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Pharmacodynamic interaction between phenytoin and sodium valproate changes seizure thresholds and pattern

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1 In this study we used cortical stimulation to assess the effects of phenytoin (PHT), sodium valproate (VPA), and their interaction on total motor seizure and on the constituent elements of the seizure.

2 PHT (40 mg kg^{-1}) was administered as an intravenous bolus infusion to animals receiving either a continuous infusion of VPA or saline. VPA plasma concentration was maintained at levels that produced no detectable anticonvulsant effect.

3 Analysis of ictal components (eyes closure, jerk, gasp, forelimb, clonus, and hindlimb tonus) and their durations revealed both qualitative and quantitative differences in drug effects.

4 The anticonvulsant effect is represented by the increase in the duration of the stimulation required to reach a given seizure threshold. PHT significantly increased the duration of the stimulation and of the motor seizure. This increase was greatly enhanced by VPA. In addition, ictal component analysis revealed that the combination of PHT and VPA causes the reduction of a specific seizure component (JERK).

5 Neither the free fraction of PHT nor the biophase equilibration kinetics changes in the presence of VPA. It is concluded that the synergism may be due to a pharmacodynamic rather than a pharmacokinetic interaction.

Keywords: Phenytoin; sodium valproate; drug interaction; ictal behaviour; cortical stimulation

Introduction

Optimization of the treatment of epilepsy with combinations of anti-epileptic drugs remains a major challenge (French, 1994; Perucca, 1996; Sabers & Gram, 1996; Reife, 1998). This is partially due to difficulties in measuring the anticonvulsant effect objectively. The lack of quantitative parameters to assess anti-epileptic drug effect represents an important obstacle even for the evaluation of a single drug (Danhof *et al*., 1992; Avanzini, 1995). Models in which epileptic seizures are induced chemically or electrically are useful for screening and classifying anti-epileptic drug effect, but are not suitable for the investigation of drug interactions (Löscher & Schmidt, 1988; Snead, 1992; Kupferberg, 1992; Velisek *et al*., 1995). This is not possible with the available models because repeated stimulation or induction of seizure activity can lead to important changes in neuronal and functional state (Leviel *et al*., 1990; Samoriski & Appelgate, 1997). Moreover, seizures are assessed in an all-or-none fashion, precluding minor changes to be detected (Swinyard, 1969; Racine, 1972; Löscher & Schmidt, 1988; Löscher & Nolting, 1991). Interindividual variability may distort the assessment of drug interactions *in vivo*, making the characterization of the anticonvulsant effect and its time course within a single animal quite desirable. In addition, most of the studies on anti-epileptic drug interactions do not comprise both pharmacokinetic and pharmacodynamic factors.

These limitations can be circumvented in a model based on direct stimulation of the frontal cortex (Voskuyl *et al*., 1989). In this approach, the induction of seizures with increasing stimulus intensity allows controlled conditions and a well-defined effect measurement. Two behavioural endpoints can be assessed simultaneously in the same animal, namely the threshold for localized seizure (TLS) and the threshold for generalized seizure (TGS). The TLS has been defined as the stimulus intensity at which mild colonic forelimb activity starts (Voskuyl *et al*., 1989). The TGS has been defined as the point at which severe clonic activity is clearly present (Voskuyl *et al*, 1992). After termination of stimulation at the TGS a short period of immobility follows without after-discharges.

The behavioural endpoints (TLS and TGS) occur at different moments during the progression of seizure activity and reveal specific effects of various anti-epileptic drugs (Hoogerkamp *et al*., 1994). We suggest that a more detailed analysis of different seizure components can be used as a tool to identify differences and selectivity in drug action and interaction. Differences in the effects of drugs on ictal components can possibly reveal some of the underlying pharmacological mechanisms of anti-epileptic drugs on seizure activity (Gale, 1988). Recently, ictal component analysis has been evaluated in our laboratory in a comparative study with the cortical stimulation model and amygdala kindling model (Della Paschoa *et al*, 1997).

