UCB, Braine-l'Alleud, Belgium) was dissolved in saline and injected i.v. at an injection volume of 1 ml/kg. (+)-Bicuculline (Fluka Chemie, Buchs, Switzerland) was locally delivered into CA3 region by simple diffusion from the tip of the recording micropipette, as described by Steward et al. (1990). The micropipettes contained 8 mM bicuculline, dissolved (with 5% acetic acid) in 0.5 M NaCl.

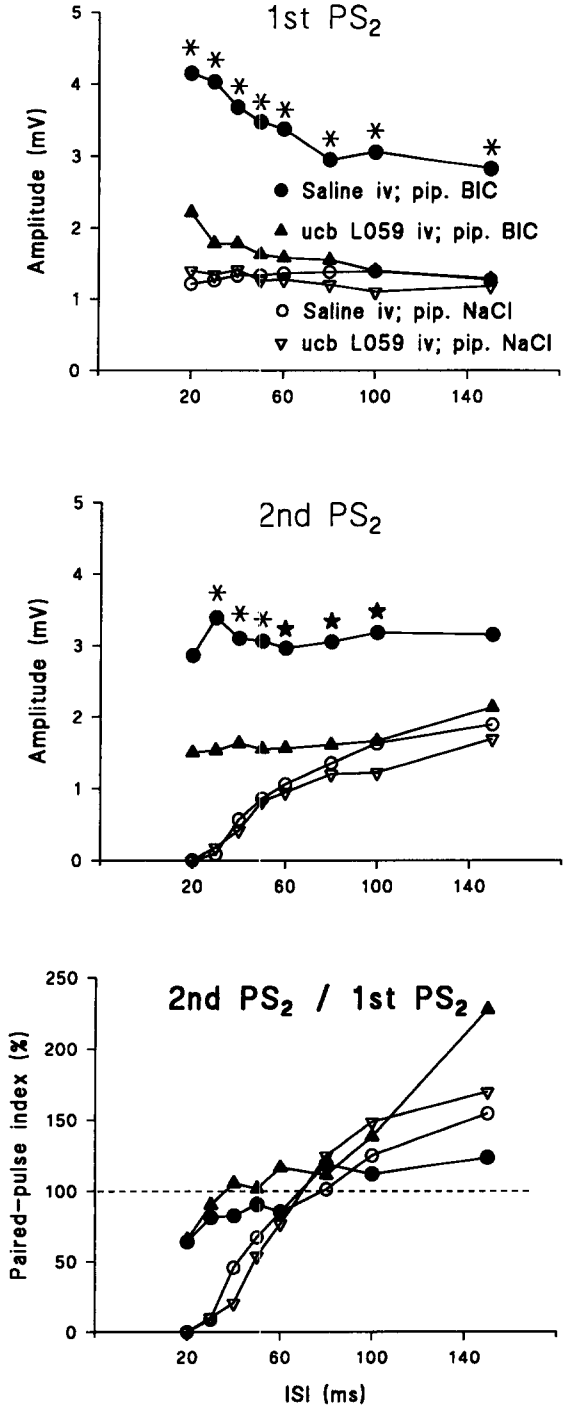

Fig. 2. Effect of intravenous administration of ucb L059 and of local administration of bicuculline on the paired-pulse inhibition of orthodromic population spikes (PS_2s), evoked in rat hippocampal CA3 region upon commissural stimulation with paired-pulses of increasing interstimulus intervals. The amplitude of PS_2 responses to the 1st (upper graph) and 2nd (middle graph) stimulus in the pair and their ratio (lower graph) are expressed as functions of interstimulus interval (ISI). 25 min after an i.v. injection of either ucb L059 5.4 mg/kg, or saline, the extracellular responses were recorded either with 0.5 M NaCl-filled (pip. NaCl), or with micropipettes containing 8 mM bicuculline in 0.5 M NaCl (pip. BIC). Symbols indicate mean values for 8 rats in each group. Significant ($P < 0.05$) differences between the two groups recorded with pip. BIC are indicated as: * – two-tailed *t*-test and ⋆ – one-tailed *t*-test. A pre-treatment control recording, not shown on the graph, indicated no difference between groups.

3. Results

3.1. Effects on stimulus-response dependence

The response of CA3 pyramidal cells to commissural stimulation was consistently increased when recorded with bicuculline-containing micropipettes compared to the control group, recorded with conventional 0.5 M NaCl-filled micropipettes (Fig. 1), and also when compared to pre-bicuculline control values in the same animals (not shown on the graph). As a global measure of the excitability of hippocampal neurons, the area under the curve (AUC) was calculated for the PS_2 amplitude as a function of I_{stim} and expressed in mV × mA. The variability of individual responses in the presence of bicuculline was large, with an AUC ranging from 9.7 to 29.3 ($n = 16$), vs. 4.5 to 10.6 when compared to controls. The CA3 response to commissural stimulation consisted of multiple population spikes (see inset Fig. 1) when recorded with bicuculline-containing micropipettes, similar to what has been reported for the dentate gyrus (Steward et al., 1990). Administration of 5.4 mg (i.e. 32 μmol)/kg i.v. ucb L059 prevented bicuculline-induced increase in PS_2 (Fig. 1), with an AUC ranging from 3.3 to 18.2 ($n = 16$). ucb

L059 had no effect on orthodromic CA3 response to commissural stimulation in the absence of bicuculline (Fig. 1).

