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Effects of anticonvulsive drugs on pentylenetetrazol kindling and long-term potentiation in freely moving rats

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Abstract

Drugs with anticonvulsive properties and different mechanisms of action were compared for their influence on long-term potentiation and pentylenetetrazol kindling in freely moving animals. Rats were chronically implanted with a stimulation electrode in the angular bundle and a recording electrode in the dentate gyrus. Field potentials in the dentate gyrus were elicited and long-term potentiation was induced by stimulation of the perforant pathway. The clinically used drugs or the potentially anticonvulsive drugs, diphenylhydantoin (50 mg/kg), diazepam (0.5 mg/kg), pentobarbital (10 mg/kg), dizocilpine (MK 801, 0.2 mg/kg) and CGP 43487 (2-amino-4-methyl-5-phosphono-3-pentenoic acid-carboxyethylester, 10 mg/kg), were injected before tetanization. In behavioural experiments pentylenetetrazol kindling was performed with pretreatment with the substances in dosages indicated above (except MK 801, 0.3 mg/kg). Field potentials recorded in the interval between drug administration and tetanization were influenced only by diphenylhydantoin which enhanced the population spike amplitude to 128% of control values. However, the substances showed different effects on long-term potentiation. MK 801, CGP 43487 and pentobarbital depressed potentiation; diazepam was without effect. Diphenylhydantoin had a minor influence on induction but significantly impaired maintenance of long-term potentiation. Furthermore, MK 801, CGP 43487, diazepam and pentobarbital differentially depressed kindling whereas phenytoin only slightly influenced it. The consequences as to hypothetical common cellular mechanisms for kindling development and long-term potentiation are discussed.

Keywords: Long-term potentiation; Pentylenetetrazol kindling; Dentate gyrus; Field potential; (Rat, freely moving); Pentobarbital; Diazepam; Diphenylhydantoin; Dizocilpine (MK 801); CGP 43487

1. Introduction

Long-term potentiation, which is an abrupt and long-lasting response enhancement of neurons after tetanic stimulation of monosynaptic afferents, is generally thought to be an elementary mechanism of memory formation (Teyler and DiScenna, 1987; Bliss and Collingridge, 1993). Moreover, there is speculation as to whether potentiation may play a role in, or may be associated with, the development of kindling (e.g., Cain, 1989) which is a model of complex-partial or generalized epilepsy (Bradford, 1995). Glutamate- as well as γ-amino-butyric-acid (GABA)-mediated transmission may be involved in kindling development (Morimoto, 1989; Bradford, 1995). However, the relationship between long-term potentiation and kindling remains to be clarified (McEachern and Shaw, 1996; Rüthrich et al., 1996; Krug et al., 1997).

There is also evidence that long-term potentiation is a complex phenomenon which can be divided into different phases, i.e., induction and maintenance (e.g., Reymann, 1993) or different forms, e.g., NMDA receptor-mediated and voltage-dependent Ca^{2+} channel (VDCC) long-term potentiation (Teyler et al., 1994). Different cellular mechanisms may be responsible for these different phases or forms of potentiation and may perhaps also be involved in kindling development.

Anticonvulsive drugs which differ in their mode of action interfere differently with kindling development in various kindling models. For example, barbiturates, diazepam and the non-competitive NMDA receptor antagonist, dizocilpine (MK 801), suppress or retard electrical

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kindling (Racine et al., 1975; Wada et al., 1976; McNamara et al., 1988; Schmutz et al., 1988; Gilbert and Mack, 1990; Mintz et al., 1990; Morimoto et al., 1991; Silver et al., 1991 Hirayama et al., 1995). Peripherally administered competitive NMDA receptor antagonists are also effective (Schmutz et al., 1990). However, diphenylhydantoin seems not to suppress electrical kindling but rather it facilitate it (Racine et al., 1975; Wada et al., 1976; Schmutz et al., 1988). Furthermore, in chemical kindling performed by repeated application of subconvulsive pentylenetetrazol dosages, the barbiturates, diazepam and MK 801 suppress or retard kindling development (Giorgi et al., 1991; Grecksch and Becker, 1992; Becker et al., 1994; Grecksch et al., 1994). Diphenylhydantoin has not yet been tested systematically in the pentylenetetrazol kindling model.