The aim of this investigation was to use ictal components to assess the pharmacological effects of phenytoin (PHT) and sodium valproate (VPA) on seizure activity and their interaction when administered in combination. PHT is a well-studied anti-epileptic drug with nonlinear pharmacokinetics (Della Paschoa *et al*., 1998), which is still prescribed in general clinical practice (Brodie & Dichter, 1997; Pimentel, 1997). VPA is often combined either with carbamazepine or with PHT in the management of severe or refractory epileptic seizures (Davis *et al*., 1994; Miller, 1994; Schmidt, 1996). However, the nature of their interaction is not completely understood. Most studies on drug interaction have assessed

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pharmacokinetic changes (Perucca, 1982; Nation *et al*., 1990; Eadie, 1991; Kimura *et al*, 1992; Riva *et al*., 1996). In pharmacodynamic studies the pharmacological response has not been quantified, but rather the absence of seizure activity (Pollack & Shen, 1985; Bourgeois, 1986; Chez *et al*., 1994). Here we assess the interaction between PHT and VPA using the pattern of different ictal components as pharmacodynamic parameters under well-defined pharmacokinetic conditions.

Methods

Animals

The study protocol was approved by the Ethical Committee for Animal Experimentation of the University of Leiden. Male adult Wistar rats (10–12 weeks old) weighing 200–250 g were used (Harlan C.P.B., Zeist, The Netherlands). The rats were housed individually in perspex cages under constant temperature (21°C) and 12:12 h light-dark cycle. Food and water were supplied *ad libitum* except during actual testing.

Surgery

Surgical procedures were carried out in animals anaesthetized by injection of 1.0 ml kg^{-1} Hypnorm® (10 mg ml^{-1} fluanisone + 0.315 mg ml^{-1} fentanyl citrate) and 1.0 ml kg^{-1} Dormicum® (5 mg ml^{-1} midazolam). Two stimulation electrodes were implanted bilaterally into the skull of the rats over the motor area of the fronto-parietal cortex as described previously (Voskuyl *et al*., 1989). Cannulas for blood sampling and drug administration were implanted, respectively, in the *vena jugularis* and in the *arteria femoralis*. A dual lumen cannula was used for simultaneous administration of VPA and PHT. Arterial rather than venous blood was sampled to circumvent the effect of metabolism and other hemodynamic factors that affect pharmacokinetic and pharmacodynamic estimates (Chiou, 1989; Gumbleton *et al*., 1994; Tuk *et al*., 1997; 1998).

Study design

Four groups of six rats each were used. Three groups received anti-epileptic drugs and will be described first. Group A (VPA) received VPA only as a continuous i.v. infusion (25 mg h^{-1}). Steady-state concentrations were rapidly reached by administering a short bolus of VPA (20 mg) at time $t=0$ (Gibaldi & Perrier, 1982; Reiter *et al*., 1992). The VPA dose was chosen in such a way that it would not cause any anticonvulsant effect on its own (Hoogerkamp *et al*., 1994). Group A also received an i.v. bolus injection of saline (as control for the PHT injection used in the other groups). Group B (PHT) received a bolus injection of PHT (40 mg kg^{-1}) in combination with an infusion of saline similar to the VPA infusion administered to group A. Group C (VPA + PHT) was administered a continuous infusion of VPA and a bolus injection of PHT. Group D (TGS++) did not receive any anti-epileptic drug, but was stimulated at current intensities that are comparable to the stimulation levels achieved in group B after administration of PHT. This group allows us to determine the effect of high intensity electric stimulation and to separate it from the pharmacological effect. The study design is schematically depicted in Figure 1. Both seizure induction and behavioural scoring were carried out and analysed off-line by a single observer, who was blinded with regard to the treatments.

