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Inhibition by levetiracetam of a non-$GABA_A$ receptor-associated epileptiform effect of bicuculline in rat hippocampus

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1 Extracellular recording of field potentials, evoked by commissural stimulation in hippocampal area CA3 of anaesthetized rats, was performed in order to study the mode of action of the novel antiepileptic drug levetiracetam (ucb LO59).

2 The amplitude of orthodromic field population spike (PS_2) markedly increased and repetitive population spikes appeared when the recording micropipette contained either bicuculline methiodide (BMI), or the specific $GABA_A$ antagonist gabazine (SR-95531).

3 BMI-induced increases in PS_2 were reduced in a dose-dependent manner by 1 to 320 μmol kg^{-1} levetiracetam i.v., with a U-shape dose-response relationship. However, levetiracetam did not reduce the increases in PS_2 produced by gabazine.

4 Clonazepam (1 mg kg^{-1}, i.p.), carbamazepine (20 mg kg^{-1}, i.p.) and valproate (200 mg kg^{-1}, i.v.) were ineffective in preventing BMI-induced increases in PS_2, while the calcium channel antagonist flunarizine, 50 μmol kg^{-1}, i.p., reduced PS_2 increments caused by BMI. The L-type calcium channel blocker nifedipine, 100 μmol kg^{-1}, i.p., was without effect. Similar to levetiracetam, flunarizine did not reduce the increases in PS_2 induced by gabazine.

5 These data suggest that the increased excitability of CA3 neurones, caused by BMI administered *in situ*, involves calcium-dependent processes not associated with blockade of $GABA_A$ receptors. The inhibition by levetiracetam of this calcium-dependent effect of BMI might contribute to the antiepileptic effects of the drug.

Keywords: Hippocampal CA3; extracellular recordings; epilepsy; bicuculline; gabazine (SR-95531); levetiracetam (ucb L059); flunarizine

Introduction

Bicuculline is a commonly used convulsant, generally thought to cause seizures via reduction of inhibitory neurotransmission through blockade of γ-aminobutyric $acid_A$ ($GABA_A$) receptors. Decreased inhibition of neuronal pathways causes an increase in the number of neurones firing simultaneously and hence hypersynchronous discharges of a large population of neurones, similar to that which can be observed in epilepsy. Antiepileptic drugs which prevent bicuculline-induced seizures are, therefore, thought to either facilitate GABA-ergic neurotransmission, or to inhibit the increases in depolarizing currents that are caused by blockade of GABA receptors. However, impaired GABA-ergic neurotransmission may not be the only cause of bicuculline-induced seizures. A non-synaptic activation by bicuculline of neuronal excitability has been suggested by several groups (Heyer *et al*., 1982; Aicardi & Schwartzkroin, 1990; Straub *et al*., 1990) and it was acknowledged that the elementary mechanisms of the epileptogenic action of bicuculline are not yet clearly understood (Mußhoff *et al*., 1994).

We have recently shown that the amplitude of the orthodromic population spike (PS_2), elicited in rat hippocampal CA3 area upon electrical stimulation of the fimbria, increased and that paired pulse inhibition (PPI) obtained at low interstimulus intervals was depressed, when the recording microelectrode contained bicuculline (Margineanu & Wülfert, 1995a). We further demonstrated that i.v. administration of a novel atypical antiepileptic drug, levetiracetam (ucb L059), prevented this increase in PS_2 without altering the decrease in PPI. This suggests that bicuculline increases neuronal excitability, in the highly seizure-prone (Scharfman, 1994) CA3 area, through a mechanism more complex than $GABA_A$ receptor blockade alone, which is thought to be the cause of the depression of PPI (Joy & Albertson, 1992). The aim of the present study was to characterize the neuronal hyperexcitability which is caused by bicuculline methiodide (BMI) in hippocampal area CA3, in order to ascertain the antiepileptic mode of action of levetiracetam. More specifically, we addressed the question as to whether BMI-induced hyperexcitability involves a calcium-dependent component not associated with $GABA_A$ receptor blockade and, finally, how levetiracetam might interfere with the activation of such processes. Some of the results presented here have been communicated in abstract form (Margineanu & Wülfert, 1995b).