In view of the presumed role of potentiation phenomena in kindling and epilepsy development, and of the multiple sites of action of anticonvulsive drugs at the cellular level, different influences on long-term potentiation may be expected. Characterization of these effects might be useful to clarify the role of potentiation phenomena in kindling development. However, the available data in the literature are not fully conclusive. Clearly, non-competitive and competitive NMDA receptor antagonists depress hippocampal long-term potentiation (Morimoto et al., 1991; Krug et al., 1993; Stan Leung and Shen, 1993; Lee et al., 1996) and, interestingly, also influence learning processes (Morris et al., 1986; Morris, 1989). However, effects of benzodiazepines on long-term potentiation have only been seen with high dosages (Del Cerro et al., 1992; McNamara et al., 1993). Diphenylhydantoin has been reported to be either ineffective (Stringer and Lothman, 1988; Birnstiel and Haas, 1991) or to inhibit long-term potentiation in the CA1 region (Lee et al., 1996). To our knowledge, the effect of barbiturates has only been investigated either in hippocampal slices (Scharfman and Sarvey, 1985; Lee et al., 1996) or at high, and therefore, anesthetic dosages in in vivo experiments (Dragunow et al., 1989; Jeffery et al., 1990). Therefore, we investigated the effects of anticonvulsive drugs on long-term potentiation in freely moving animals using dosages which were also applied in chemical pentylenetetrazol kindling. These dosages do not interfere with the waking behaviour of the animals. It is hypothetized that different anticonvulsive drugs may interfere specifically with the different phases or forms of long-term potentiation, and that comparison of the effects gives a novel insight into common cellular mechanisms which underlie both phenomena of neuronal plasticity.

2. Materials and methods

Ethical approval for all procedures followed was sought prior to the experiments, according to the requirements of the National Act on the Use of Experimental Animals (Germany).

2.1. Animals

The experiments were performed on 197 male Wistar rats, (Shoe:Wist (Shoe)) from Tierzucht Schönwalde weighing 220–240 g (8 weeks old) at the time of operation or the beginning of the kindling procedure. The animals were kept under controlled laboratory conditions (light regime of 12 h light/12 h dark, temperature 20 ± 2°C, air humidity 55–60%). They had free access to commercial rat pellets (Altromin 1326) and tap water. For the kindling experiments the animals were housed in groups of five per cage.

2.2. Kindling procedure

Forty milligrams per kilogram pentylenetetrazol (the ED_{16} for an acute tonic-clonic seizure in our rat strain) was administered intraperitoneally 10 times, once every 48 h. After each injection the behaviour was classified, using a modified rating scale according to Racine (Becker et al., 1995). Pretreatment time was: MK 801 30 min, diazepam, phenytoin and pentobarbital 60 min and CGP 43487 120 min before each kindling injection, according to pharmakokinetic data from the literature. Control animals received physiological saline (10 ml/kg) before the pentylenetetrazol injection.

2.3. Surgery

The animals were anesthetized with Nembutal (40 mg/kg, i.p.) and mounted in a David Kopf stereotaxic instrument (lambda 1 mm below bregma). At the stereotaxic coordinates AP −2.8 mm, lateral 1.8 mm and AP −6.9 mm, lateral 4.1 mm (adopted from Paxinos and Watson, 1982) small holes were drilled into the bone. A monopolar recording electrode and a bipolar stimulation electrode (stainless steel wire, 125 μm diameter, polyurethane-insulated) were positioned at these coordinates into the dentate gyrus slightly below the granular cell layer and into the angular bundle. Electrodes were adjusted, according to depth, by monitoring the evoked field potentials during implantation (mean = 3.6–4.0 mm from bregma for recording electrodes and 4.0–4.5 mm from bregma for stimulating electrodes). The electrodes were fixed to the bone with acrylic dental cement and connected loosely to two miniature plastic sockets. Silver wires connected to miniature screws and inserted into the nasal bone served as ground and indifferent electrode. The sockets were connected to the bone with acrylic cement. In order to diminish the risk of socket loss the animals were housed individually after surgery.

2.4. Potential recording and data processing

The experiments were performed at least 10 days after surgery. The animals were connected to an AC-coupled

amplifier (frequency range 2 Hz–10 kHz) and to the stimulation unit (A-M systems model 2100) via a flexible shielded cable and a swivel, thus allowing them free movement with only minimal restrictions. A personal computer, a MUSYCS interface and FAMOS software (IMC Meßsysteme, Berlin) were used to record the field potentials, to generate pulses for triggering the stimulation unit and calibration signals at the amplifier input and to average and store the field potentials. For recording of test potentials, series of eight biphasic square wave pulses with a duration of 100 μs per half-cycle were generated with a frequency of 0.2 Hz. The potentials were digitized with a resolution time of 100 μs, averaged and stored on a hard disk. From these averaged potentials the 'slope function' of the field e.p.s.p. (mV/ms) and the amplitude of the population spike (mV) were calculated as depicted in Fig. 1.

Long-term potentiation was induced by tetanizing the perforant pathway with 20 trains of 15 biphasic impulses of the same intensity and duration as the test impulses. The frequency within the trains was 200 Hz and the distance between them, 5 s.