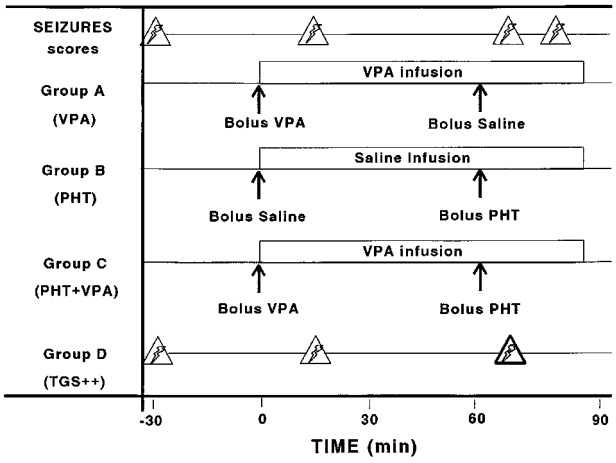

Figure 1 Experimental set-up for the assessment of pharmacodynamic drug interaction. The effect of VPA on the anticonvulsant effect of PHT (40 mg kg^{-1}) was investigated by administering a continuous i.v. infusion of VPA at concentrations which produced no effect on its own in this model (ca. 150 mg l^{-1}). The influence of different current intensities during stimulation was controlled in group D, which was stimulated to the same levels required to reach the TGS in the group B.

Administration of the drugs

The drugs were administered intravenously by infusion into the jugular vein. 100 mg kg^{-1} h^{-1} VPA was given as a continuous infusion for 6 h (25 mg h^{-1}, solution: 55 mg ml^{-1}) after a loading dose (80 mg kg^{-1}, solution: 65 mg ml^{-1}) in order to maintain VPA plasma concentrations around 150 μg ml^{-1}. VPA (Sanofi, Manchester, U.K.) was dissolved in sterile demineralized water. PHT was administered 1 h after the start of the infusion of VPA as a single bolus infusion of 40 mg kg^{-1} at a rate of 0.1 ml min^{-1}. Phenytoin sodium (Sigma, Amsterdam, The Netherlands) was dissolved in water alkalinized with 0.1 N sodium hydroxide (pH = 8.0). An infusion of saline was used as control both for the steady-state infusion of VPA when only PHT was administered and for the bolus infusion of PHT when only VPA was administered (Figure 1).

Blood sampling, pharmacokinetics and protein binding

To determine the pharmacokinetics of VPA and PHT, blood samples were collected before and at several intervals after drug administration, namely 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240 and 300 min. Plasma levels of PHT were measured by a HPLC technique slightly modified from Lolin *et al*. (1994). Within-day precision was 1.7% for a 10 μg ml^{-1} control sample ($n=8$). Limit of detection was 0.25 μg ml^{-1} and the assay was linear in the range from 1–100 μg ml^{-1}. VPA concentrations were measured by a gas chromatographic method (Durozard & Bavarel, 1987). Within-day precision was 0.9% for a 470 μg ml^{-1} control sample ($n=10$). Limit of detection was 1.4 μg ml^{-1} and the assay was linear in the range from 1.4–500 μg ml^{-1}. The validation of both PHT and VPA assays were carried out with rat plasma samples previously spiked either with 150 μg ml^{-1} VPA or with 25 μg ml^{-1} PHT, respectively, mimicking the actual experiments. In addition, protein binding of PHT was determined by ultrafiltration, as previously described (Mandema *et al*., 1991). Residual blood was collected by aorta puncture after completion of the

experiments and centrifuged at 500 r.p.m. for 10 min. Plasma was separated and stored at −30°C until assay. The protein binding was determined for each individual animal by ultrafiltration at 37°C, using the Amicon Micropartition System (Amicon Division, Danvers, MA, U.S.A.). To evaluate a pharmacokinetic interaction, protein binding of PHT was assessed in a wide concentration range (5–100 μg ml^{-1}), both in the absence and presence of circa 150 μg ml^{-1} VPA. Separation of free drug from protein bound drug was carried out at 37°C by filtration of 400 μl plasma through a YMT ultrafiltration membrane (Amicon) at 1090 × *g* for 10 min. The ultrafiltrate was then analysed for free drug concentrations.

Cortical stimulation

The electric stimulation consisted of a ramp-shaped bipolar pulse train increasing linearly in intensity (50 pulses s^{-1}, 0–2400 μA in 20 s, maximum current set at 3000 μA (Voskuyl *et al*., 1989). Stimulation continued until the threshold for generalized seizure activity was reached. Stabilization of the baseline threshold was obtained by stimulating the animals twice daily for 2 weeks before the experiment. The threshold for localized seizure activity (TLS) and the threshold for generalized seizure activity (TGS) were determined during a single seizure in the same animal. Baseline thresholds were determined before drug administration and after the start of infusion of VPA (group A and C) or saline (group B). Following the bolus injection of phenytoin (group B and C) or saline (group A) seizure thresholds were determined at several intervals up to 5 h thereafter, namely, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270 and 300 min. Group D received a single supra-threshold stimulation, with current intensity reaching up to 1000 μA. This current intensity is comparable to the stimulation required to reach the TGS after the administration of PHT alone.