Methods

The experimental procedure of recording in anaesthetized rats the field potentials evoked in hippocampal CA3 area by commissural stimulation (Margineanu & Wülfert, 1995c) and the protocol used when recording with drug-containing microelectrodes (Margineanu & Wülfert, 1995a; Wülfert & Margineanu, 1996) have been described recently in detail.

Animals and surgery

Adult male Sprague-Dawley rats weighing 300 to 350 g were used; they were kept on a 12 h light : 12 h dark cycle, with free access to water and to a standard cube diet. They were anaesthetized with 1.6 g kg^{-1} urethane i.p., and procaine was injected at the incision site and at the stereotaxic pressure points. The i.v. injections were via a catheter introduced in the left jugular vein.

Electrical stimulation and recording

Field potentials were recorded extracellularly in the CA3 region of the hippocampus with glass microelectrodes introduced at coordinates: antero-posterior (AP) = −3.4 mm from bregma, lateral (L) = 3.2 mm from the midline, dorso-ventral (DV) about −3.3 mm from brain surface. The recording microelec-

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trodes were horizontally pulled borosilicate glass micropipettes, capillary-filled with 0.5 M NaCl and broken back to a tip diameter of about 10 μm. These microelectrodes will be subsequently termed 'pip.NaCl'. Commissural stimulation, with monophasic rectangular pulses delivered by a Grass S88 stimulator, was produced with a twisted Pt-Ir wire (0.2 mm diameter) bipolar stimulating electrode, placed in the contralateral fimbria at coordinates: AP = −1.7 mm from bregma, L = 1 mm from the midline and DV about −3.3 mm from brain surface. The characteristic field potential, elicited in CA3 area by commissural stimulation, is known (Andersen, 1975) to consist of two population spikes (PS): a shorter (<2 ms) latency antidromic PS_1 and a longer (>3 ms) latency orthodromic PS_2 (see examples of responses in Figure 1). Stimulus-response (S-R) curves were established by varying the stimulus-strength (I_{stim}) and charting the amplitudes of population spikes. The S-R curve (I_{stim} ranging between 0.25 and 6 mA) recorded with the pip.NaCl provided the pretreatment control for each animal. After i.v. or i.p. drug injection, pip.NaCl was replaced with a recording micropipette containing either BMI, 8 mM in 0.5 M NaCl, or the selective $GABA_A$ antagonist gabazine (SR-95531), 5 mM in 0.5 M NaCl. These microelectrodes will be subsequently termed 'pip.BMI' and 'pip.GBZ', respectively. The input impedance of all microelectrodes was around 2 MΩ. The tip of the drug-containing micropipette was positioned at the same coordinates as that of the pip.NaCl, within the 0.01 mm accuracy limit of the Kopf 1760 stereotaxic instrument, for the DV depth and with no change in AP and L positions. When sham replacing pip.NaCl in 12 control animals, both PS_1 and PS_2 recorded with the second micropipette were within the variability range of the control recording with the first micropipette. Fifteen minutes after the positioning of pip.BMI or pip.GBZ, a S-R curve was recorded with the same I_{stim} values and timing as the control. To verify the persistence of the effects, a S-R curve was again recorded, 40 min later, with the same I_{stim} values given in random succession.

Data analysis

The acquisition software averaged on-line three successive samples of evoked field potential and calculated the amplitudes and latencies of population spikes. The amplitude of each PS was measured from the negative peak to a tangent drawn between the preceding and the following maxima of the waveform. The response of each animal was consistently expressed by the average of the population spikes elicited with stimuli of 1, 1.5, 2 and 3 mA. Mean values and standard deviations of each parameter were obtained for groups of at least 6 rats and statistical significance of differences was assessed by use of *t* tests. Statistical analysis of the data presented in Figure 2 was performed by ANOVA, with Dunnett's test for multiple comparisons.