2.5. Experimental protocol

Potentiation experiments were performed over 3 days. On the first day the animals were habituated for 20 min. After that an input/output (I/O) curve was determined and the stimulus intensity was calculated which evoked a population spike of 40% of its maximal amplitude. This

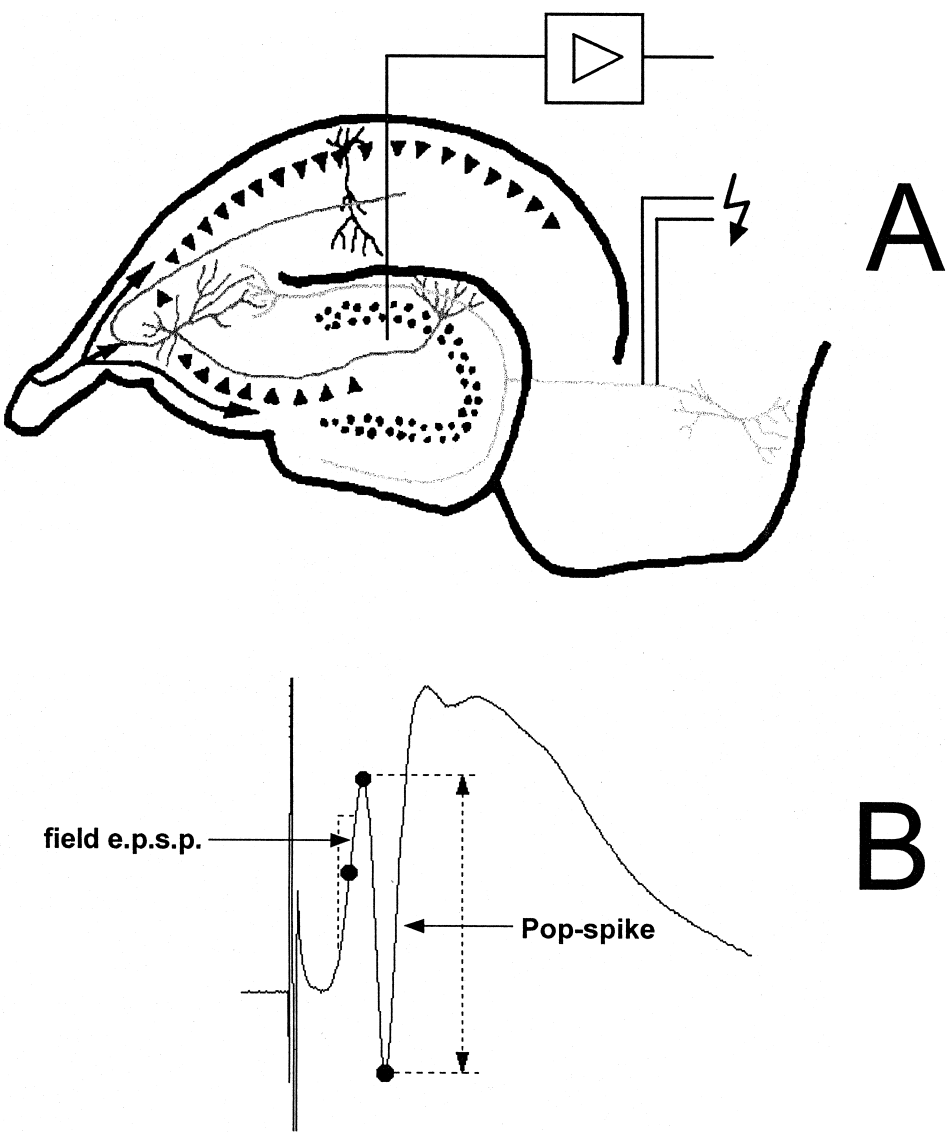

Fig. 1. Schematic diagram of the position of the stimulating electrode in the perforant pathway and the recording electrode in the dorsal blade of the dentate gyrus slightly below the granular cell layer (A) and an analogous example of a field potential recorded in the dentate gyrus together with markings for calculation of the population spike amplitude and the 'slope' of the field e.p.s.p. (B).

intensity was used to evoke test potentials and for tetanization. On the next day the animals were again habituated for 20 min. Following this, three to four series of test potentials were recorded with an interval of 5 min between each series. Test substances were injected 5 min after recording of the last test potential series. Further test potentials were recorded 30 min after injection of MK 801, pentobarbital or diazepam; 60 min after injection of phenytoin; and 120 min after injection of CGP 43487. Long-term potentiation was induced immediately thereafter. The time-course of potentiation was followed until 48 h after tetanization. Two groups of animals, injected with an identical volume of physiological saline, served as control groups and were randomly intermingled with the substance-injected rats: one for pentobarbital, MK 801 and CGP 43487 and another for diazepam and diphenylhydantoin.