Anticonvulsant effect measurement

The measurement of the anticonvulsant effect in the CSM differs in an important aspect from that measurement in other models of electrically induced seizure activity, such as kindling. In these models a standard stimulus is applied and the anticonvulsant effect is assessed by the ability of drugs to suppress the frequency or duration of the induced seizures. In contrast, the CSM uses a stimulus increasing in intensity until a defined response occurs, e.g., self-sustained seizure activity. The anticonvulsant effect of drugs is measured by the stimulus intensity that is required to induce the same response (TLS, TGS). This may produce seemingly paradoxical results because anticonvulsant properties lead to higher stimulation and long seizure activity. In this study the rats of the first three groups were stimulated until the TGS was reached. Antiepileptic drugs that protect against generalized epileptic activity increase the time to reach the TGS and prolong duration of the motor seizure (Figure 2).

Behavioural scores

Seizures and the post-ictal period were recorded on video-tape (JVC-recorder, 25 frames per second). Behaviour was scored at baseline, 15 min after the start of the valproate infusion, 10 and 20 min after the administration of phenytoin. The motor seizure was scored from the first observable motor sign of convulsive activity after the beginning of stimulation up to post-ictal immobility, as done in previous investigations in this laboratory (Della Paschoa *et al*., 1997). The individual components scored are described in the ethogram (Table 1). Encoding of ictal components from video-recorder to computer was carried out by a frame-by-frame analysis (Camera®, IEC-ProGamma, The Netherlands) (Van der Vlugt *et al*., 1992). Ictal components in the used ethogram can occur simultaneously.

Data analysis

Seizure activity was split in two parts: during and after the electric stimulus. Ictal behaviour in the post-stimulation period was usually very short and highly variable. We have not suited ictal behaviour in the post-stimulation period in detail and the results presented here included only ictal behaviour during the stimulation period.

The duration of the ictal components has been shown to be a suitable parameter for seizure activity and the anticonvulsant effect (Della Paschoa *et al*., 1997). To measure the contribution of each ictal component to the seizure pattern we made use of the summation of the duration of successive bouts (total duration). The duration of the seizures differed between baseline and drug conditions due to longer electric stimulation following drug administration. Since individual seizure durations varied between animals and drug conditions, the

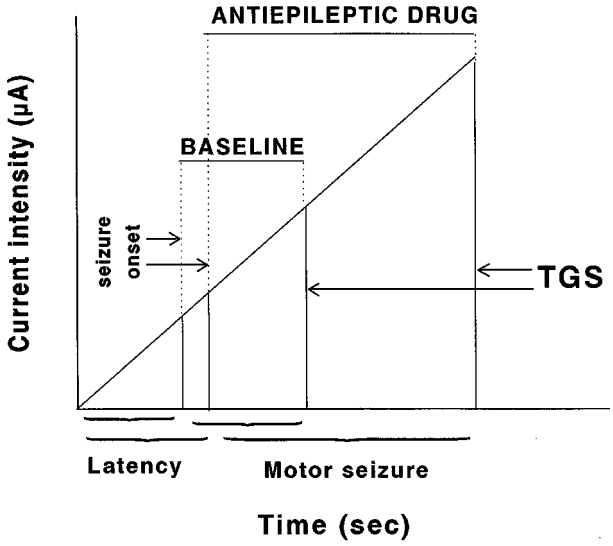

Figure 1 Schematic representation of the correlation between current intensity and duration of the stimulation to reach the threshold for generalized seizure activity (TGS). The increase in current intensity attests anticonvulsant properties. Latency and duration of the motor seizure were selected as parameters for the behavioral analysis.

