Anticonvulsant Drug Effects in the Direct Cortical Ramp-Stimulation Model in Rats: Comparison with Conventional Seizure Models

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ABSTRACT

A modified cortical ramp stimulation (CRS) model has been developed allowing repeated determinations of seizure threshold at short time intervals in individual rats without inducing postictal threshold increases. Anticonvulsant potency of the standard antiepileptic drugs carbamazepine, phenytoin, phenobarbital, valproate, diazepam and ethosuximide in the CRS model was compared with respective drug potencies in two more traditional seizure models with transcorneal stimulus application, i.e., the minimal electroshock seizure threshold (minEST) and the maximal electroshock seizure threshold (maxEST). In the CRS model, two different types of threshold were determined, the threshold for localized seizures (TLS) and the threshold for generalized seizures (TGS). When screw electrodes were implanted over the primary motor cortex, TLS was characterized by unilateral forelimb clonus, tonic abduction of contralateral forelimb, and head adversion. When ramp-shaped stimulation was continued above the TLS current, bilateral clonic forelimb seizures with loss of posture developed, which was defined as TGS. In contrast to TLS, TGS could not be repeatedly determined at short time intervals because of postictal threshold increase. TLS was dose-dependently increased by carbamazepine, phenobarbital, valproate and diazepam, although phenytoin showed a truncated dose-response, and ethosuximide was ineffective. In comparison to TLS, drug-induced increases in TGS were more marked. All drugs dose-dependently increased minEST and, except ethosuximide, maxEST. For comparison of drug potencies, doses increasing seizure thresholds by 20 or 50% were calculated from dose-response curves. Respective comparisons showed marked differences in drug potencies between models, indicating that the CRS method presents a model of another, more pharmacoresistant seizure type than seizure types induced in traditional models, such as transcorneal electroshock. Based on the location of electrodes in the frontal neocortex, the characteristic seizure pattern, and the low pharmacological sensitivity of the seizures to standard antiepileptics, the modified CRS model most likely represents a new model of localization-related seizures occurring in frontal lobe epilepsy and may thus be used in the search for novel drugs with higher efficacy against this difficult-to-treat type of epilepsy.

Seizures induced by electrical stimulation in rodents, such as in the MES test or kindling, are widely used to detect and quantify the anticonvulsant effect of new compounds during antiepileptic drug development (Löscher and Schmidt, 1988; Fisher, 1989; White et al., 1995; White, 1997). One drawback of such seizure models is that anticonvulsant activity can be reliably determined at only one time point after drug administration in the same animal, because postictal increases in seizure threshold inhibit subsequent seizure induction and alter anticonvulsant drug activity (Mucha and Pinel, 1977; Freeman and Jarvis, 1981; Green, 1986; Löscher and Hönnack, 1990). For instance, after the administration to rats of a single electroshock for 1 sec, there is a marked rise in seizure threshold that lasts for at least 3 hr (Green, 1986). Thus, for determination of the time-course of anticonvulsant effects, separate groups of animals have to be used per time point after drug administration so that considerable numbers of animals are needed in this respect. A further disadvantage of most conventional electrical seizure models is that, because of postictal refractoriness, seizure threshold can not be reliably determined in individual animals, which for instance prohibits to measure seizure threshold before and after drug administration in the same animal. In 1989, Voskuyl et al. (1989) described a new seizure model in which the threshold for convulsions in individual rats can be repeatedly determined by applying ramp-shaped pulse trains via bilaterally implanted screw electrodes directly to the cerebral cortex. Because in this model the repeated threshold determination

ABBREVIATIONS: CRS, cortical ramp-stimulation; maxEST, maximal electroshock seizure threshold; MES, maximal electroshock seizures; minEST, minimal electroshock seizure threshold; PEG, polyethylene glycol; PTZ, pentylentetrazol; TGS, threshold for generalized seizures; TID, threshold increasing dose; TLS, threshold for localized seizures; EEGs, electroencephalograms.
at short intervals is possible without concomitant changes in threshold or characteristics of seizures, it appears to be ideally suited to study the potency and time-course of anticonvulsant drug effects in individual rats (Voskuyl et al., 1989). In a subsequent study, Voskuyl et al. (1992) reported that the CRS model allows the distinction of two different types of seizure threshold, a threshold for localized and for generalized seizure activity (TLS and TGS, respectively). In analogy to one of the test endpoints used in the minimal electroshock seizure threshold test described by Swinyard (1972), the start of forelimb clonus during ramp-shaped stimulation was arbitrarily defined as the TLS (Voskuyl et al., 1992; Hoogerkamp et al., 1994). If ramp-shaped stimulation was continued to about 30% above the TLS current, clonic activity became more severe and more generalized, which was defined as TGS (Voskuyl et al., 1992; Hoogerkamp et al., 1994). As pointed out by Hoogerkamp et al. (1994), this method was developed to combine various forms of the classical electroshock test (minimal and maximal electroshock seizures) and the determination of their thresholds in a single test. Based on the effect of several antiepileptic drugs on TLS and TGS in the CRS model, Voskuyl's group concluded that the model allows differentiation in a single test between a drug's effect on seizure initiation (TLS) and seizure propagation (TGS) in a quantitative way, a feature not offered by any other seizure model (Voskuyl et al., 1992; Hoogerkamp et al., 1994).