2.6. Substances

All substances were injected intraperitoneally as follows: Dizocilpine (MK 801, (+)-5-Methyl-10.11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5.10-imine maleate, Merck Sharp and Dohme Research Laboratories), 0.2 or 0.3 mg/kg; CGP 43487 (the (D)-enantiomer from DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid-carboxyethylester, a generous gift from Dr. Olpe, CIBA-Geigy, Basel), 10 mg/kg; phenytoin (Phenhydan®, Desitin Arzneimittel), 50 mg/kg; pentobarbital sodium (Sigma) 10 mg/kg; diazepam (Faustan®, Arzneimittelwerk Dresden), 0.5 mg/kg.

2.7. Statistical evaluation

The values for the amplitude of the population spike after tetanization were expressed as % deviation from the baseline (100%). Statistical comparison of the potentiation data was performed with the Mann–Whitney *U*-test. The significance of kindling development was assessed with the 'repeated measurement' model. The significance level was fixed at 0.05.

3. Results

3.1. Kindling experiments

As Fig. 3A, Fig. 4A and Fig. 5A show, in control animals (saline/pentylenetetrazol) the kindling procedure induced behavioural seizures of increasing severity, culminating in tonic-clonic seizures of stages 4 to 5 after 10 injections. Some differences in kindling development between the control groups were seen after injection 4 and 5. These variations were not significant, however and, all animals reached stable stage four to five seizures in the last kindling stimulations. Drug pretreatment influenced the kindling development differently. The NMDA receptor antagonists, MK 801 and CGP 43487, considerably depressed kindling development. After 10 injections only seizure stages of 2.4 ± 0.18 and 2.2 ± 0.44 were obtained ($F(1,14) = 13.06$, $P < 0.003$ and $F(1,25) = 52.26$, $P < 0.0001$). Pentobarbital at a non-sedative dosage was more effective. Even after 10 kindling injections a mean seizure stage of only 0.2 ± 0.14 was obtained ($F(1,29) = 114.95$, $P < 0.0001$). Diazepam at the comparatively small dosage of 0.5 mg/kg also reduced seizure occurrence ($F(2,30) = 10.82$, $P < 0.0001$). On the other hand, phenytoin had no influence. After 10 injections seizure stage 4 was still observed. The differences from the saline control group were not significant.

3.2. Field potentials and long-term potentiation in the dentate gyrus

As Fig. 2 indicates, the substances had minor effects on the population spike amplitude. Only after injection of

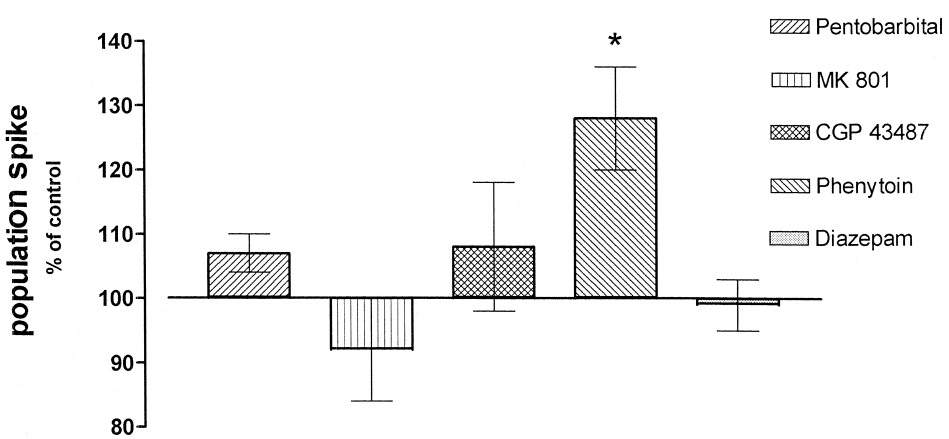

Fig. 2. Effects of dizocilpine (MK 801), CGP 43487, pentobarbital, diazepam and diphenylhydantoin (phenytoin) on the population spike amplitude of the evoked potential 30 min, 60 min or 120 min after application, respectively. The values express % deviation from control potentials before application. Bars indicate S.E.M.; $* P < 0.05$. Number of animals as indicated in Figs. 3–5.