Drugs

BMI (Sigma, St. Louis, MO) and gabazine (RBI, Natick, MA) were dissolved in 0.5 M NaCl and introduced in the recording microelectrodes, levetiracetam (UCB s.a., Braine-l'Alleud, Belgium) and valproate (Sigma) were dissolved in saline and injected i.v., clonazepam (Hoffmann-La Roche), flunarizine, nifedipine and carbamazepine (Sigma) were suspended with 5% (w/v) arabic gum in saline and injected i.p. Injection volumes were 1 ml kg^{-1} i.v. and 5 ml kg^{-1} i.p. All injections, both i.v. and i.p. were made 10 min before the positioning of the pip.BMI or the pip.GBZ.

Results

Hyperexcitability induced by BMI and gabazine in situ

A single commissural stimulation caused both an increase in PS_2 and multiple population spikes when the recording microelectrode contained either BMI or gabazine. These effects were observed in the absence of any concomitant increase in the antidromic PS_1 (see traces in Figure 1). Pilot experiments indicated that increasing concentrations of BMI in the recording micropipette dose-dependently increased PS_2, reaching a plateau at 8 mM. Accordingly, a concentration of 8 mM BMI was used for further experiments. A concentration of 5 mM gabazine was selected on the basis of similar experiments. The graphs in Figure 1 show that *in situ* diffusion of both BMI and gabazine produced significant increases in the PS_2 amplitude (Figure 1b) and also caused sizeable repetitive spikes PS_3 (Figure 1c). The effects of BMI and gabazine were comparable, although BMI tended to produce less effects with time. Recordings carried out at 40 min intervals with pip.BMI showed that both PS_2 and PS_3 decreased ($P<0.05$, two-tailed *t* test), although PS_2 remained significantly above control. However, no noticeable decrease of either PS_2, or PS_3 was observed in the second recording with pip.GBZ (40 min later).

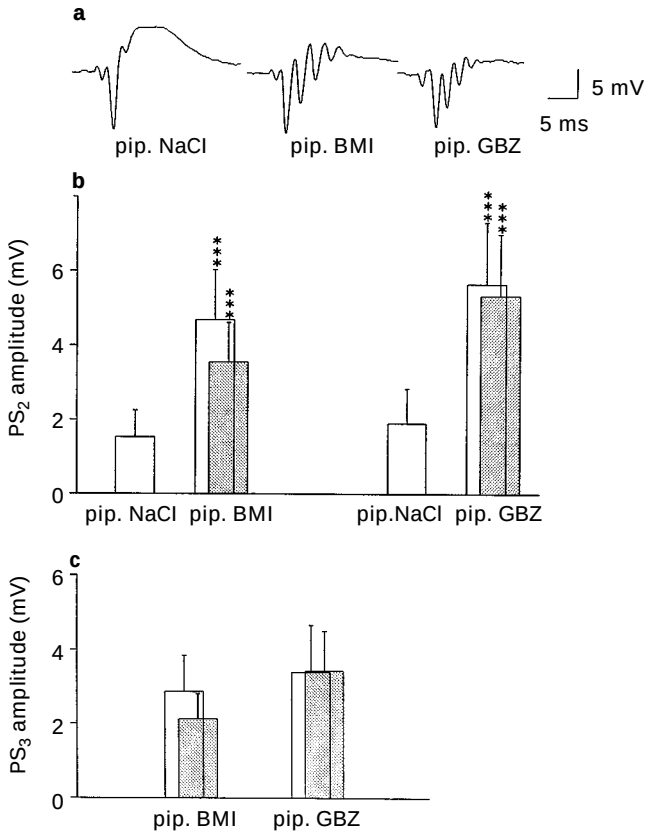

Figure 1 Effect of bicuculline methiodide (BMI) and of gabazine (GBZ) on the field potentials evoked in rat hippocampal CA3 region upon commissural stimulation. (a) Representative field potentials (averages of 3 subsequent samples), recorded either with a standard microelectrode (pip.NaCl; trace at left), or with a BMI (8 mM)-containing microelectrode (pip.BMI; trace at middle), in the same animal, and with a gabazine (5 mM)-containing microelectrode (pip.GBZ; trace at right), in another animal. The responses were evoked by stimuli of equal strengths (I_{stim}) and the calibration bars apply to the three records. The first downward peak, immediately following the stimulation artifact, is in each case the antidromic population spike and the following peak is the orthodromic population spike (PS_2). Repetitive orthodromic peaks (PS_3 and subsequent) appeared only in the presence of BMI or gabazine. (b) and (c) Average amplitudes (mean ± s.d.) of PS_2 (b) and PS_3 (c) in a group of 18 rats, initially recorded with pip.NaCl, then twice with pip.BMI, and in a group of 14 rats, initially recorded with pip.NaCl, then twice with pip.GBZ. The second recordings with pip.BMI or pip.GBZ, indicated by stippled columns, were performed after 40 min. Significant differences between the two successive recordings with either pip.BMI or pip.GBZ, with respect to control recordings with pip.NaCl in the same animals, are indicated by $***P<0.0005$, two-tailed *t* test.