Table 1 Ethogram. Definition of ictal components scored by behavioural analysis. The analytical frame resolution was 40 ms

Component	*Description*
Eyes	(bi or unilateral) eye closure or blinking
Jerk	sudden upward or backward twitch (paroxysm) of the head and/or body
Gasp	gasping (jaw clonus, vibrissae and ear twitching) or grimace-like movement of facial muscles
Forelimb clonus	clonic movements of the forelimbs
Hindlimb clonus	tonic extension of the hindlimbs

relative contribution of a particular ictal component was expressed as the fraction (percentage) of the duration of the seizure in which it occurred.

Differences between groups were assessed using parametric one-way ANOVA. Variance homogeneity was tested by Levene's test. *Post hoc* Bonferroni test was applied if necessary.

Results

Single administration of VPA

In the control group, mean (SE) plasma concentration of VPA during the ictal measurements was 122.7 ± 6.4 μg ml^{-1}. This steady-state concentration of VPA had no significant effect on seizure thresholds or the stimulation duration. No effect was observed on the total duration of any of the ictal components or their relative contribution to the seizure (data not shown). The values did not differ from baseline measurements. In the group receiving the drug combination, similar concentrations of VPA were reached (115.5 ± 4.9 μg ml^{-1}). These results were in agreement with a previous study in which VPA concentrations within the same range had no significant effect on its own (Hoogerkamp *et al*, 1994).

Pharmacokinetics and biophase equilibration

To exclude a pharmacokinetic drug interaction the pharmacokinetics of PHT was assessed in the presence and absence of VPA (Figure 3). The plasma concentrations and TGS measurements obtained up to 5 h after drug administration were used for this purpose. The pharmacokinetics could be fitted to a Michaelis-Menten elimination model with a biophase equilibration delay, as previously reported (Della Paschoa *et al*., 1998). The use of a pharmacokinetic model with biophase equilibration supplies information about drug concentration at the effect-site and explains why plasma concentrations do not correlate directly with the pharmacodynamics. The results in Figure 3 show that the pharmacokinetics of PHT are not affected by the presence of VPA. Table 2 shows that VPA had no acute effect on PHT plasma concentrations during the behavioural assessment. Furthermore, VPA had no significant effect on the protein binding of PHT nor on the biophase equilibration constant (ke0). These results demonstrate that the transfer of unbound PHT to the effect-site was not changed in the presence of VPA.

Seizure duration

Upon administration of PHT alone the duration of stimulation, the current intensity, and consequently the seizure duration, increased. The increase in the duration of stimulation reflects the anticonvulsant activity of PHT. It increased from 6.2 ± 0.4 s at baseline to 19.3 ± 2.0 s and 22.2 ± 3.4 s ($P < 0.05$) at 10 and 20 min after administration of PHT, respectively. The combination of PHT and VPA enhanced the effect observed upon administration of PHT alone ($P < 0.01$). In that group, a value of 42.3 ± 5.1 s was reached 20 min after administration of PHT (Figure 4).

The latency from the beginning of the stimulation to the first sign of the motor seizure also changed following the administration of PHT. It increased from 4.1 ± 0.4 s at baseline conditions to 6.6 ± 0.5 s at 10 min after administration of PHT ($P < 0.05$). However, latency was not significantly changed by concomitant administration of VPA, as compared to the effects of PHT alone.

The action of PHT on the duration of the motor seizure was more manifest than its effect on the latency. The duration of motor seizure activity increased from 2.1 ± 0.5 s at baseline conditions to 12.7 ± 1.9 s ($P < 0.05$) and 15.9 ± 3.5 s ($P < 0.01$) 10 and 20 min after the administration of PHT, respectively. This effect was even more evident following the administration of the drug combination. As depicted in Figure 4, the duration of the motor seizure reached 36.8 ± 5.2 s at 20 min after administration of PHT in the presence of VPA ($P < 0.01$). The increase in duration corresponded to an increase in current intensity of 796 ± 155 *versus* 1704 ± 209 μA at 10 min and 785 ± 130 *versus* 1898 ± 170 μA at 20 min.

