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Levetiracetam has no significant γ-aminobutyric acid-related effect on paired-pulse interaction in the dentate gyrus of rats

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Abstract

γ-Aminobutyric acid (GABA)ergic mechanisms of the novel antiepileptic drug, levetiracetam (*Keppra*®), have been both favored and rejected. Since paired-pulse interaction is accepted in functionally assessing GABAergic mechanisms, we investigated whether levetiracetam affects the paired-pulse inhibition/facilitation of the field potentials, evoked in the dentate gyrus of urethane-anesthesized rats. This model revealed a strong paired-pulse inhibition at 20-ms interstimulus interval, a noteworthy paired-pulse facilitation at 80-ms interstimulus interval, and a moderate paired-pulse inhibition at 500-ms interstimulus interval. Bicuculline (3 mg/kg/h, i.v.) and baclofen (10 mg/kg, i.v.) markedly depressed paired-pulse inhibition at 20-ms interstimulus interval, while clonazepam (1 mg/kg, i.p.), diazepam (10 mg/kg, i.v.), and phenobarbital (40 mg/kg, i.v.) enhanced it. Bicuculline also depressed paired-pulse inhibition at 500-ms interstimulus interval. Bicuculline, baclofen, and diazepam reduced paired-pulse facilitation at 80-ms interstimulus interval. Distinct from these $GABA_A$ receptor- and $GABA_B$ receptor-related drugs, levetiracetam (17 and 540 mg/kg, i.v.) had no significant effect on either paired-pulse interaction in this model, a result not favoring any major role of GABAergic mechanisms in its antiseizure action.

Keywords: Levetiracetam; Paired-pulse inhibition/facilitation; GABAergic mechanism; Antiepileptic drug; Dentate gyrus, rat

1. Introduction

Stimulation with paired (conditioning and test) pulses is largely used to assess convulsant and anticonvulsant drugs, with the rationale that the γ-aminobutyric acid (GABA), liberated by the conditioning stimulus from inhibitory interneurons, causes either a postsynaptic depression of the response to the test stimulus, when this one comes at a low interstimulus interval, or a facilitation of the test response at higher interstimulus interval, upon a presynaptic inhibition of GABA release (Brucato et al., 1995; Tasker and Dudek, 1991). Levetiracetam is a novel antiepileptic drug (*Keppra*®) with repeatedly proven clinical efficacy (Marson et al., 2001; Leppik, 2002), which has shown a preclinical profile distinct from all other antiepileptic drugs in use (Klitgaard, 2001). Levetiracetam is not chemically related to, and does not act via, any cellular mechanism accepted for any established antiepileptic drug, but it was reported to exert several nonconventional effects on neurons (Margineanu and Klitgaard, 2002a). However, published results both favor (Löscher et al., 1996) and reject (Sills et al., 1997; Tong and Patsalos, 2001) putative GABAergic mechanisms of levetiracetam.

A previously reported absence of an effect of levetiracetam on paired-pulse inhibition at low interstimulus interval in hippocampal CA3 area of anesthesized rats (Margineanu and Wülfert, 1995) led to a questioning of the GABAergic nature of the antibicuculline effect of levetiracetam. However, since the paired-pulse inhibition occurring at the lowest ($\approx$ 20 ms) interstimulus interval is complete in the CA3 area (Margineanu and Wülfert, 2000), recording in that area is unfit for revealing drug-induced increases in inhibition. In contrast to this, in the dentate gyrus of anesthesized rats, the low interstimulus interval paired-pulse inhibition is strong, but not complete, allowing the study of both an enhancement and a decrease of inhibition by a drug systemically administered. Indeed, it is in the dentate gyrus of urethane-anesthesized rats that effects of newer antiepileptic drugs on paired-pulse inhibition were assessed (Stringer, 2000;

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Stringer and Taylor, 2000). Accordingly, with the aim of functionally assessing putative GABAergic mechanisms of levetiracetam, we investigated whether this drug influences either paired-pulse inhibition, or paired-pulse facilitation in the dentate gyrus of urethane-anesthesized rats. Some of the results have been communicated in abstract form (Margineanu and Klitgaard, 2002b). The experiments described below have been approved by the local ethics committee for animal experimentation, according to Belgian law.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 300–350 g were used, which were kept on a 12 L:12 D cycle, with free access to water and a standard cube diet. They were anesthesized with 1.6 g/kg, i.p. urethane, and procaine and epinephrine were injected at the incision site. In those rats to be further injected intravenously, a polyethylene tubing (1.09 mm outer diameter) was introduced in the left jugular vein.