Anti-BMI effects of levetiracetam and flunarizine

To assess an anti-BMI effect of levetiracetam, groups of rats were administered 1 to 320 μmol kg^{-1} levetiracetam i.v., according to previous studies in animal models (Gower *et al.*, 1992; 1995; Löscher & Hönack, 1993). Intravenous administration of levetiracetam, 10 min before the positioning of the BMI-containing microelectrode, dose-dependently reduced both the increase in PS_2 by BMI, and the amplitude of the third orthodromic spike (PS_3), which was only observed in the presence of BMI, without modifying the antidromic spike PS_1. The data in Figure 2a show a U-shaped dose-response relationship for levetiracetam. The amplitude of repeated spikes (PS_3) caused by BMI was also inhibited by levetiracetam, with a similar U-shaped dose-dependency (data not shown). The anti-BMI effect of levetiracetam on both parameters remained significant and kept the same dose-dependency, when field potentials were evoked 40 min later in the same animals, with the pip.BMI left in place. Levetiracetam administration did not affect excitability in the absence of BMI, even at 320 μmol kg^{-1}, i.v. ($P<0.83$, two-tailed t test; $n=6$ rats). In contrast to its anti-BMI effect, levetiracetam did not reduce either the increase in PS_2 (Figure 2b), or the amplitude of the repeated orthodromic spike (PS_3), caused by gabazine in hippocampal area CA3.

In order to assess further the pharmacological responsiveness of BMI-induced epileptiform activity in rat hippocampal CA3 area, groups of rats were administered either clonazepam, valproate and carbamazepine, or the calcium antagonists flunarizine and nifedipine, before the recording with pip.BMI. Paired groups of rats were administered vehicle in each case, for comparison, followed by recording with pip.BMI.

Clonazepam, 1 mg kg^{-1}, i.p., caused no reduction of BMI-induced increases in PS_2 ($P<0.96$; $n=6$ rats/group), and valproate, 200 mg kg^{-1}, i.v. ($n=8$ rats/group) and carbamazepine, 20 mg kg^{-1}, i.p. ($n=6$ rats/group) presented only minor, non-significant anti-BMI effects ($P<0.62$ and $P<0.41$, respectively).

In contrast, 50 μmol kg^{-1} flunarizine, i.p., consistently reduced the hyperexcitability caused by BMI *in situ* (Figure 3), while the L-type Ca^{2+} channel blocker nifedipine, 100 μmol kg^{-1}, i.p., had no significant effect ($P<0.61$; $n=6$ rats/group). However, flunarizine did not reduce either the increase in PS_2 (Figure 3), or the amplitude of PS_3 caused by gabazine.