However, until now, the predictive value of the CRS model in terms of drug potency and efficacy against specific types of epileptic seizures or types of epilepsy is not known. With respect to drug potencies, no dose-response experiments in the CRS model were published which allow comparison with drug potencies in more traditional tests, such as the MES test. Our main aim was to directly compare anticonvulsant potencies of various antiepileptic drugs in the CRS seizure threshold model, using TLS and TGS as endpoints, with drug potencies in two conventional electroshock seizure models with bilateral transcorneal stimulation, i.e., the minEST and the maxEST (cf., Swinyard, 1972). Furthermore, based on the clinical characteristics of the seizures, on the location of the cortical stimulation electrodes and on antiepileptic drug potency, the study should clarify for which type(s) of seizure the CRS model may yield predictive pharmacological data. All experiments were performed in the same rat strain, i.e., female Wistar rats, as used in the experiments of Voskuyl's group, to avoid that strain differences in seizure susceptibility affect the comparison with previous pharmacological studies in the CRS model (Voskuyl and van Rijn, 1996).

**Methods**

**Animals.** Female Wistar rats (Harlan-Winkelmann, Borchern, Germany), weighing 190 to 220 g, were used. The animals were purchased from the breeder at a body weight of about 200 g. After arrival in the animal colony, the rats were kept in a vivarium under controlled environmental conditions (ambient temperature 22-24°C, humidity 50-60%, 12/12 hr light/dark cycle, artificial light on at 6:00 a.m.) for at least 1 wk before being used in the experiments. Standard laboratory food (Altromin 1324 standard rat diet) and tap water were allowed ad libitum. All experiments were done in a laboratory with the same environmental conditions as the vivarium. Temperature and humidity were continuously controlled in both vivarium and laboratory. All drug or vehicle applications were done at about the same time the morning to minimize circadian influences. Rats were adapted to the conditions of the vivarium and laboratory for at least 10 days before being used for experiments.

**Electrode implantation.** For implantation of electrodes, rats were anesthetized with chloral hydrate (380 mg/kg i.p.) and placed in a stereotaxic frame according to the method of Paxinos and Watson (1986). The skull surface was exposed and after trepanation two small stainless steel screw electrodes of 1.2 mm diameter were implanted bilaterally at different locations, based on preliminary experiments with verification of locations after necropsy. In a first series of experiments, the electrodes were implanted over the frontoparietal (somatosensory) neocortex as described by Voskuyl's group (Hoogerkamp et al., 1994 and 1996) at the following coordinates (relative to Bregma in mm): AP -1.0, L 3.5 (fig. 1A). Some rats were implanted with L ±3.0, corresponding to the initial description of Voskuyl et al. (1989). In a second series of experiments, these coordinates were modified to reach a more reliable endpoint for seizure threshold determinations (see below). Based on the organization of motor and somatosensory neocortex of the rat (Hall and Lindholm, 1974; Neafsey et al., 1986), the screw electrodes were implanted over the projection field of the forelimb, the caudal forelimb area considered to be the equivalent of the forelimb area of the primate primary motor cortex (Rouiller et al., 1993), in the frontal neocortex at the following coordinates: AP +1.0, L ≥3.5 (see fig. 1B). The screw electrodes were lowered approximately 1 mm below the surface of the bone to penetrate the dura without lesioning the cortex. To form the screw electrodes, a 0.1-mm Teflon-insulated stainless steel wire with a standard microelectronic connector was soldered to the head of the screws. The electrode assembly was combined to form a female connector and was affixed to the skull with dental acrylic cement, using three additional anchor screws for fixation to the skull (see fig. 1). Determination of cortical electrical threshold was initiated after at least 10 days of recovery after surgery. The integrity of the cortical surface was controlled after the experiments using standard histological techniques.

In some rats, bipolar electrodes (one per rat) were implanted at a depth of 2 mm (V -2.0) at different locations in the frontoparietal cortex (see fig. 1C) for recording of EEGs before and after electrical stimulation via the screw electrodes. The anchor screw over the olfactory bulb was used as grounding electrode. Based on the technique used for ramp stimulation (see below), it was not possible to record the EEG directly at the location of electrical stimulation.

**Ramp generator for seizure threshold determinations.** Because a ramp generator as used by Voskuyl et al. (1989) was not commercially available, a digital controlled generator was custom-designed to produce the ramp-shaped pulse trains (for details see Rundfeldt et al., 1995). When starting the ramp stimulation, a digital pulse counter was reset to zero and the pulse train was enabled to increment the counter. The stimulation could be stopped at any time with an internal or external stop pulse. This pulse stopped the counter and shut down the pulse train. The reading of the counter was preserved on a four digit display and used to calculate the peak to peak current of the last pulse applied to the animal (Rundfeldt et al., 1995). Maximal 4095 pulses were applied for one stimulation train. At a rate of 50 bipolar pulses each second the maximal stimul- ation duration was 81.9 sec. Duration of each bipolar pulse was 2 msc, and current intensity linearly increased with 1.95 µA/pulse. Because the stimulus was stopped at the first sign of a TLS or TGS convulsive behavior, the stimulus duration varied from animal to animal and between control and drug trials. The current setting was selected to produce a stimulus duration between 10 and 30 sec for control and drug experiments.

**Determination of seizure threshold in the cortical ramp stimulation model.** For determination of TLS or TGS, a single train of bipolar rectangular pulses (total pulse duration 2 msc, 50 pulses/sec) with steadily increasing (ramp-shaped) current amplitude was applied directly to the cortex through the two screw electrodes in freely moving rats, using a flexible cable that connected the
stimulator to the rat during stimulation and concurrent and subsequent observation. For determination of TLS, stimulation was interrupted at the onset of the first clear sign of convulsive behavior, and the current of the last pulse applied was defined as TLS. For determination of TGS, the ramp-shaped stimulation was continued until more severe signs of seizure activity, e.g., prolonged generalized clonic activity, appeared.