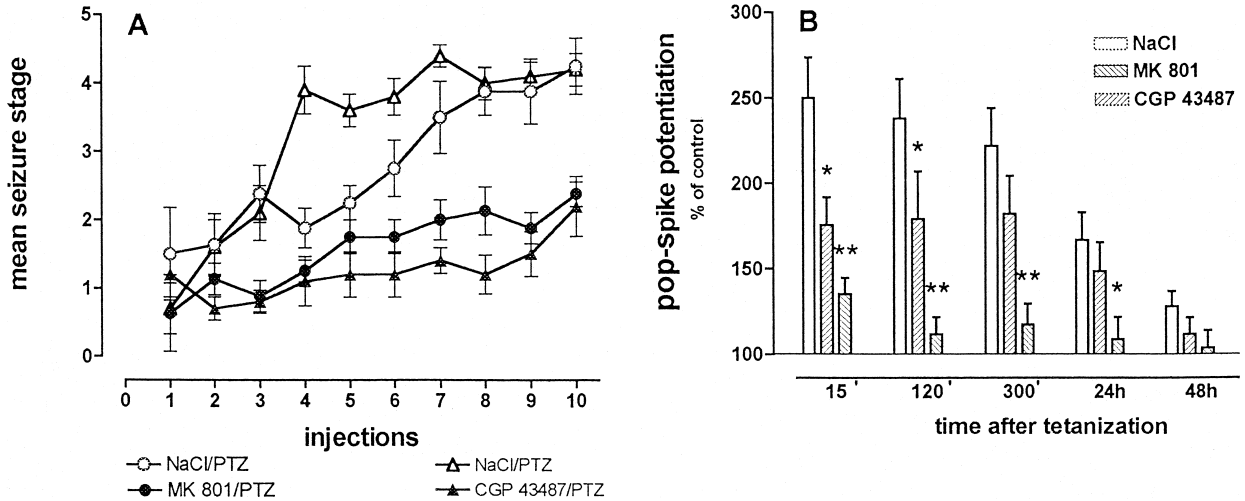

Fig. 3. Effects of the non-competitive NMDA receptor antagonist, dizocilpine (MK 801), and the competitive NMDA receptor antagonist, CGP 43487, on kindling development in a pentylenetetrazol kindling schedule and on long-term potentiation in the dentate gyrus. (A) Influence on kindling development: pretreatment with MK 801 = 30 min before PTZ, CGP 43487 = 120 min before PTZ: Number of animals = $n = 8$ and $n = 14$ for NaCl/PTZ, $n = 8$ for MK 801/PTZ, $n = 13$ for CGP 43487/PTZ. Bars indicate S.E.M. Open symbols for control groups correspond to those for the pretreated groups. (B) Influence on long-term potentiation: pretreatment with MK 801 = 30 min before tetanization, pretreatment with CGP 43487 = 120 min before tetanization. Number of animals $n = 10$ for NaCl control group, $n = 8$ for MK801, $n = 10$ for CGP 43487. Abscissa = % deviation of the population spike amplitude from control potentials taken 5 min before tetanization (100%). Bars indicate S.E.M., $**P < 0.02$, $*P < 0.05$.

50 mg/kg did diphenylhydantoin increase population spike amplitude, to 128% of the control records ($P < 0.05$). In preliminary experiments ($n = 8$ animals) this significant increase persisted for 2 h. Pentobarbital, MK 801, CGP 43487 and diazepam had no effect.

Long-term potentiation was differently influenced by the substances. As can be seen in Fig. 3B, in animals injected with physiological saline, tetanization induced a strong potentiation of the population spike to $249 \pm 24\%$ of the control value. This enhancement was also seen 24 h ($167 \pm 16\%$) and 48 h ($128 \pm 9\%$) after tetanization.

As Fig. 3B shows further, the non-competitive NMDA receptor antagonist, MK 801, markedly depressed potentiation at the low dose of 0.2 mg/kg. Immediately after tetanization an amplitude increase to $135 \pm 9\%$ of the control value was observed, and after 24 h and 48 h, the control levels were almost reached. The orally active competitive NMDA receptor antagonist, CGP 43487, also de-