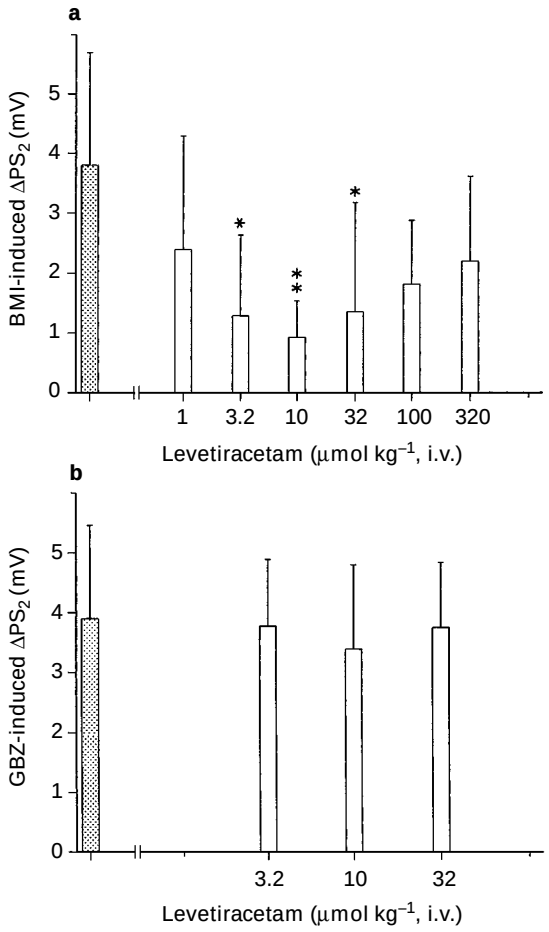

Figure 2 Dose-dependence of the effect of levetiracetam on the increases in amplitude of the field orthodromic population spike (PS_2), induced by (a) bicuculline methiodide (BMI) and by (b) gabazine (GBZ) in the recording microelectrodes. The columns (mean$\pm$s.d.) represent the increases (ΔPS_2) with respect to the amplitudes recorded in the same animal with standard (NaCl-filled) microelectrodes. (a) Groups of 6 rats received an i.v. injection of either saline (stippled column) or a dose of levetiracetam between 1 and 320 μmol kg^{-1}, followed by the recording with BMI (8 mM)-containing microelectrodes in all groups. (b) Groups of 7 rats received an i.v. injection of either saline (stippled column) or a dose of levetiracetam between 3.2 and 32 μmol kg^{-1}, followed by the recording with gabazine (5 mM)-containing microelectrodes in all groups. Significant differences with respect to control group in (a), assessed by ANOVA with Dunnett's test for multiple comparisons, are indicated by $*P<0.05$ and $**P<0.01$.

Discussion

The main finding of the present study is that the two prototypic $GABA_A$ receptor antagonists BMI and gabazine produce apparently similar epileptiform responses in rat hippocampus *in vivo*, but which differ profoundly in their response to pharmacological agents.

From a methodological point of view, it should be observed that both BMI and gabazine are hydrosoluble molecules, not suitable for systemic administration, as they hardly cross the blood-brain barrier (Wermuth & Bizière, 1986). Accordingly, the inclusion of these drugs in the recording micropipettes provides a useful way of studying their effects on neurones *in*

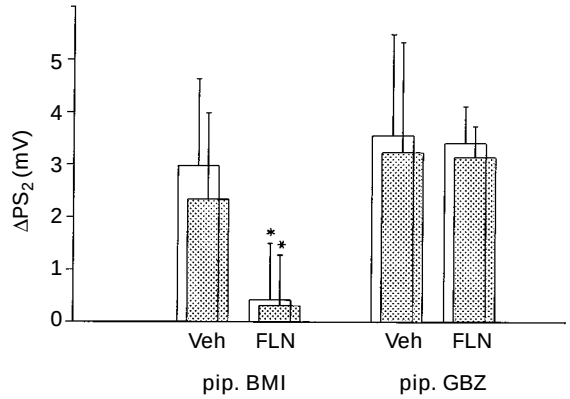

Figure 3 Effect of flunarizine (FLN) on the increases in amplitude of the field orthodromic population spike (PS_2), induced by either bicuculline methiodide (8 mM), or gabazine (5 mM) in the recording microelectrodes (pip.BMI and pip.GBZ, respectively). The columns (mean$\pm$s.d.) represent the increases (ΔPS_2), with respect to the amplitudes recorded in the same animal with standard (NaCl-filled) microelectrodes. Two groups of rats were administered 50 μmol kg^{-1} FLN i.p. and were subsequently recorded twice with either pip.BMI or pip.GBZ, at 40 min intervals. Paired groups of rats were administered vehicle (Veh; 5% arabic gum in saline), then equally recorded twice with either pip.BMI, or pip.GBZ. The second recordings are indicated by the stippled columns. The number of rats was 6 in each group. Significant differences between time-matched recordings in the paired groups (FLN vs vehicle) are indicated by $*P<0.05$, two-tailed t test.