Using the electrode location described by Hoogerkamp et al. (1994, 1996), i.e., 3.5 mm left and right of the midline of the skull and 1.0 mm posterior to bregma (fig. 1A), i.e., above the frontoparietal (somatosensory) neocortex, no consistent seizure pattern was observed on ramp stimulation, but the individual convulsions differed from rat to rat. The same was true when the coordinates initially described by Voskuyl et al. (1989), i.e., 3.0 mm left and right of the midline and 1.0 mm posterior to bregma were used. In the same rat, the induced seizures were strictly reproducible on repeated determination of seizure threshold. Furthermore, seizure severity recorded at threshold currents did not change over the period of numerous threshold determinations, and seizure activity stopped immediately when ramp-shaped pulse trains stimulation was interrupted, indicating that seizure duration did not change upon repeated TLS determinations. In most rats, the first signs of seizure activity (which was used as endpoint for TLS determinations) were postural symptoms with loss of posture, vocalization and/or backward or stooping movements of the body, sometimes preceded by arrest reactions. Forelimb clonus was not consistently seen in most rats at TLS currents. If stimulation was continued, bilateral clonic seizures appeared after the initial convulsive signs, but this more generalized seizure activity induced prolonged postictal threshold increases and could therefore not be used for repeated threshold determinations.

To obtain a more consistent seizure endpoint for TLS determinations, the screw electrode location was changed to 1.0 mm anterior to bregma as shown in figure 1B. This resulted in a much higher incidence of forelimb clonus and a more consistent reaction to ramp stimulation. About 70% of all rats stimulated via this modified electrode location exhibited a short repetitive unilateral forelimb clonus as first convulsive sign upon ramp stimulation. The unilateral forelimb clonus was associated with tonic abduction of the contralateral forelimb and adhesion of the head. This combination of unilateral forelimb clonus, tonic abduction of contralateral forelimb and head adhesion was the typical seizure pattern induced as earliest sign of convulsive activity by ramp-stimulation at this electrode location in the frontal (primary motor) neocortex, and consequently this seizure pattern was defined as TLS. In a minority of animals, isolated myoclonic seizures, sometimes followed by bilateral asymmetrical tonic abduction of forelimbs, in part associated with rearing, were the earliest signs of convulsive activity and were therefore used for TLS determination. Often, the seizures were associated with eyelid closure. In contrast to the more posterior electrode position shown in figure 1A, no loss of posture or vocalization was seen at TLS currents. The individual characteristics of seizure activity at TLS current were highly reproducible in each animal. All these signs of seizure activity aborted immediately when stimulation was stopped at this point, and animals resumed normal behavior without any signs of postictal changes. The TLS was often preceded by repetitive motor activity, such as circling, whisker movements or backward movements. EEG recordings during the convulsive symptoms described above did not show paroxysmal alterations in cortical locations posterior from the location of stimulation (see fig. 1C), indicating that seizure activity was relatively localized to the focus of stimulation, so that the term TLS is adequate.

When ramp-shaped stimulation was continued above the TLS current, clonic activity became more severe and more generalized with bilateral forelimb clonus, face and ear clonus, and, in part, rearing and falling, thus resembling secondarily generalized seizures in the kindling model. Tonic extension of fore- or hindlimbs was not seen at the maximally applied currents of 6 mA. In contrast to the TLS, seizure activity continued for 10 to 40 sec after stimulation was stopped at the occurrence of generalized seizure activity. EEG recordings showed epileptiform spiking activity at the locations of EEG electrodes shown in figure 1C, demonstrating the generalization of epileptic activity from the foci in the neocortex. However, in the absence of EEG recording, it was often difficult to accurately determine the onset of generalized convulsive activity. Thus, to allow a clear and reproducible differentiation from TLS, TGS was defined as bilateral forelimb clonus with loss of posture.

The TLS current decreased by 30 to 40% during the first 2 wk of twice daily threshold determination, but thereafter continued testing did not result in further marked changes in threshold. Seizure pattern or seizure severity at TLS currents did not change obviously during the stabilization period. After stabilization, TLS currents determined via stimulation at the location shown in figure 1B were about 1500 to 2500 μA, which was considerably higher than TLS currents determined via electrodes locations shown in figure 1A, which ranged up to about 1000 μA. Even higher currents were needed when the dura was not penetrated, whereas TLS currents decreased when the tip of the screw electrode reached into the neocortex. In all experiments described in ‘Results,’ rats with screw electrodes at the neocortical locations shown in figure 1 with electrodes penetrating the dura but not the neocortex were used.

Antiepileptic drug testing in the cortical ramp stimulation model

Threshold determinations were started after a post-surgery recovery time of at least 10 days. In the next 2 wk, TLS was determined twice daily with an interval of 4 to 6 hr until stable TLS currents were reached. Before the first drug experiments in a group of rats, several TLS determinations with vehicle application were done to test stability of the threshold responses. The protocol used for these vehicle controls and all subsequent drug experiments was as follows: In each rat, the individual pre-injection (baseline) TLS was determined three times at 15-min intervals, followed by i.p. injection and subsequent TLS determinations at 25, 5, 1, 1.5, 2, 2.5, 3 and 4 hr after injection. In case of prolonged drug activity, further threshold determinations were done up to 24 hr after drug (or vehicle) injection. Groups of 6 to 16 rats were used for drug and vehicle trials. During the first drug experiments in a group of animals, each drug trial was preceded by a vehicle control trial (usually 2 days before the drug trial) with the same fixed time intervals after injection. After several vehicle control trials were performed in this way without any evidence of significant vehicle effects, further drug experiments were undertaken without separate control trials for each dose of the respective drug, but at least one vehicle control trial was done per drug. At least 5 to 7 days (depending on the dose) were interposed between two drug experiments in the same group of rats.

In case of TGS, the experimental protocol used for drug testing had to be changed because, in contrast to TLS, it was not possible to repeatedly determine TGS at short time intervals in the same rat without postictal increases in seizure threshold (see ‘Results’). Furthermore, because of these postictal changes, it was not possible to determine TGS before and after drug administration on the same day in the same group of animals. Therefore, control TGS after vehicle injection was determined 2 days before drug administration, and in the subsequent drug trial TGS was determined at only one time point after drug injection. In all experiments with TGS determination, TLS was also quantified. In experiments on TLS and TGS in the CRS model, at least three different doses were tested per drug to allow quantification and comparison of drug potency (see ‘Statistics’).