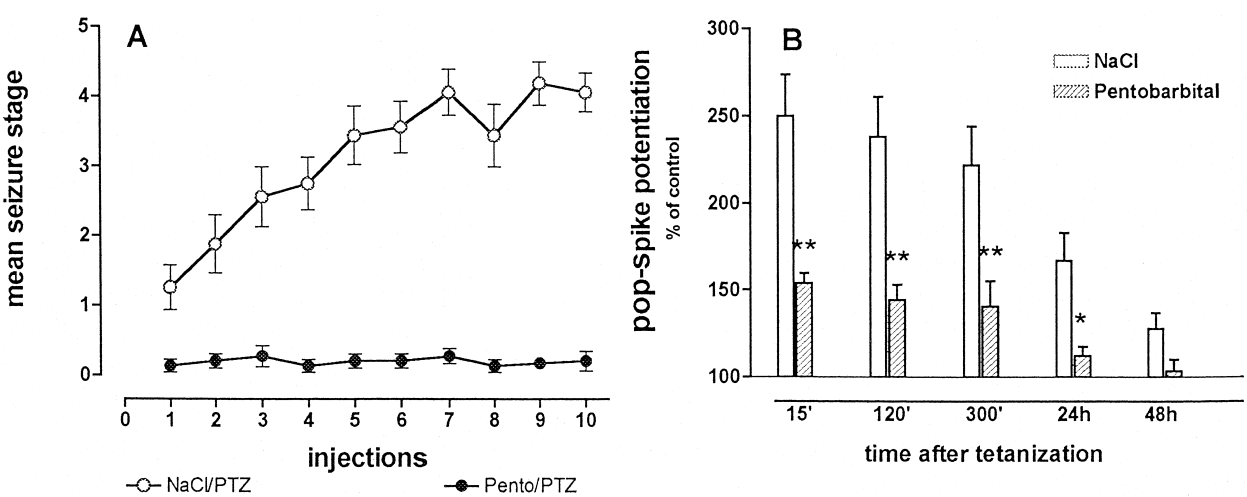

Fig. 4. Effect of pentobarbital on pentylenetetrazol kindling development and on long-term potentiation in the dentate gyrus. (A) Influence on kindling development: pretreatment with pentobarbital = 60 min before PTZ. $n = 16$ for NaCl/PTZ, $n = 15$ for pentobarbital/PTZ. Bars indicate S.E.M. (B) Influence on long-term potentiation: pretreatment with pentobarbital = 30 min before tetanization. $n = 10$ for NaCl control group, $n = 10$ for pentobarbital group. Abscissa = % deviation of the population spike amplitude from control records taken 5 min before tetanization (100%). Bars indicate S.E.M., $**P < 0.02$, $*P < 0.05$.

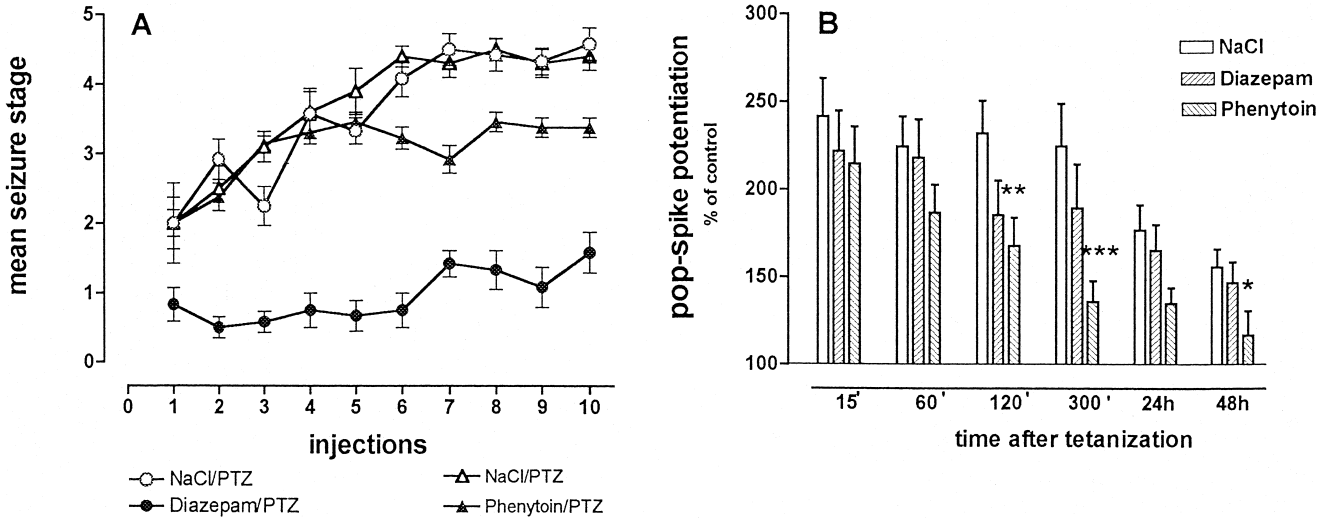

Fig. 5. Effects of diazepam and diphenylhydantoin (phenytoin) on pentylenetetrazol kindling development and on long-term potentiation in the dentate gyrus. (A) influence on kindling development: pretreatment with diazepam = 60 min before PTZ, pretreatment with phenytoin = 60 min before PTZ. Number of animals $n = 12$ and $n = 10$ for NaCl/PTZ, $n = 12$ for diazepam/PTZ, $n = 13$ for phenytoin. Open symbols for the control groups correspond to those for the pretreated groups. (B) Influence on long-term potentiation: pretreatment with diazepam = 30 min before tetanization, pretreatment with phenytoin = 60 min before tetanization. $n = 12$ for the NaCl control group, $n = 9$ for diazepam, $n = 9$ for phenytoin. Abscissa = % deviation of the population spike amplitude from control records taken 5 min before tetanization. Bars indicate S.E.M., $***P < 0.002$, $**P < 0.02$, $*P < 0.05$.

pressed long-term potentiation. Shortly after tetanization the population spike amplitude increased to 171 ± 16% of the control values. Twenty-four hours after tetanization, 148 ± 17% of control amplitude was seen and 111 ± 10% after 48 h. However, the depression of potentiation was not as strong as that with MK 801. A significant difference from the control group was found only in the first 2 h after tetanization.