vivo since high local concentrations of the drugs can be obtained exclusively in the area to be studied (Steward *et al*., 1990). The extracellular recordings of the population spikes revealed that BMI and gabazine caused similar epileptiform responses in hippocampal area CA3, where epileptiform bursting has been shown to originate (Schwartzkroin & Prince, 1978). The increases in PS_2 amplitude are caused by a larger number of neurones firing simultaneously (neuronal hypersynchrony) and the appearance of repetitive PS_3 reflects an increase in the number of action potentials fired by individual neurones in the presence of BMI or gabazine (Figure 1).

The finding that the increase in PS_2, as well as PS_3, induced by BMI, declined with time, while the effect of gabazine remained unchanged (Figure 1), may be due to the instability of bicuculline under physiological conditions (Olsen *et al*., 1975). Loss of activity might also reflect different mechanisms of the two drugs.

The results in Figure 2a extend our previous observation that levetiracetam inhibited the hyperexcitability caused by bicuculline in rat hippocampus (Margineanu & Wülfert, 1995a). Our data show that the more hydrosoluble bicuculline derivative BMI causes increased neuronal hypersynchrony and bursting, and that levetiracetam exerts potent inhibition of these effects, with a U-shaped dose-response relationship. The cause of the progressive loss of inhibition by levetiracetam, observed at doses higher than 32 μmol kg^{-1}, i.v., is not clear and it warrants further investigation. One possibility could be that BMI-induced hyperexcitability does not arise from a single mechanism and that levetiracetam exerts opposite effects, with different dose-dependencies, on the different components. The lack of effect *per se* of levetiracetam on PS_2 is in agreement with recent observations by Birnstiel *et al*. (1997), who demonstrated that levetiracetam does not alter basic cell characteristics or normal synaptic transmission in area CA3. This finding is also consistent with the observations that levetiracetam, in contrast to most known antiepileptic drugs, does not cause sedative effects or express CNS-depressant properties in the rat (Gower *et al*., 1992).

Since gabazine is a highly selective $GABA_A$ receptor antagonist (Wermuth & Bizière, 1986), the absence of any significant effect of levetiracetam on gabazine-induced hyperexcitability (Figure 2b) is in agreement with the apparent lack of effect of levetiracetam on GABA-related neurochemistry in mouse brain (Sills *et al*., 1997). The anti-BMI effect of levetiracetam, demonstrated in this study (Figure 2a), therefore suggests that BMI-induced hyperexcitability may comprise a non-GABAergic component. The finding that clonazepam did not alter hyperexcitability caused by BMI in our model further supports this hypothesis.

Flunarizine has been repeatedly shown to exert potent anticonvulsant effects in various animal models of epilepsy (De Sarro *et al*., 1988; Popoli *et al*., 1990; Mack & Gilbert, 1992). Flunarizine has also been found to inhibit the epileptiform activity induced by bicuculline in human neo-cortical slices *in vitro* (Straub *et al*., 1996). The anti-BMI effect, together with the observation that flunarizine did not inhibit gabazine in our model (Figure 3), support the conclusion that the epileptiform effect caused by BMI in hippocampal CA3 area involves a non-$GABA_A$-mediated component. These findings also indicate that the effect of BMI, which is antagonized by levetiracetam, might be calcium channel-dependent. The lack of any significant reduction of BMI-induced hyperexcitability by carbamazepine and by valproate, two antiepileptic drugs thought to act mainly on sodium and potassium channels (Macdonald & Kelly, 1995), also supports the contention that calcium currents may be involved in BMI-induced PS_2 increases.

Flunarizine is not a selective blocker of a single type of calcium channel. However, flunarizine has been shown to block selectively low-threshold T-type calcium channels in neuroblastoma cells (Wang *et al*., 1990) and to be very effective in blocking T-type calcium channels in freshly isolated hypothalamic neurones (Akaike *et al*., 1989). Hippocampal CA3 pyramidal neurones are particularly rich in T-type calcium channels (Johnston *et al*., 1992). The finding that flunarizine inhibited BMI-induced increases in excitability in CA3 area might therefore indicate that activation of T-channels does contribute to BMI-induced increases in neuronal excitability in this area. The lack of effect of nifedipine, a typical L-type calcium channel antagonist (e.g. Ito *et al*., 1994), contra-indicates an effect of BMI on L-type channels in area CA3.