Determination of minimal and maximal electroshock seizure threshold via corneal electrodes. For determination of conventional seizure thresholds via bilateral transcorneal stimulation, a stimulator (BMT Medizintechnik GmbH, Berlin, Germany) was used which delivered a constant current (adjustable from 1-200 mA) regardless of impedance of the test object; self-adjusting stimulus
Results

Characteristics of the CRS model in rats. Using the modified CRS model with electrode position as shown in figure 1B and characterized by unilateral forelimb clonus, tonic abruption of contralateral forelimb and head adversion...
as endpoint for TLS, once TLS had stabilized, repeated TLS determinations at short intervals were possible without any postictal increase in seizure threshold (fig. 2A), a prerequisite for using this model for drug testing with repeated TLS determinations before and after drug administration. However, this was not possible in case of TGS. As shown in figure 2B, repeated determination of TGS in the same rats at short intervals led to significant increases in TGS and TLS. We tried to resolve this problem by decreasing the TGS current to values at which just generalized clonic activity without loss of posture occurred, but this again led to postictal threshold increases in most animals (not illustrated) and problems in clear separation of TGS from TLS. Therefore, in contrast to TLS, TGS was not repeatedly determined in the same rats on the same experimental day, but only once per day, which led to reproducible values.

In some groups of rats, a decrease of TLS was seen during repeated TLS determinations in the forenoon (see for instance data from vehicle control in fig. 3A), but values returned to initial values in the afternoon, possibly indicating diurnal alterations in seizure threshold as known from animal models (Woodbury, 1969). This, however, was not seen in all groups used in the present experiments. It is important to note that rats even after 200 TLS determinations did not show any signs of anxiety, aggression or pain before, during or after TLS or TGS determinations.

Time course and potency of anticonvulsant effects of antiepileptic drugs on TLS in the CRS model. Except otherwise indicated, all data described in the following for the CRS model are from rats with ramp-shaped stimulation via bilateral screw electrodes over the frontal neocortex as illustrated in figure 1B. Figure 3 illustrates typical trials
with repeated TLS determinations after i.p. vehicle or drug administration in the same group of rats. In this example, three doses of carbamazepine were tested and data were compared either with individual preinjection control data determined on the same day before injection of carbamazepine or with control data from two separate vehicle control experiments performed in the same group of rats 2 days before drug injection. At all three doses tested (5, 10 and 20 mg/kg i.p.), carbamazepine significantly increased TLS when data were compared to preinjection baseline (fig. 3). Peak threshold increases were seen after 30 min. Threshold increases at 30 min after 10 and 20 mg/kg were not only significantly different from predrug control values of the same day but also from the TLS determined 30 min after vehicle injection in the vehicle control experiments (P < .001). For comparison, we also determined the effect of carbamazepine, 20 mg/kg, on TLS in a group of 10 rats with screw electrodes at 1.0 mm posterior (instead of anterior) to bregma, i.e., the electrode location used in the studies of Voskuyl’s group. Carbamazepine significantly increased TLS with similar magnitude and time course as in the experiments with the modified electrode location (not illustrated); at 30 min after drug injection a significant threshold increase of 15.3% above predrug baseline was seen (P < .01). With respect to adverse effects of carbamazepine, no behavioral alterations were seen at 5 mg/kg, although ataxia was seen at the higher doses.

In figure 4A, the anticonvulsant effect of carbamazepine is illustrated as TLS-increase in μA above mean predrug baseline to demonstrate the dose-dependent increase in TLS seen after this drug. Data for vehicle control in this figure are means from the two vehicle control experiments.

In contrast to carbamazepine, phenytoin was less potent to increase TLS (fig. 4B). Although significant threshold increases were seen at all four doses tested (12.5, 25, 50 and 75 mg/kg i.p.) when data were compared to predrug baseline of the same day, there was no clear dose-dependence of phenytoin’s effect, and peak threshold increases reached after about 60 min were only about 10% above predrug control. After administration of 5 mg/kg in a group of 16 rats, there was no significant increase in TLS above preinjection control (not illustrated). Compared to vehicle experiments, significant threshold increases were seen 60 min after phenytoin doses of 12.5, 25 and 50 mg/kg (P < .05). At doses of 25 mg/kg and above, phenytoin induced ataxia and hypothermia.

Phenobarbital was more potent than phenytoin to increase TLS (fig. 4C). At all three doses tested (10, 20 and 40 mg/kg i.p.), the threshold was significantly and dose-dependently increased above predrug baseline, peak increases being reached at 30 min (fig. 4C). Compared to vehicle control experiments, the threshold increase induced by phenobarbital at 30 min was significant after 20 and 40 mg/kg (P < .01). In contrast to the experiments with other drugs, in which TLS steadily returned towards control after peak increases had been reached, the effect of phenobarbital appeared to be biphasic with a second peak of activity after 8 to 24 hr (fig. 4C). At 48 and 96 hr after drug administration, TLS was not significantly different from predrug baseline (not illustrated). Phenobarbital induced ataxia after doses of 20 or 40 mg/kg. Furthermore, transient hyperactivity was seen about 15 to 30 min after 40 mg/kg phenobarbital.