2.2. Field potentials recording

Field potentials were recorded extracellularly in the dentate gyrus with glass microelectrodes stereotaxically introduced at the approximate coordinates, according to the atlas by Paxinos and Watson (1998): antero-posterior −4 mm from bregma, lateral 2.5 mm from the midline, dorso-ventral −3 mm from brain surface. The microelectrodes were borosilicate glass micropipettes, capillary-filled with 0.5 M NaCl, with an input impedance around 2 MΩ. The field potentials were evoked upon stimulation of the ipsilateral perforant path, via 0.2-mm-diameter Pt wire bipolar electrodes, stereotaxically introduced at approximate coordinates: antero-posterior −7.8 mm, lateral 4 mm, dorso-ventral −3 mm. The stimulation was with pairs of conditioning and test pulses, separated by interstimulus intervals of between 20 and 500 ms, delivered by a Grass S88 stimulator, via a PSIU6 constant current unit. The paired pulses were identical, monophasic, and rectangular; of 0.2 ms duration; and with the strength of 0.5 mA, producing field potentials with a population spike about 50% of the maximal value. The field potentials were amplified by a differential amplifier (AM502; Tektronix) with the filters set to 1 Hz and 1 kHz, visualized on a digital oscilloscope (TDS 430A; Tektronix) and acquired via an internally developed software with a sampling frequency of 2.5 kHz. The acquisition software averaged on-line three samples of field potentials evoked at 20-s intervals and calculated the amplitudes of population spikes, measured from the negative peak to a tangent drawn between the preceding and the following maxima of the waveform (as illustrated on the upper traces in Fig. 1).

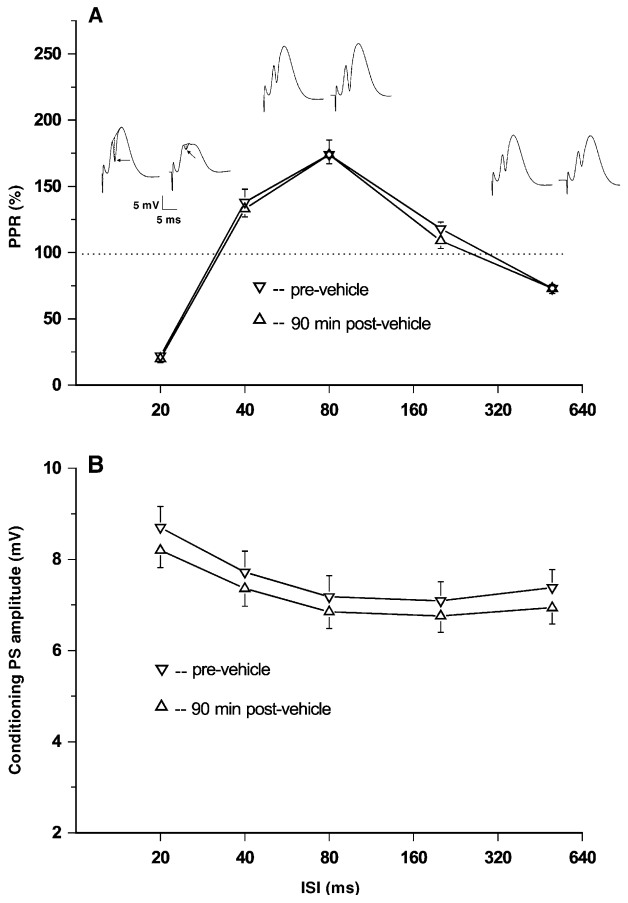

Fig. 1. Interstimulus interval (ISI) dependence of (A) the paired-pulse ratio (PPR) of the amplitude of the population spikes respectively evoked by paired test vs. conditioning stimuli, and (B) the amplitude of the population spike (PS) in the conditioning responses, evoked in the dentate gyrus of urethane-anesthesized control rats, before (▽) and 90 min after (△) vehicle injection. The data recorded at both 30 and 60 min are nearly identical with the two sets on the graph and were not represented, to avoid blurring. The symbols indicate mean ± S.E.M. for the ensemble of 40 control rats involved in this study (i.e., five groups of eight rats per group, pooled together). The traces are recordings from a single animal, showing typical examples of field potentials evoked with pairs of conditioning (left trace, in each pair) and test (right trace, in each pair) pulses, with interstimulus intervals of either 20 ms (leftmost pair), 80 ms (middle pair), or 500 ms (rightmost pair). The arrows on the leftmost pair of traces point at the population spikes, and the dotted lines indicate how the acquisition software measured their amplitudes.

After positioning the stimulation and recording electrodes at the previously mentioned approximate coordinates, their positions were finely adjusted until a single stimulus of 0.2 ms duration and a strength about 0.1 mA could elicit a field potential with a population spike of about 1 mV (which we considered a ‘‘standard’’ dentate gyrus response). Then the response to paired pulses of fixed duration (0.2 ms) and strength (0.5 mA), with interstimulus intervals of either 20, 40, 80, 200, and 500 ms, was repeatedly recorded, once before injecting the drug (predrug control), then repeated in the same animal at 30, 60, and 90 min after drug administration.