As is shown further in Fig. 4B, 10 mg/kg pentobarbital, which completely prevented kindling development, depressed long-term potentiation in the same way as did the NMDA receptor antagonists. Fifteen minutes after tetanization the population spike increased to 153 ± 6% of the control, whereas 24 h afterwards, it was 112 ± 5% of the control and 48 h afterwards the control levels were reached again. The differences from potentiation in the control group were significant, with $P < 0.002$ or $P < 0.02$ during the whole observation period.

On the other hand, diazepam, which depressed seizure development during kindling, had no influence on long-term potentiation. As can be seen in Fig. 5B, the population spike increased to 241 ± 22% of the control values in the saline-injected group and to 221 ± 23% in the substance-treated group. There were no significant differences between groups over time. However, after injection of 50 mg/kg diphenylhydantoin, which only incompletely prevented seizures during kindling development, there was a different effect. Shortly after tetanization the population spike amplitude increased to 215 ± 21% of the control value. During the first 2 h post-tetanization there were no significant differences from the control group. However, 2 h after tetanization potentiation declined gradually to baseline values and there were significant differences from the control group.

4. Discussion

The relationship between kindling and long-term potentiation still remains a matter for discussion. In earlier publications (e.g., Cain, 1989) differences between the two phenomena were clearly described. However, potentiation of field potentials during and after electrical kindling has frequently been reported (e.g., Racine and Cain, 1991; Sutula, 1991). A connection between kindling and long-term potentiation has also recently been proposed by McEachern and Shaw (1996). Our previous results obtained in the dentate gyrus and the CA1 region also point to close connections between the two phenomena (Rüthrich et al., 1996; Krug et al., 1997). This led to our assumption that cellular mechanisms responsible for induction and maintenance of long-term potentiation are also involved in the processes underlying kindling.

In order to further substantiate such a proposed relationship we compared the potency of anticonvulsive drugs, which possess different mechanisms of action, to influence both pentylenetetrazol kindling and long-term potentiation in freely moving animals. Therefore, we used dosages which induced no serious side-effects,in other words, the dosages were of physiological relevance.

The results presented here confirm and extend findings of others, indicating similar influences of NMDA receptor antagonists and pentobarbital, but different actions of diazepam, on kindling development and on long-term poten-

tiation. Diphenylhydantoin had another profile of action. Kindling was not influenced by this compound and only the late maintenance of long-term potentiation was decreased. In particular, it is not surprising that MK 801, a non-competitive NMDA receptor antagonist, and CGP 43487, an orally active competitive NMDA receptor antagonist, depress kindling development and long-term potentiation similarly. These results agree well with others obtained with electrical or chemical kindling (e.g., McNamara et al., 1988; Mintz et al., 1990; Schmutz et al., 1990; Giorgi et al., 1991; Morimoto et al., 1991) or long-term potentiation (e.g., Morris et al., 1986; Gilbert and Mack, 1990; Maren et al., 1991; Krug et al., 1993; Stan Leung and Shen, 1993; Lee et al., 1996). The weak action of CGP 43487 in our potentiation experiments may have been a result of the relatively low dosage used. Higher dosages cause behavioural side-effects. The precursor of the substance, CGP 39551, which is well characterized as a competitive NMDA receptor antagonist (Pozza et al., 1990) influenced long-term potentiation in the dentate gyrus at a dose of 30 mg/kg (Maren et al., 1992). Thus, the similarity of effects in both experimental protocols appears to support our proposals.