Valproate did not significantly increase TLS at 50 mg/kg, but significantly and dose-dependently increased TLS at 100 and 200 mg/kg with peak effects reached after 15 min (fig. 4D). The anticonvulsant effect was only short-lasting, so that TLS reached control values within 3 hr after injection. Threshold increases at 15 min after 100 and 200 mg/kg valproate were not only significantly different from predrug baseline but also from values determined 15 min after vehicle application in separate vehicle control experiments (P < .05). For comparison, we also determined the effect of valproate, 200 mg/kg, on TLS in two groups of 10 rats with screw electrodes at 1.0 mm posterior (instead of anterior) to bregma, i.e., the electrode location illustrated in figure 1A. Valproate significantly increased TLS with similar magnitude and time course as in the experiments with the modified

Fig. 4. Effect of carbamazepine, phenytoin, phenobarbital and valproate on TLS in the CRS model in rats. Data are shown as TLS increase (in μA) above mean preinjection baseline. In addition to the drug experiments, the mean values from two to three vehicle control experiments are illustrated per drug. Data are means and S.E. of 6 to 13 rats per drug and dose. Analysis of variance with post hoc testing indicated that, compared to individual preinjection control TLS, all drugs induced significant TLS increases (P at least < .05) at all doses shown, except the lowest dose of valproate. Statistical differences to individual vehicle control experiments are described in the text. Absolute TLS preinjection control values (means) in the two groups of rats used for the experiments ranged between 1432 to 1583 and 2294 to 2432 μA, respectively.
electrode location (not illustrated); at 15 min after drug injection a significant threshold increase of 22.3 and 20.7 above predrug baseline was seen (P < .01). With respect to adverse effects of valproate, ataxia, ‘wet dog shakes’ and hyperactivity were seen after 100 and 200 mg/kg.

To study the reproducibility of anticonvulsant drug effects in the CRS model, several experiments with carbamazepine, phenytoin, phenobarbital and valproate were repeated in other groups of 6 to 11 rats (not illustrated), yielding significant anticonvulsant effects of similar magnitude and time-course than the experiments described above.

Diazepam was tested at five doses in two different groups of rats (fig. 5, A and B). In a first series of experiments, diazepam was administered i.p. at 1.5, 3.0 and 5.0 mg/kg (fig. 5A). All three doses significantly increased TLS, both when compared to predrug baseline and (at 15 min) to separate vehicle control experiments (P < .05). As with valproate, the peak effect was reached 15 min after drug injection. In a second series of experiments in another group of rats, diazepam was administered at 0.2, 1.5, 5.0 and 10 mg/kg (fig. 5B).

Again, all doses significantly increased TLS compared to predrug baseline. Compared to vehicle controls, TLS increase 15 min after diazepam was significant for all doses (P < .05) except 0.2 mg/kg. When the percent TLS increases in the two experiments were compared for the same doses, comparable increases were obtained: 1.5 mg/kg, 11.2 and 12.7% above predrug baseline; 5 mg/kg, 15.4 and 16.4% above predrug baseline. Thus, percent TLS increase in response to drug administration was more reproducible between experiments than TLS increase expressed in μA above baseline (fig. 5), which was also seen in the other experiments with repeated testing of drugs in different groups of rats with different baseline TLS values. With respect to adverse effects, doses of 1.5 mg/kg and above induced transient hyperactivity, followed by sedation and ataxia.

Compared to individual predrug baseline, ethosuximide induced significant increases in TLS at 100, 200 and 400 mg/kg (fig. 5C). However, peak increases above predrug baseline were only in the range of 4 to 9%, which was not significantly different from vehicle control data. Although 100 mg/kg ethosuximide did not induce behavioral alterations, ataxia and ptosis were observed at 200 mg/kg, and hyperactivity, more intense ataxia, and ptosis at 400 mg/kg, which prohibited the use of higher doses.

Because in the experiments described above the same groups of rats had been used for several drug experiments, it was tested whether this procedure affects determination of anticonvulsant potency in the CRS model, using two groups of age-matched rats. One group of eight rats was repeatedly tested over 8 wk with vehicle injections up to a total of 200 TLS determinations before the first drug administration, whereas another group of eight rats was tested with drugs directly after TLS stabilization, and then drug testing was repeated after 8 wk with 200 TLS determinations. Thus, both groups received the same number of stimulations over the same period, the only difference was the testing with either drug or vehicle after TLS stabilization. Two drugs, carbamazepine (20 mg/kg) and valproate (200 mg/kg), were used for this experiment (not illustrated). The data from this experiment showed that the same group of rats can be used for repeated testing of antiepileptic drugs without any significant alteration in drug potency between experiments. Furthermore, even a high number of TLS determinations in the absence of drug does not seem to affect subsequent drug potency determinations.

Anticonvulsant effect of antiepileptic drugs on TGS in the CRS model. As described above, because of postictal changes in seizure threshold, determination of TGS could not be used for time course studies with antiepileptic drugs, but only one TGS determination was done after drug administration. The time after drug injection chosen for each drug was based on the time-course studies in the TLS experiments, being the time of peak drug effect. Data after drug administration were compared with a control TGS determined after vehicle 2 days before the respective drug experiment. Three drugs, valproate, carbamazepine and phenytoin, were tested in two different groups of rats (not illustrated in A and B). The data from this experiment showed that the same group of rats can be used for repeated testing of antiepileptic drugs without any significant alteration in drug potency between experiments. Furthermore, even a high number of TLS determinations in the absence of drug does not seem to affect subsequent drug potency determinations.

**Fig. 5.** Effect of diazepam and ethosuximide on TLS in the CRS model in rats. Data are shown as TLS increase (in μA) above mean preinjection baseline. In addition to the drug experiments, the mean values from respective vehicle control experiments are illustrated. Data are means and S.E. of 9 to 12 rats per drug and dose. Diazepam was evaluated in two different groups of rats (illustrated in A and B). Analysis of variance with post-hoc testing indicated that, compared to individual pre-injection control TLS, all drugs induced significant TLS increases (P at least <.05) at all doses shown. Statistical differences to individual vehicle control experiments are described in the text. Absolute TLS preinjection control values (means) in the two groups of rats ranged between 1497 to 1557 and 2390 to 2421 μA, respectively.
oin, were evaluated at different doses. In addition to TGS, TLS was determined in each experiment. As shown in figure 6, the three drugs significantly increased both TLS and TGS, but the effect on TGS was clearly more pronounced.