2.3. Drugs and administration

Levetiracetam (UCB Pharma Sector, Braine-l'Alleud, Belgium), dissolved in saline, was injected as an intravenous bolus at doses of either 17 mg/kg, in a volume of 1 ml/kg; or 540 mg/kg, in a volume of 2 ml/kg. (+)-Bicuculline (Fluka Chemie, Buchs, Switzerland), dissolved in saline with a drop of acetic acid, was administered by continuous intravenous infusion of 3 mg/kg/h, in a volume of 1 ml/h. (±)-Baclofen (RBI, Natick, MA, USA), dissolved in saline, was injected as an intravenous bolus at a dose of 10 mg/kg, in a volume of 1 ml/kg. Diazepam (vials of 2 ml containing 10 mg of diazepam; Roche, Brussels, Belgium), was injected as an intravenous bolus at a dose of 10 mg/kg, in a volume of 2 ml/kg. Clonazepam (Roche, Basel, Switzerland), solubilized with 0.1% Tween 80 in saline, was injected as an intraperitoneal bolus at a dose of 1 mg/kg, in a volume of 5 ml/kg. Phenobarbital (Federa, Brussels Belgium), dissolved in saline, was bolus-injected intravenously in a volume of 2 ml/kg. All the solutions were freshly prepared.