3.2. Effects on paired-pulse inhibition

Paired-pulse stimulation of the commissural pathway produced either inhibition or facilitation of the response to the second stimulus in the pair, depending on the interstimulus interval. In the control group, the second orthodromic PS_2 was completely abolished at 20 ms interstimulus interval, but reappeared at increasing interstimulus interval and even became significantly higher than the first PS_2 at 150 ms (Fig. 2). Accordingly, the paired-pulse index, defined as the ratio of the two PS_2s elicited by the paired stimuli, increased from 0 to approximately 150%, at an interstimulus interval of 150 ms. Intravenous administration of 5.4 mg/kg ucb L059 neither affected paired-pulse inhibition nor facilitation.

When the response to paired pulses was recorded with bicuculline-containing micropipettes, both the 1st (Fig. 2, upper part) and the 2nd (Fig. 2, middle part) stimulus elicited significantly ($P < 0.05$) higher PS_2s, and paired-pulse inhibition at interstimulus interval less than 30 ms was significantly ($P < 0.001$) reduced (Fig. 2, lower part). Intravenous administration of 5.4 mg/kg ucb L059, prior to positioning the bicuculline-containing micropipette, decreased the amplitude of the orthodromic responses elicited by both stimuli in the pair (Fig. 2, upper and middle parts), when recording with bicuculline-containing micropipettes. The extent of paired-pulse inhibition at low interstimulus interval was, however, not different from inhibition in the group receiving saline prior to positioning the bicuculline-containing micropipette (Fig. 2, lower part), demonstrating that the drug did not alter paired-pulse inhibition, neither in the presence nor in the absence of bicuculline. Paired-pulse facilitation observed at an interstimulus interval of 150 ms tended to increase following administration of ucb L059.

4. Discussion

Bicuculline has been used repeatedly as an antagonist of $GABA_A$ receptors, to study electrophysiological processes associated with epileptiform activity in the hippocampus (Sloviter, 1991) and we have recently described its effect on CA3 response to commissural stimulation (Margineanu and Wülfert, 1995). Although both i.p. and i.v. administration of bicuculline increases the response to electrical stimulation in local areas of the brain, the short half-life of the drug (Olsen et al., 1975) makes the interpretation of data problematic. Using recording micropipettes containing bicuculline allows local administration of the drug through diffusion and hence the study of in situ effects of the $GABA_A$ receptor antagonist (Steward et al., 1990). Whether such effects are caused only by inhibition of $GABA_A$ receptors is, however, not clear. It was suggested years ago (Heyer et al., 1981) that bicuculline may exert part of its convulsant effects via mechanisms other than $GABA_A$ receptor blockade. More recently, the calcium antagonist verapamil was reported to suppress the paroxysmal depolarization shifts induced by bicuculline in CA3 neurons of hippocampal slices, while having no effect on postsynaptic potentials (Straub et al., 1990). This finding would imply that at least a part of the epileptogenic action of bicuculline could be due to increases in membrane calcium conductance, independently of $GABA_A$ receptor blockade. In the same vein, bicuculline has also been reported to enhance the stimulus-dependent rise in intracellular calcium in CA1 hippocampal neurons, without changing baseline calcium levels (Van der Linden et al., 1993). The inhibition of bicuculline-induced increases in PS_2 by ucb L059, shown in Fig. 1, could therefore have been caused by facilitation of GABAergic mechanisms and/or by inhibition of bicuculline-induced increases in calcium conductance of pyramidal neuronal membranes.

The interstimulus interval dependence of paired-pulse inhibition of the orthodromic PS_2, elicited in the CA3 region by commissural stimulation (Fig. 2), is in good agreement with studies in the dentate gyrus and in area CA1, both in vivo (Joy and Albertson, 1992; Sloviter, 1991) and in vitro (Leung and Fu, 1994). The reduction of low-interstimulus interval paired-pulse inhibition by bicuculline, observed in our study, is consistent with the hypothesis that paired-pulse inhibition is caused by the release of GABA from interneurons activated by the first stimulus and that bicuculline inhibits paired-pulse inhibition via blockade of postsynaptic $GABA_A$ receptors. Our finding that ucb L059 did neither affect paired-pulse inhibition nor the reduction of paired-pulse inhibition by bicuculline argues against facilitation of $GABA_A$ receptor-mediated mechanisms as an explanation of the anticonvulsant effect of the drug and suggests that ucb L059 may inhibit some depolarizing ion currents activated directly or indirectly by bicuculline. Further studies are warranted to determine the depolarizing current inhibited by ucb L059.

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