As described above, a bilateral clonic forelimb seizure with loss of posture was used as endpoint for TGS determinations in these experiments. In separate experiments in another group of eight rats (not illustrated), we examined whether the endpoint used for TGS affects drug potency. In these experiments, vehicle or carbamazepine, 10 mg/kg, were injected and the duration of ramp-shaped stimulation (started 30 min after injection) was recorded for both first appearance of bilateral forelimb clonus without loss of posture and with loss of posture in the same rats. Comparison of drug-induced increases in TGS in the absence of falling with TGS associated with falling indicated that both types of TGS were significantly increased by carbamazepine, but that TGS with loss of posture was more sensitive. TGS (mean ± S.E. of eight rats) without falling was 1857 ± 182 μA after vehicle and 2087 ± 210 μΑ after carbamazepine (P < .01), whereas TGS with falling was 2154 ± 143 μA after vehicle and 2659 ± 219 μA after carbamazepine (P < .01), respectively.

Anticonvulsant effect of antiepileptic drugs on the minEST with transcorneal stimulation. Similar to TGS in the CRS model, the minEST could be only determined at one time point after drug administration, because of postictal refractoriness. Thus, data after drug administration were compared to data from separate experiments with vehicle injection (figs. 7 and 8). As shown in figures 7 and 8, all six drugs tested significantly increased minEST in rats, but the effect of phenytoin, diazepam and particularly ethosuximide was weak and not clearly dose dependent. Furthermore, phenytoin increased severity of the threshold seizures with higher incidence of generalized seizures with loss of posture. Some experiments were repeated in another group of rats to examine reproducibility of the drug-induced threshold increases. Respective experiments are also illustrated in figure 7 and demonstrate that drug effects on minEST were quite reproducible.

Anticonvulsant effect of antiepileptic drugs on the maxEST with transcorneal stimulation. As with the minEST, the maxEST could be only determined at one time point after drug administration, because of postictal refractoriness. Thus, data after drug administration were compared to data from separate experiments with vehicle injection (figs. 8 and 9). All 6 drugs significantly increased maxEST, and the increase was dose-dependent except for ethosuximide, which induced only weak threshold increases at all doses tested. Again, some experiments were repeated in another group of rats to examine reproducibility of the drug-induced threshold increases. Respective experiments are illustrated in figure 9 and demonstrate that drug effects on maxEST were reproducible.

Comparison of anticonvulsant drug potencies in the different seizure threshold models. Drug-induced increases in seizure threshold models were used for comparative illustration of dose-responses and calculation of doses increasing the respective threshold by 20 and 50% (TID$_{20}$ and TID$_{50}$). Figures 10 to 12 show that linear dose-responses were obtained with most drugs in most models, thus allowing calculation of TID$_{20}$ or TID$_{50}$ by log-linear regression analysis. Some drugs were to weak or showed no linear dose-response in a model, e.g., phenytoin, ethosuximide and diazepam in case of TLS, thus prohibiting the calculation of TIDs. Furthermore, in case of TLS a TID$_{50}$ could not be determined for any drug, because threshold increases did not reach the 50% level. TIDs calculated from the data shown in figures 10 to 12 are given in table 1. Both the data in figures 10 to 12 and the data in table 1 clearly demonstrate that data obtained on anticonvulsant dose-response and potency in the CRS model differ markedly from data obtained in the EST models with transcorneal stimulation. With all drugs, the lowest anticonvulsant potency was obtained with TLS in the CRS model. There was no clear correlation between data from TLS and data from minEST. Compared to TLS, anticonvulsant potency was higher in case of TGS. However, except for ethosuximide, the highest drug potencies were obtained with maxEST. Comparison of TIDs from TGS and maxEST determinations showed that both phenytoin and carbamazepine were much more potent to increase maxEST. In case of valproate no pronounced difference in TID$_{50}$ was obtained in the two models, but because of difference in slope of the dose-response curves (fig. 11), TID$_{50}$ of valproate in case of TGS was almost two times higher compared to maxEST, again demonstrating that seizure thresholds determined in the CRS model are more resistant to anticonvulsant drug effects than seizures in conventional electroshock models with transcorneal stimulation.

**Discussion**

Our experiments demonstrate that the CRS model is useful to determine time of peak effect and duration of antiepileptic activity in individual rats, but that both dose-response characteristics and drug potencies obtained in this
model clearly differ from more commonly used seizure models. This was not due to the modified electrode localization over the frontal neocortex used in the present study, but the same dose-response characteristics and drug potencies were also obtained with the electrode position over the frontoparietal neocortex described by Voskuyl’s group (Hoogerkamp et al., 1994, 1996), as shown by direct comparison of drug data from groups with the two different electrode positions (Rundfeldt et al., 1995; present data; Rundfeldt C, Gerecke U and Lösch W, unpublished experiments). Thus, these data indicate that the CRS model presents a model of another, more pharmacoresistant seizure type than seizure types induced in traditional models, such as transcorneal electroshock. Based on the location of the electrodes in the frontal neocortex, the characteristic seizure pattern obtained at TLS currents, and the low pharmacological sensitivity of these seizures to standard antiepileptics, TLS in the CRS model most likely represents a model of localization-related seizures occurring in frontal lobe epilepsy (cf., Chauvel and Bancaud, 1994).