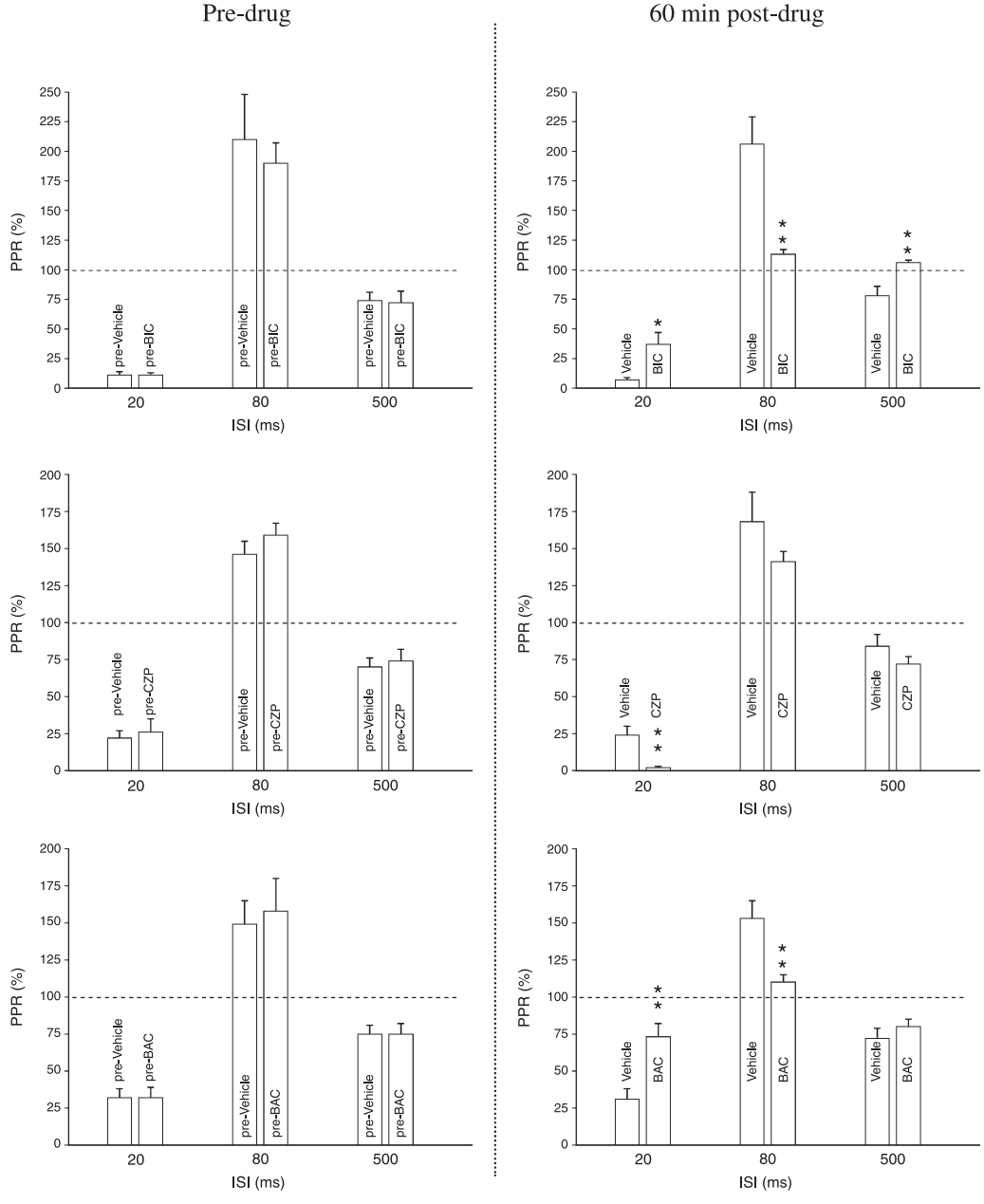

Fig. 2. Paired-pulse ratio (PPR) of the amplitude of the population spikes respectively, evoked by paired test vs. conditioning stimuli, with interstimulus intervals (ISI) of 20, 80, and 500 ms, before (Predrug) and 60 min after administration of either bicuculline 3 mg/kg/h, i.v. (BIC), clonazepam 1 mg/kg, i.p. (CZP), or baclofen 10 mg/kg, i.v. (BAC), or the corresponding vehicle. PPR values, in percent of the response to the conditioning test, are given as mean ± S.E.M. for groups of eight rats receiving each treatment. Significant differences with respect to the corresponding vehicle-receiving group, at each recording time, assessed with two-tailed t tests, are indicated with * ($P<0.05$) or ** ($P<0.005$). The same effects as shown for 60 min have been also recorded 30 and 90 min following drug administration (data not shown to avoid redundancy).

2.4. Data processing

Paired-pulse interaction was quantified by means of the ratio of the amplitude of the population spike evoked by the test stimulus and the one evoked by the conditioning stimulus (see the leftmost pair of traces reproduced in Fig. 1A), termed "paired-pulse ratio." Mean values, S.D. values, and S.E.M. values were consistently obtained for groups of eight rats and the statistical significance of the differences between the groups was assessed using two-tailed *t* tests.

3. Results

The graph in Fig. 1A shows the threefold pattern of paired-pulse interaction we recorded in the dentate gyrus of anesthesized rats: a strong paired-pulse inhibition at low (20 ms) interstimulus interval, a noteworthy paired-pulse facilitation at middle (80 ms) interval, and a less pronounced paired-pulse inhibition at long (500 ms) interval. These paired-pulse interactions—through which the pharmacological effects are subsequently quantified in Figs. 2–4, are respectively illustrated by the three pairs of traces inserted in Fig. 1A. Although the conditioning pulses, considered in isolation, are identical irrespective of the interstimulus interval, they elicited population spikes of slightly higher amplitudes at lower interstimulus intervals (Fig. 1B). The interstimulus interval-dependent variation of the amplitude of conditioning responses was modest, but it appeared regularly in all groups of rats and remained similar in the repeated recordings (Fig. 1B).

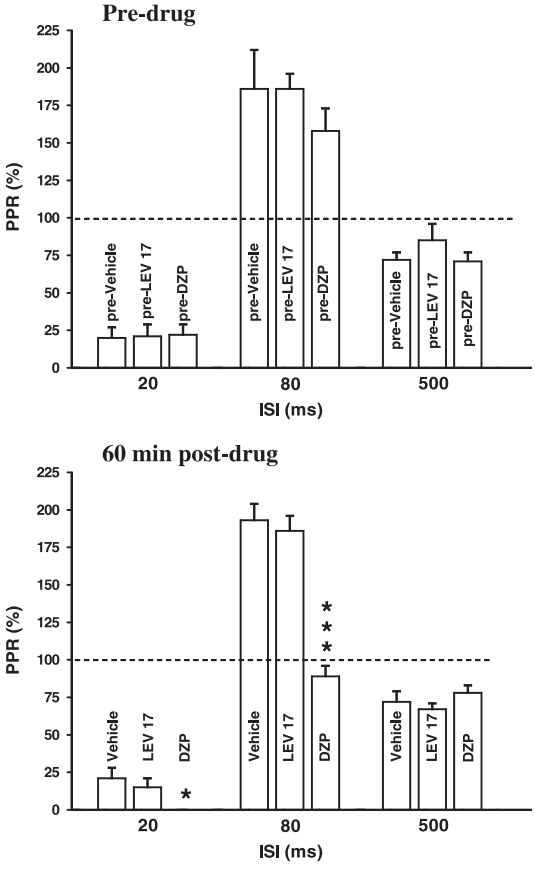

Fig. 3. Effects of levetiracetam 17 mg/kg, i.v. (LEV 17) and of diazepam 10 mg/kg, i.v. (DZP) on the paired-pulse ratio (PPR) of the population spikes evoked in the dentate gyrus of anesthesized rats by perforant path stimulation with paired pulses with interstimulus intervals (ISI) of 20, 80, and 500 ms. The columns represent mean ± S.E.M., for eight rats per group, and of the recordings made before (upper graph) and 60 min following drug administration (lower graph). Significant differences with respect to the group receiving vehicle, assessed with two-tailed *t* tests, are indicated with * ($P<0.05$) or *** ($P<0.0005$). The same effects as in the lower graph have been also recorded 30 and 90 min following drug administration (data not shown).