In conclusion, the present study provided pharmacological data suggesting that the hyperexcitability caused in rat hippocampal CA3 area by *in situ* application of BMI involves non-$GABA_A$ receptor-associated activation of calcium channels. Our data further indicate that levetiracetam may inhibit neuronal hyperexcitability via inhibition of these channels. However, further *in vitro* studies are needed to characterize the possible effect of BMI on calcium channels and to determine how levetiracetam prevents these effects.

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References

AICARDI, G. & SCHWARTZKROIN, P.A. (1990). Suppression of epileptiform burst discharges in CA3 neurons of rat hippocampal slices by the organic calcium channel blocker, verapamil. *Exp. Brain Res*., **81,** 288 – 296.

AKAIKE, N., KOSTYUK, P.G. & OSIPCHUK, Y.V. (1989). Dihydropyridine-sensitive low-threshold calcium channels in isolated rat hypothalamic neurons. *J. Physiol*., **412,** 181 – 195.

ANDERSEN, A. (1975). Organization of hippocampal neurons and their interconnections. In *The Hippocampus*, Vol. 1. ed. Isaacson, R.L. & Pribram, K.H. pp. 155 – 175. New York: Plenum.

BIRNSTIEL, S., WÜLFERT, E. & BECK, S.G. (1997). Levetiracetam (L059) affects in vitro models of epilepsy in CA3 pyramidal neurons without altering normal synaptic transmission. *Naunyn-Schmiedeberg's Arch. Pharmacol*. (in press).

DE SARRO, G.B., MELDRUM, B.S. & NISTICÓ, G. (1988). Anticonvulsant effects of some calcium entry blockers in DBA/2 mice. *Br. J. Pharmacol*., **93,** 247 – 256.

GOWER, A., NOYER, M., VERLOES, R., GOBERT, J. & WÜLFERT, E. (1992). ucb L059, a novel anti-convulsant drug: pharmacological profile in animals. *Eur. J. Pharmacol*., **222,** 193 – 203.

GOWER, A., HIRSCH, E., BOEHRER, A., NOYER, M. & MARESCAUX, C. (1995). Effects of levetiracetam, a novel antiepileptic drug, on convulsant activity in two genetic rat models of epilepsy. *Epilepsy Res*., **22,** 207 – 213.

HEYER, E.J., NOWAK, L.M. & MACDONALD, R.L. (1982). Membrane depolarization and prolongation of calcium-dependent action potentials of mouse neurons in cell culture by two convulsants: bicuculline and penicillin. *Brain Res*., **232,** 41 – 56.

ITO, C., IM, W.B., TAKAGI, H., TAKAHASHI, M., TSUZUKI, K., LIOU, S.-Y. & KUNIHARA, M. (1994). U-92032, a T-type Ca^{2+} channel blocker and antioxidant, reduces neuronal ischemic injuries. *Eur. J. Pharmacol*., **257,** 203 – 210.

JOHNSTON, D., FISHER, R. & GRAY, R. (1992). Voltage-gated calcium channels in adult hippocampal neurons. In *Ionic Channels*, Vol. 3. ed. Narahashi, T. pp. 39 – 62. New York: Plenum Press.

JOY, R.M. & ALBERTSON, T.E. (1992). In vivo assessment of the importance of GABA in convulsant and anticonvulsant drug action. *Epilepsy Res*., Suppl. **8**, 63 – 75.

LÖSCHER, W. & HÖNACK, D. (1993). Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. *Eur. J. Pharmacol.*, **232,** 147–148.

MACDONALD, R.L. & KELLY, K.M. (1995). Antiepileptic drug mechanisms of action. *Epilepsia*, **36**, Suppl. **2,** S2–12.

MACK, C.M. & GILBERT, M.E. (1992). An examination of the anticonvulsant properties of voltage-sensitive calcium channel inhibitors in amygdala kindled seizures. *Psychopharmacology*, **106,** 365–369.