In most CRS experiments of our study, an electrode position over the caudal forelimb area of the rat motor cortex as
defined by Hall and Lindholm (1974) was used. Stimulation at this location led in most rats to a reproducible seizure pattern with unilateral forelimb clonus, tonic abduction of contralateral forelimb and head adersion. Alternatively, some rats exhibited asymmetrical tonic abduction of both forelimbs, associated with myoclonic jerks, as first convulsive signs on CRS stimulation. Further increase of current led to rapid generalization of seizure activity with bilateral clonic activity, rearing and loss of posture. This seizure pattern is remarkably similar to seizures occurring in frontal lobe epilepsy in humans (Broglin et al., 1992; Chauvel and Bancaud, 1994; Chauvel et al., 1995; Salanova et al., 1995). In frontal lobe epilepsy, seizures often arise from the precentral and premotor areas and are characterized by abrupt onset of unilateral or bilateral tonic and postural seizures, predominantly in upper limbs, associated with unilateral clonic seizures and adersion of head and eyes (Chauvel and Bancaud, 1994). Frontal lobe seizures usually begin in one hemisphere, but spread to the contralateral frontal lobe is very fast. In preliminary experiments, we stimulated eight rats with ramp-shaped pulses via screw electrodes over the rostral (3.5 mm anterior to bregma) and caudal forelimb area (1.0 mm anterior to bregma) of one hemisphere and observed the same seizure pattern as with bilateral electrodes over the caudal

Fig. 9. Effect of carbamazepine, phenytoin, phenobarbital and valproate on the maximal electroshock seizure threshold (maxEST) determined via transcorneal stimulation in rats. For each drug and dose, the maxEST of the vehicle control experiment and the respective drug experiment are shown. Vehicle and drug experiments were done in the same group of 15 to 20 rats with a time interval of at least 2 days between experiments. Pretreatment times for drug and vehicle injections were 15 min (valproate), 30 min (carbamazepine, phenobarbital) or 60 min (phenytoin). Significant differences to control are indicated by asterisk (*P < .01; **P < .001).

Fig. 10. Dose-response curves for carbamazepine and phenytoin in the CRS ('ramp') model with direct cortical stimulation, and the minEST and maxEST models with transcorneal stimulations. Data are shown as percent threshold increase above control on a semi-logarithmic scale. In case of TGS, minEST and maxEST percent increases were calculated in comparison to separate vehicle control experiments in the same rat group, whereas TLS increases were calculated in comparison to individual preinjection control (baseline) values of each drug experiment. The correlation coefficients from log-linear regression analyses of data are indicated in the figures. Because of truncated dose-response, no regression coefficients are shown for phenytoin in the TLS and minEST models. The 20% increase which was used for calculation of TID20 (see table 1) is indicated by the hyphenated horizontal lines. If an experiment was repeated, the data from the respective experiments are illustrated separately. The pretreatment times, after which dose-responses were calculated in the different models, are indicated in the figure.
forelimb area of both hemispheres (Krupp E, unpublished observations), indicating that spread to the contralateral forebrain and within the ipsilateral forebrain is as rapid as reported for human frontal lobe seizures (Chauvel and Bana-caud, 1994). This was also indicated by EEG recordings contralateral to the stimulation sites in rats with unilateral screw electrodes, in which paroxysmal spiking coincided with the clinical seizure activity (Krupp E, unpublished observations).

Similar to the observations in the CRS model in rats, frontal lobe seizures in humans may secondarily generalize to bilateral clonic or tonic seizures, which is due to rapid seizure spread to other cortical and subcortical structures (Broglia et al., 1992; Chauvel et al., 1995). Phenytoin, phenobarbital, carbamazepine and valproate are first-line drugs for frontal lobe seizures with or without secondary tonic-clonic generalizations (Broglia et al., 1992). These antiepileptics are also effective drugs for primarily generalized tonic-
clonic seizures (Mattson, 1995). However, in contrast to convulsive generalized seizures, frontal lobe epilepsies are often resistant to drug treatment (Broglin et al., 1992). This difference in efficacy of antiepileptic drugs between generalized convulsive seizures and frontal lobe seizures is also exemplified by the present experimental data. The threshold for MES (maxEST), a model of primarily generalized tonic-clonic seizures (Lösch and Schmidt, 1988; White et al., 1995), was very sensitive to all antiepileptic drugs, except ethosuximide that is ineffective against this type of epileptic seizures (Mattson, 1995). Similarly, generalized seizures occurring at TGS currents in the CRS model were sensitive to antiepileptics, also less so than MES. In contrast, the TLS with frontal lobe seizures was much less sensitive to antiepileptic drugs, except ethosuximide that is ineffective against this type of epileptic seizures (Mattson, 1995). Therefore, generalized seizures occurring at TGS currents in the CRS model were sensitive to antiepileptics, also less so than MES. In contrast, the TLS with frontal lobe seizures was much less sensitive to antiepileptic drugs, except ethosuximide that is ineffective against this type of epileptic seizures (Mattson, 1995).

In contrast to reports from Voskuyl's group on the CRS model (Voskuyl et al., 1992; Hoogerkamp et al., 1994), it was not possible in our hands to determine the TGS repeatedly without inducing postictal increases in both TLS and TGS. We tried to resolve this problem by stopping ramp stimulation at the first signs of bilateral clonic activity without loss of posture, but this again resulted in postictal threshold increases and introduced problems in separating TGS from TLS. Nevertheless, when TGS determinations are done in the same way as minEST or maxEST determinations, i.e., with only one threshold determination per rat and day, this type of seizure threshold may be used as an additional endpoint in the CRS model with pharmacological sensitivity that differs from TLS as discussed above. However, it is important to note that the suggestion of Voskuyl's group (Voskuyl et al., 1992; Hoogerkamp et al., 1994) that there is a correlation between a selective effect in the MES test and on the TGS, as well as between anticonvulsant activity in the PTZ test and in the TGS test does not hold true, but that TGS rather represents a model of secondarily generalized seizures and TLS a model of frontal lobe seizures. Thus, TLS and TGS in the CRS model cannot replace other seizure tests but rather represent a valuable addition to other seizure models, particularly because there is no current model of frontal lobe seizures in common use during antiepileptic drug development.