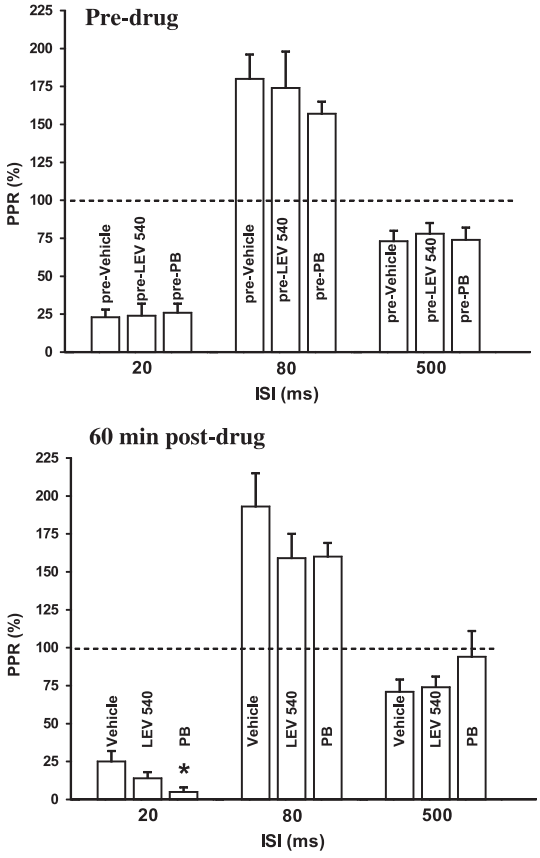

Fig. 4. Effects of levetiracetam 540 mg/kg, i.v. (LEV 540) and of phenobarbital 40 mg/kg, i.v. (PB) on the paired-pulse ratio (PPR) of the population spikes evoked in the dentate gyrus of anesthesized rats by perforant path stimulation with paired-pulses with interstimulus intervals (ISI) of 20, 80, and 500 ms. Similar presentation as in Fig. 3, for groups of eight rats per group. At 30 and at 90 min, the effects (not shown) have been the same as shown for 60 min following drug administration (lower graph).

The $GABA_A$ receptor antagonist, bicuculline, consistently reduced both the low (20 ms) interstimulus interval paired-pulse inhibition and the middle (80 ms) interval paired-pulse facilitation, and eliminated the long (500 ms) interval paired-pulse inhibition (Fig. 2, upper). In contrast, the benzodiazepine, clonazepam, consistently enhanced paired-pulse inhibition at 20-ms interstimulus interval, while not influencing the other paired-pulse interactions (Fig. 2,

Table 1
Amplitude of the population spikes evoked by conditioning stimuli (from the pairs with 80-ms interstimulus interval), before and at different time intervals after administration of either: levetiracetam 17 mg/kg, i.v. (LEV 17); or 540 mg/kg, i.v. (LEV 540); or bicuculline 3 mg/kg/h, i.v. (BIC); or clonazepam 1 mg/kg, i.p. (CZP); or baclofen 10 mg/kg, i.v. (BAC); or diazepam 10 mg/kg, i.v. (DZP); or phenobarbital 40 mg/kg, i.v. (PB)

	Predrug	Time (min) after drug injection		
		30	60	90
LEV 17	6.25 ± 0.50	6.28 ± 0.63	6.70 ± 0.61	6.83 ± 1.14
LEV 540	7.47 ± 0.97	7.99 ± 1.00	7.57 ± 1.01	7.44 ± 1.24
BIC	7.12 ± 0.91	17.20 ± 0.61*	17.68 ± 0.88*	17.38 ± 0.65*
CZP	7.44 ± 0.92	7.15 ± 0.65	7.56 ± 0.70	7.71 ± 0.73
DZP	8.28 ± 1.08	9.17 ± 1.10	9.36 ± 0.99	9.33 ± 1.16
PB	8.33 ± 1.14	7.35 ± 1.19	6.50 ± 1.18**	6.40 ± 1.17**
BAC	8.02 ± 0.89	8.24 ± 1.27	7.88 ± 1.13	7.28 ± 1.16

The values (in mV) are given as mean ± S.E.M. for groups of eight rats receiving each treatment.

* Highly significant ($P < 0.00005$; two-tailed t test) difference with respect to the corresponding predrug control.