Interestingly, at a dose with only minimal sedative effects, pentobarbital also depressed kindling development and long-term potentiation in the same manner and was as effective as the NMDA receptor antagonists. Effects on electrical kindling have been reported (Wada et al., 1976; Schmutz et al., 1988; Silver et al., 1991; Hirayama et al., 1995), but barbiturates have been tested only occasionally with pentylenetetrazol kindling (Becker et al., 1995). Barbiturates also have been shown to decrease long-term potentiation in the CA1 region of hippocampal slices (Scharfman and Sarvey, 1985; Lee et al., 1996) and in in vivo preparations at anesthetic dosages (Dragunow et al., 1989; Jeffery et al., 1990). However, to our knowledge, a similar effect of pentobarbital on kindling and long-term potentiation at such a weak dose has not been reported before. In our opinion, the results are of interest for two reasons: Firstly, the depression of long-term potentiation highlights the risks in the interpretation of experimentally induced synaptic plasticity in anesthetized animals (see Riedel et al., 1994). Secondly, pentobarbital has antikindling effects at a non-sedative dose, which, in preliminary experiments, did not affect locomotor activity. The beneficial effect of pentobarbital on kindling at a dose without strong side-effects might reflect therapeutic potential. It also blocks long-term potentiation, a process which is initiated by activation of glutamate receptors. The main mechanism of action of barbiturates is considered to be GABA receptor-agonistic. Effects on modulatory transmitter systems were also described (for review, see Rogawski and Porter, 1990). However, barbiturates also block or diminish glutamatergic transmission (Morgan, 1991). Pentobarbital seems to act predominantly at the kainate/quisqualate receptor (Morgan et al., 1991; Cai and McCaslin, 1993) but a direct influence on NMDA receptor-coupled processes has also been observed (Charlesworth et al., 1995). Moreover, L-type and N-type Ca^{2+} channels are also influenced (Gross and MacDonald, 1988). Therefore, it could be assumed that the depression of long-term potentiation is due to a combined action on different glutamate receptors and Ca^{2+} channels, thus preventing the rise of intracellular Ca^{2+}, which is the key event in LTP induction. This mechanism may also be involved in kindling depression. Diazepam, a GABA receptor-agonistic substance did not influence long-term potentiation but did influence kindling development. Others have also reported either no influence on potentiation or weak effects at higher dosages, (e.g., Birnstiel and Haas, 1991; Del Cerro et al., 1992; McNamara et al., 1993; Seabrook et al., 1997). These different effects of diazepam on kindling development and long-term potentiation are of special interest. Although seizure development during kindling was depressed, which demonstrates acute anticonvulsive effects of diazepam, kindling-related behavioural disturbances were not prevented. Also, kindling-induced potentiation phenomena in the hippocampus were not influenced (Rüthrich et al., 1992). This suggests that kindling is a multifactorial complex which involves plastic adaptive changes at different levels, e.g., convulsions, neurophysiological and neurochemical alterations and changes in behaviour. It might be hypothetized that each of these components can be specifically modified by pharmacological treatment.

The effects of diphenylhydantoin are difficult to interpret. Kindled seizures did not appear to be sufficiently suppressed or retarded by this compound. Thus, the effects seen with electrical kindling models are also confirmed with chemical kindling (e.g., Racine et al., 1975; Wada et al., 1976; Schmutz et al., 1988). Concomitantly, the effect on long-term potentiation differs from that of the other substances. The maintenance phase rather than the initial potentiation was interfered with. Thus, the findings of Stringer and Lothman (1988) and Birnstiel and Haas (1991) must be reconsidered since an absence of effect in these studies was the conclusion on the basis of a short recording period after tetanization. Instead, the finding of Lee et al. (1996) in the CA1 region was confirmed. The relatively strong and long-lasting increase of the field potential after phenytoin in our experiments might have interfered with and masked the effect on potentiation. However, 4–5 h after tetanization when the effect of phenytoin on the field potential was normalized, there was a significant difference in potentiation between the saline-injected controls and the phenytoin injected animals. Teyler et al. (1994) reported that, besides an early potentiation which depends on NMDA receptor activation, a kind of potentiation also exists which is induced by gating voltage-dependent Ca^{2+} channels (so called VDCC long-term potentiation). This kind of potentiation was influenced by phenytoin (Lee et al., 1996). With our tetanization protocol (200 Hz without

blocking of NMDA receptors) a combination of the two kinds of long-term potentiation would be induced. Considering the effects of diphenylhydantoin on various voltage-gated ionic channels (for review see Rogawski and Porter, 1990), also including Ca^{2+} channels (Kobrinsky et al., 1994), it might be that the decline of potentiation reflected a preferential influence on that kind of potentiation with minimal effects on NMDA receptor-dependent potentiation.

Taken together, our results show that NMDA receptor antagonists and pentobarbital applied in comparable dosages depress chemical kindling development and long-term potentiation similarly in the dentate gyrus.This indicates that, as is the case with induction of long-term potentiation, a glutamatergic component also plays an important role in pentylenetetrazol kindling. Considering the results reviewed by McEachern and Shaw (1996) which indicate changes in glutamatergic transmission after kindling also, it can be hypothetized that, during kindling development, mechanisms are activated and persistently changed which are also responsible for induction and maintenance of long-term potentiation. However, the different effects of a benzodiazepine seen in our experiments further indicate that kindling cannot be reduced to long-term potentiation, but is indeed a more complex phenomenon which involves changes in very different neuronal systems which in turn lead to the general changes seen in behaviour.

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