As reported previously (Voskuyl et al., 1989), during initial repeated TLS determinations there was a marked decrease in TLS, which reached a steady-state level after about 20 stimulations, resembling the decrease in focal seizure threshold in the kindling model (Sato et al., 1990). However, in contrast to kindling, this decrease in seizure threshold was not associated with an increase in seizure severity or duration. Only after a high number of stimulations, such as 200 or more stimulations in an individual animal, rarely a secondary generalization of seizures was observed after the stimulation was stopped at TLS (Krupp E, unpublished observations). This clearly separates the CRS model from kindling of the anterior neocortex, which leads to rapid increases of seizure severity and duration at repeated application of 800 μA for 1 sec via depth electrodes (Albright and Burnham, 1980). Furthermore, the pharmacological sensitivity of neocortical kindled seizures differs markedly from those in the CRS model (Albright and Burnham, 1980). Interestingly, the seizure types resulting from neocortical kindling also differ from those seen at TLS or TLS currents in the CRS model (Albright and Burnham, 1980), so that neocortical kindling

### TABLE 1
Anticonvulsant drug potencies in the different seizure threshold models in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time of Pretreatment (min)</th>
<th>TID20 (mg/kg i.p.)</th>
<th>TID50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ramp stim.</td>
<td>Transcorneal stim.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TLS</td>
<td>TGS</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>30</td>
<td>24.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>60</td>
<td>n.e.</td>
<td>24.6</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>30</td>
<td>39.8</td>
<td>n.d.</td>
</tr>
<tr>
<td>Valproate</td>
<td>15</td>
<td>148</td>
<td>95.7</td>
</tr>
<tr>
<td>Diazepam</td>
<td>15</td>
<td>n.e.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>30</td>
<td>n.e.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Doses increasing the respective threshold by 20% (TID20) or 50% (TID50) were calculated by log-linear regression analysis of the data shown in figures 10 to 12. In case of phenytoin in the minEST model, TID50 was calculated from the linear portion of the truncated dose-response curve (see fig. 10). n.e., not effective, indicates that a TID could not be determined, because the maximal drug-induced threshold increase was less than 20 or 50% above control threshold, although high doses inducing adverse effects were used. In case of TLS, no TID20 could be determined for any drug, because drug-induced threshold increases did not reach 50% above control. n.d., not determined.

As reported previously (Voskuyl et al., 1989), during initial repeated TLS determinations there was a marked decrease in TLS, which reached a steady-state level after about 20 stimulations, resembling the decrease in focal seizure threshold in the kindling model (Sato et al., 1990). However, in contrast to kindling, this decrease in seizure threshold was not associated with an increase in seizure severity or duration. Only after a high number of stimulations, such as 200 or more stimulations in an individual animal, rarely a secondary generalization of seizures was observed after the stimulation was stopped at TLS (Krupp E, unpublished observations). This clearly separates the CRS model from kindling of the anterior neocortex, which leads to rapid increases of seizure severity and duration at repeated application of 800 μA for 1 sec via depth electrodes (Albright and Burnham, 1980). Furthermore, the pharmacological sensitivity of neocortical kindled seizures differs markedly from those in the CRS model (Albright and Burnham, 1980). Interestingly, the seizure types resulting from neocortical kindling also differ from those seen at TLS or TLS currents in the CRS model (Albright and Burnham, 1980), so that neocortical kindling...
might be considered a model of the complex (psychomotor) type of frontal lobe seizures, whereas TLS represents a model of the focal motor type of frontal lobe seizures, the most common seizure type in frontal lobe epilepsy (Chauvel and Bancaud, 1994; Chauvel et al., 1995; Salanova et al., 1995). An important advantage of TLS in the CRS model is that because of the lack of a clear kindling-like increase in seizure severity and duration upon repeated TLS determinations, there is no postictal increase in seizure threshold, thus allowing repeated determinations at short intervals, which is not possible in the kindling model (Freeman and Jarvis, 1981; Löschner and Honack, 1990). A further difference to the kindling model is that even high numbers of seizures in the CRS model do not affect the subsequent determination of drug potency (see our experiments), whereas drug potency markedly increases after high numbers of seizures in the kindling model (Mace and Burnham, 1987), which limits the repeated use of kindled rats for drug potency determinations. Furthermore, whereas repeated anticonvulsant testing in kindled rats may lead to marked loss of drug potency (contingent tolerance; cf., Tietz, 1992), repeated testing of the same antiepileptic drugs in rats using the CRS model led to reproducible data on drug potency. Nevertheless, as recently pointed out by Voskuyl and van den Beukel (1996), the marked decline in TLS during initial ramp stimulations and the fact that the TLS remains at this decreased level even if stimulation is discontinued for several weeks indicate chronic brain alterations in the CRS model, which need to be characterized further.

In conclusion, our study indicates that the CRS model can be used for two different purposes. First, as demonstrated before by Voskuyl’s group (Dingemanse et al., 1990; Voskuyl et al., 1992; Hoogerkamp et al., 1994 and 1996), the TLS model can be used to obtain valid information about onset and duration of an anticonvulsant effect as well as time of peak effect in a single experiment in individual rats, thus markedly reducing the number of animals and time that is needed when other models are used in this respect. Second, as indicated by seizure pattern and pharmacology, the CRS model represents a new model of frontal lobe seizures and may thus be used in the search for novel drugs with higher efficacy against this difficult-to-treat type of epilepsy. However, it remains to be demonstrated that efficacy in a model of frontal lobe seizures would give any more information about efficacy of novel drugs in frontal lobe epilepsy than more conventional seizure models currently used in antiepileptic drug development.

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References


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