** Significant ($P < 0.05$) difference with respect to the corresponding predrug control, if assessed with paired t test.

middle). The $GABA_B$ receptor agonist, baclofen, reduced paired-pulse inhibition at 20-ms interstimulus interval and paired-pulse facilitation at 80-ms interstimulus interval (Fig. 2, lower).

The data in Fig. 3 show that levetiracetam, at a dose of 17 mg/kg, i.v.—which is anticonvulsant in rats (Gower et al., 1995; Klitgaard et al., 1998), did not influence any paired-pulse interaction (either inhibition or facilitation) in the three postdrug recording periods. Unlike levetiracetam, diazepam 10 mg/kg, i.v. enhanced paired-pulse inhibition at 20-ms interstimulus interval, no population spike being evoked by the test pulse, and it eliminated the facilitation at 80-ms interstimulus interval, both effects being stable. Levetiracetam did not produce any significant modification of the paired-pulse interaction even at a very high dose of 540 mg/kg, i.v. (Fig. 4), although a minor tendency to enhance inhibition at 20-ms interstimulus interval and to reduce facilitation at 80-ms interval was seen at this dose. Phenobarbital 40 mg/kg, i.v. enhanced paired-pulse inhibition at 20-ms interstimulus interval and tended (nonsignificantly) to reduce it at 500-ms interval.

The data in Table 1 show that both doses of levetiracetam did not induce, up to 90 min postadministration, any noticeable change of the field potentials evoked by the conditioning pulses, with difference from phenobarbital (which induced a steady decrease of population spikes amplitude), and in sharp contrast with bicuculline (which caused, as expectedly, a huge increase in population spike amplitude).

4. Discussion

The triphasic pattern of paired-pulse interaction, illustrated in Fig. 1, is in full agreement with numerous earlier studies performed in the dentate gyrus of rats anesthesized with either urethane (e.g., Brucato et al., 1992, 1995; Robinson and Racine, 1986) or halothane (e.g., Criado et al., 1994; Steffensen and Henriksen, 1991). Urethane—the decades-old anesthetic most widely used in electrophysiological studies in vivo, in spite of a persisting lack of mechanistic understanding of its activity—was reported to reduce paired-pulse inhibition in the dentate gyrus of rats, at interstimulus intervals up to 60 ms, but not higher (Shirasaka and Wasterlain, 1995). Accordingly, an intrinsic effect of urethane would, indeed, shift the values of paired-pulse ratio we measured at low interstimulus interval. However, since all groups of rats received similar anesthesia, the shift is expectedly similar in all groups and cannot obscure an effect of another drug, unless that one would share the mechanisms of urethane. The strongly substantiated conclusion of Shirasaka and Wasterlain (1995) that urethane has no agonist effect on the $GABA_A$ system implies that $GABA_A$ receptor-related effects are on top of the effect of urethane, which merely shifts the baseline. Our results confirm, indeed, the possibility to record both increases and reductions of low interstimulus interval paired-pulse inhibition, induced by various GABA-related drugs.

The values of paired-pulse ratio corresponding to the ensemble of control (vehicle-injected) rats shown in Fig. 1, as well as to each control group appearing in Figs. 2–4, show that the three components of this pattern (i.e., the inhibition at both short and long interstimulus intervals, and the facilitation at middle range interstimulus interval) remained remarkably stable when recording repeatedly in the same animals on a time interval of 90 min postinjection. We decided to assess levetiracetam at such a time interval, in this rat model in vivo, since the time of the peak effect in rats of this drug was reported to be 60 min (Löscher and Hönack, 1993). Consequently, in order to separate the effects of the administered drugs on paired-pulse interaction from a putative effect of long-term repeated recording, and also to monitor any possible day-to-day variability, we repeatedly included control groups. In each of the five control groups taken separately (Figs. 2–4), one observes the same pattern of interstimulus interval-dependent paired-pulse interaction (i.e., strong inhibition, then facilitation, then moderate inhibition), which assesses a fairly good reproducibility of the model. Conversely, the existence of some quantitative differences between the various control groups confirms the advisability to include contemporary controls. As the field potentials we recorded are averages of several successive responses, the slight, but not accidental, interstimulus interval dependence of the response to conditioning stimuli (Fig. 1B), visible only at lower interstimulus intervals (see the traces included in Fig. 1A), reflects a frequency-like potentiation, as previously described by Sloviter (1991).