MARGINEANU, D.G. & WÜLFERT, E. (1995a). ucb L059, a novel anticonvulsant, reduces bicuculline-induced hyperexcitability in rat hippocampal CA3 in vivo. *Eur. J. Pharmacol.*, **286,** 321–325.

MARGINEANU, D.G. & WÜLFERT, E. (1995b). ucb L059, a novel anticonvulsant, reduces bicuculline-induced neuronal hyperexcitability, possibly through Ca-dependent mechanisms. *Pharmacol. Res.*, **31S,** 347.

MARGINEANU, D.G. & WÜLFERT, E. (1995c). Input-output dependence reveals the components of GABAergic modulation in rat hippocampal CA3 in vivo. *Arch. Physiol. Biochem.*, **103,** 115–121.

MUBHOFF, U., MADEJA, M., BLOMS-FUNKE, P. & SPECKMANN, E.-J. (1994). Effects of the epileptogenic agent bicuculline methiodide on membrane currents induced by *N*-methyl-D-aspartate and kainate (oocyte; *Xenopus laevis*). *Brain Res.*, **639,** 135–138.

OLSEN, R.W., BAN, M., MILLER, T. & JOHNSTON, G.A.R. (1975). Chemical instability of the GABA antagonist bicuculline under physiological conditions. *Brain Res.*, **98,** 383–387.

POPOLI, P., PÈZZOLA, A. & SCOTTI DE CAROLIS, A. (1990). Possible involvement of the adenosinergic system in flunarizine anticonvulsant activity in rats. *Arch. Int. Pharmacodyn.*, **306,** 45–56.

SCHARFMAN, H.E. (1994). Synchronization of area CA3 hippocampal pyramidal cells and non-granule cells of the dentate gyrus in bicuculline-treated rat hippocampal slices. *Neuroscience*, **59,** 245–257.

SCHWARTZKROIN, P.A. & PRINCE, D.A. (1978). Cellular and field potential properties of epileptogenic hippocampal slices. *Brain Res.*, **147,** 117–130.

SILLS, G.J., LEACH, J.P., FRASER, C.M., FORREST, G., PATSALOS, P.N. & BRODIE, M.J. (1997). Neurochemical studies with the novel anticonvulsant levetiracetam in mouse brain. *Eur. J. Pharmacol.*, **325,** 35–40.

STEWARD, O., TOMASULO, R. & LEVY, W.B. (1990). Blockade of inhibition in a pathway with dual excitatory and inhibitory action unmasks a capability for LTP that is otherwise not expressed. *Brain Res.*, **516,** 292–300.

STRAUB, H., SPECKMANN, E.-J., BINGMANN, D. & WALDEN, J. (1990). Paroxysmal depolarization shifts induced by bicuculline in CA3 neurons of hippocampal slices: suppression by the organic calcium antagonist verapamil. *Neurosci. Lett.*, **111,** 99–101.

STRAUB, H., KÖHLING, R., LÜCKE, A., FAUTECK, J.-D., SPECKMANN, E.-J., MOSKOPP, D., WASSMANN, H., TUXHORN, I., WOLF, P., PANNEK, H. & OPPEL, F. (1996). The effects of verapamil and flunarizine on epileptiform activity induced by bicuculline and low Mg^{2+} in neocortical tissue of epileptic and primary non-epileptic patients. *Brain Res.*, **733,** 307–311.

WANG, R., KARPINSKI, E., WU, L. & PANG, P.K.T. (1990). Flunarizine selectively blocks transient calcium channel currents in N1E-115 cells. *J. Pharmacol. Exp. Ther.*, **254,** 1006–1011.

WERMUTH, C.G. & BIZIÈRE, K. (1986). Pyridazinyl-GABA derivatives: a new class of synthetic $GABA_A$ antagonists. *Trends Pharmacol. Sci.*, **7,** 421–424.

WÜLFERT, E. & MARGINEANU, D.G. (1996). Effects of in situ manipulation of nitric oxide on rat hippocampal CA3 neurone excitability. *NeuroReport*, **7,** 2737–2742.

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