This study did not show any significant effect of levetiracetam on either paired-pulse interaction, not only at an anticonvulsant dose of 17 mg/kg, i.v. (Fig. 3), but also at a

high dose of 540 mg/kg, i.v. (Fig. 4), in contrast to the $GABA_A$ receptor-related anticonvulsants diazepam (Fig. 3), clonazepam (Fig. 2), and phenobarbital (Fig. 4). However, it should be not overlooked that at 540 mg/kg, i.v., levetiracetam slightly tended to enhance low interstimulus interval paired-pulse inhibition (Fig. 4). Beyond not significantly influencing paired-pulse plasticity in the dentate gyrus, levetiracetam did not produce any noticeable modification of the conditioning responses in this brain region (Table 1), in full agreement with previous observations in the hippocampal CA3 area (Margineanu and Wülfert, 1995). On the other hand, the fact that also clonazepam, diazepam, and baclofen did not significantly modify the conditioning responses (Table 1), while all of them strongly affected paired-pulse interaction (Figs. 2 and 3), highlights the attractiveness of using paired pulses when exploring putative GABA-related effects.

The effects found with the reference compounds are in agreement with previously published studies. Indeed, effects similar to these results have been reported by Steffensen and Henriksen (1991) for bicuculline and baclofen, by Brucato et al. (1995) for baclofen, and by Joy and Albertson (1992) for diazepam and phenobarbital. Accordingly, apart from noting the ability of this model to reveal modulatory effects on paired-pulse interaction, we refrain from discussing at length the cellular mechanisms by which the various GABA-related reference compounds exert such modulations.

Moreover, the amount of inhibition/facilitation after a particular stimulus is known to depend on stimulus intensity (Burdette and Gilbert, 1995; Margineanu and Wülfert, 2000). Furthermore, not only GABA, but also adenosine (Higgins and Stone, 1996) and calcium channels (Stringer and Taylor, 2000), may contribute to short latency paired-pulse inhibition. In view of these, and being aware of the high risk of biased mechanistic simplifications when interpreting paired-pulse results (Tasker and Dudek, 1991), we do not speculate on how the absence of effect of levetiracetam on paired-pulse interaction could be explained by what is already known on the neuronal effects of this drug (Margineanu and Klitgaard, 2002a). Nevertheless, beyond any mechanistic uncertainty, the difference between the clear-cut effects on paired-pulse interaction of the antiepileptic drugs known to enhance GABAergic neurotransmission (benzodiazepines and barbiturates) and the lack of effect of levetiracetam strongly suggests that the antiseizure activity of this drug does not derive from any conventional form of GABAergic facilitation, at least in the hippocampus. This is in agreement with the reported absence of affinity of levetiracetam for $GABA_A$/benzodiazepine and $GABA_B$ receptors (Noyer et al., 1995), absence of effects of this drug on brain concentration (Sills et al., 1997) and release (Tong and Patsalos, 2001) of GABA, on the activities of both GABA-synthesizing and GABA-degrading enzymes (Löscher et al., 1996; Sills et al., 1997), and on neuronal voltage (Poulain and Margineanu, 2002) and current (Rigo et al., 2002) responses to GABA. On the other hand, the already older antibicuculline effect (Margineanu and Wülfert, 1995) and the newer data showing that levetiracetam opposes inhibition by negative allosteric modulators of GABA-induced currents (Rigo et al., 2002) and voltage responses (Poulain and Margineanu, 2002) suggest an interaction of levetiracetam with the $GABA_A$ receptors, which still remains to be characterized. While our results, gathered on normal rats, cannot suffice to substantiate extrapolations to pathologic conditions, they highlight the fact that the reversal by levetiracetam of the effects of $GABA_A$ receptor antagonists/negative allosteric modulators (Poulain and Margineanu, 2002; Rigo et al., 2002) does not represent any conventional GABAergic facilitation, in any sense similar to the GABA-related agonists presented above.

In conclusion, our results show an absence of any significant effect of levetiracetam on paired-pulse interaction in the dentate gyrus of urethane-anesthesized rats, in contrast to clear-cut effects of reference $GABA_A$ receptor-related antiepileptic drugs. These results on hippocampal responsiveness, while not sufficing to discard putative effects of levetiracetam on the inhibitory neurotransmission in pathological conditions, do not favor any major role for GABA-enhancing mechanisms in the antiseizure action of this new antiepileptic drug.

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References

Brucato, F.H., Morrisett, R.A., Wilson, W.A., Swartzwelder, H.S., 1992. The $GABA_B$ receptor antagonist, CGP-35348, inhibits paired-pulse disinhibition in the rat dentate gyrus in vivo. Brain Res. 588, 150–153.

Brucato, F.H., Mott, D.D., Lewis, D.V., Swartzwelder, H.S., 1995. $GABA_B$ receptors modulate synaptically-evoked responses in the rat dentate gyrus, in vivo. Brain Res. 677, 326–332.

Burdette, L.J., Gilbert, M.E., 1995. Stimulus parameters affecting paired-pulse depression of dentate granule cell field potentials: I. Stimulus intensity. Brain Res. 680, 53–62.

Criado, J.R., Steffensen, S.C., Henriksen, S.J., 1994. Ethanol acts via the ventral tegmental area to influence hippocampal physiology. Synapse 17, 84–91.

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.

Higgins, M.J., Stone, T.W., 1996. The contribution of adenosine to paired-pulse inhibition in the normal and disinhibited hippocampal slice. Eur. J. Pharmacol. 317, 215–223.

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.

Klitgaard, H., 2001. Levetiracetam: the preclinical profile of a new class of antiepileptic drugs? Epilepsia 42 (Suppl. 4), 13–18.

Klitgaard, H., Matagne, A., Gobert, J., Wülfert, E., 1998. Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. Eur. J. Pharmacol. 353, 191–206.

Leppik, I.E., 2002. Levetiracetam: clinical use. In: Levy, R.H., et al. (Eds.), Antiepileptic Drugs, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 433–441.

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, 147–158.

Löscher, W., Hönack, D., Bloms-Funke, P., 1996. The novel antiepileptic drug levetiracetam (ucb L059) induces alterations in GABA metabolism and turnover in discrete areas of rat brain and reduces neuronal activity in substantia nigra pars reticulata. Brain Res. 735, 208–216.

Margineanu, D.G., Klitgaard, H., 2002a. Levetiracetam: mechanisms of action. In: Levy, R.H., et al. (Eds.), Antiepileptic Drugs, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 419–427.

Margineanu, D.G., Klitgaard, H., 2002b. Levetiracetam contrasts $GABA_A$- and $GABA_B$-related drugs by its lack of effect on paired-pulse interaction in the dentate gyrus of rats. Pharmacologist 44 (Suppl. 1), A99.

Margineanu, D.G., Wülfert, E., 1995. 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., 2000. Differential paired-pulse effects of gabazine and bicuculline in rat hippocampal CA3 area. Brain Res. Bull. 51, 69–74.

Marson, A.G., Hutton, J.L., Leach, J.P., Castillo, S., Schmidt, D., White, S., Chaisewikul, R., Privitera, M., Chadwick, D.W., 2001. Levetiracetam, oxcarbazepine, remacemide and zonisamide for drug resistant localization-related epilepsy: a systematic review. Epilepsy Res. 46, 259–270.

Noyer, M., Gillard, M., Matagne, A., Henichart, J.-P., Wülfert, E., 1995. The novel antiepileptic drug levetiracetam (ucb L059) appears to act via a specific binding site in CNS membranes. Eur. J. Pharmacol. 286, 137–146.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, 4th ed. Academic Press, San Diego.

Poulain, P., Margineanu, D.G., 2002. Levetiracetam opposes the action of $GABA_A$ antagonists in hypothalamic neurones. Neuropharmacology 42, 346–352.

Rigo, J.-M., Hans, G., Nguyen, L., Rocher, V., Belachew, S., Malgrange, B., Leprince, P., Moonen, G., Selak, I., Matagne, A., Klitgaard, H., 2002. The anti-epileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated currents. Br. J. Pharmacol. 136, 659–672.

Robinson, G.B., Racine, R.J., 1986. Interactions between septal and entorhinal inputs to the rat dentate gyrus: facilitation effects. Brain Res. 379, 63–67.

Shirasaka, Y., Wasterlain, C.G., 1995. The effect of urethane anesthesia on evoked potentials in dentate gyrus. Eur. J. Pharmacol. 282, 11–17.

Sills, G.J., Leach, J.P., Frazer, 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.

Sloviter, R.S., 1991. Feedforward and feedback inhibition of hippocampal principal cell activity evoked by perforant path stimulation: GABA-mediated mechanisms that regulate excitability. Hippocampus 1, 31–40.

Steffensen, S.C., Henriksen, S.J., 1991. Effects of baclofen and bicuculline on inhibition in the fascia dentata and hippocampus regio superior. Brain Res. 538, 46–53.

Stringer, J.L., 2000. A comparison of topiramate and acetazolamide on seizure duration and paired-pulse inhibition in the dentate gyrus of the rat. Epilepsy Res. 40, 147–153.

Stringer, J.L., Taylor, C.P., 2000. The effects of gabapentin in the rat hippocampus are mimicked by two structural analogs, but not by nimodipine. Epilepsy Res. 41, 155–162.

Tasker, J.G., Dudek, F.E., 1991. Electrophysiology of GABA-mediated synaptic transmission and possible roles in epilepsy. Neurochem. Res. 16, 251–262.

Tong, X., Patsalos, P.N., 2001. A microdialysis study of the novel antiepileptic drug levetiracetam: extracellular pharmacokinetics and effect on taurine in rat brain. Br. J. Pharmacol. 133, 867–874.