Ictal components

The seizure pattern consisted of the ictal components presented in the ethogram. All ictal components observed at baseline conditions were also found after drug administration. The total duration of most ictal components increased after the administration of PHT, since both stimulation and seizure activity also increased (data not shown). After normalizing for differences in duration, upon administration of PHT no

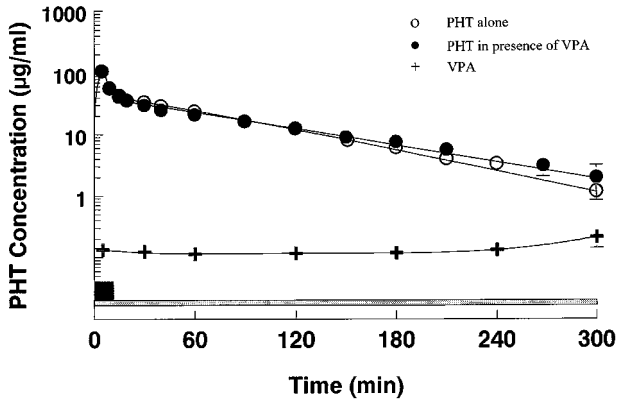

Figure 3 Pharmacokinetics of PHT after single administration alone (○) and in combination with a continuous infusion of VPA (●). PHT data were fitted to a two-compartment pharmacokinetic model with Michaelis-Menten elimination. The scatters (+) represent the time course of VPA concentrations during infusion. The line represents the best fit to experimental data and the bars the infusion duration of PHT and VPA. Data points are means ± s.e.mean ($n = 6$).

Table 2 Total plasma concentration, free fraction and biophase equilibration kinetics of PHT in absence and presence of VPA. The values do not differ significantly ($P < 0.05$, one-way ANOVA, $n = 6$, Levene's test for homogeneity of variance)

	Total concentration (μg ml^{-1})		*Free fraction* (%)		*Biophase equilibrium* (K_{e0})
Time	*10 min*	*20 min*	*10 min*	*20 min*	(min^{-1})
Group B (PHT)	62.5 ± 3.7	37.5 ± 2.0	22.3 ± 1.1	21.2 ± 1.2	0.1069 ± 0.0210
Group C (PHT + VPA)	60.1 ± 2.3	38.2 ± 1.4	25.2 ± 0.8	24.2 ± 0.6	0.1280 ± 0.0197

difference was observed in the relative contribution to the seizure pattern of most particular ictal components (Figure 5). The exception being a specific suppression of jerks (JERK), both at 10 ($P<0.05$) and at 20 min ($P<0.05$) for the combination of PHT and VPA.

Supra-threshold stimulation

In order to control the effect of high intensity electric stimulation and separate it from the pharmacological action, the effect of supra-threshold stimulation on the fraction factor of each ictal component was measured (Figure 6). Indeed, during supra-threshold stimulation, the relative contributions of particular ictal components to the seizure pattern was not significantly different from baseline or PHT conditions (both at 10 and 20 min after administration). EYES, GASP and HLTO were slightly higher in the group that received the drug combination.

The relative contribution of JERK was not affected by current intensity. These observations seem to confirm the pharmacological nature of the effect on the ictal component JERK, both at 10 and 20 min after drug administration ($P<0.01$, $P<0.01$).

Discussion

This study demonstrates that VPA potentiates the PHT-induced increase in the threshold for generalized seizure activity and changes the seizure pattern by suppressing JERK, one of the most evident components. No such interaction was found in the threshold at which seizure activity started. These acute effects do not seem to be caused by pharmacokinetic changes.

Seizure pattern

In this study, cortical stimulation was continued until the threshold for self-sustained activity was reached, i.e., the threshold for generalized seizure activity (TGS) (Voskuyl *et al*., 1989). The duration of seizure activity and its motor components were measured within this time frame. Following the administration of PHT, the stimulation time needed to reach the TGS was longer, because of the linear temporal increase in current intensity. Stimulation lasted significantly longer for the group in which the combination of PHT and VPA was administered (Figure 4). Apparently, such effect is an indication of synergism between the two anti-epileptic drugs (Berenbaum, 1989), since the administration of VPA alone did not cause any change in the duration of stimulation. In contrast to the effect on the total duration of motor seizure,

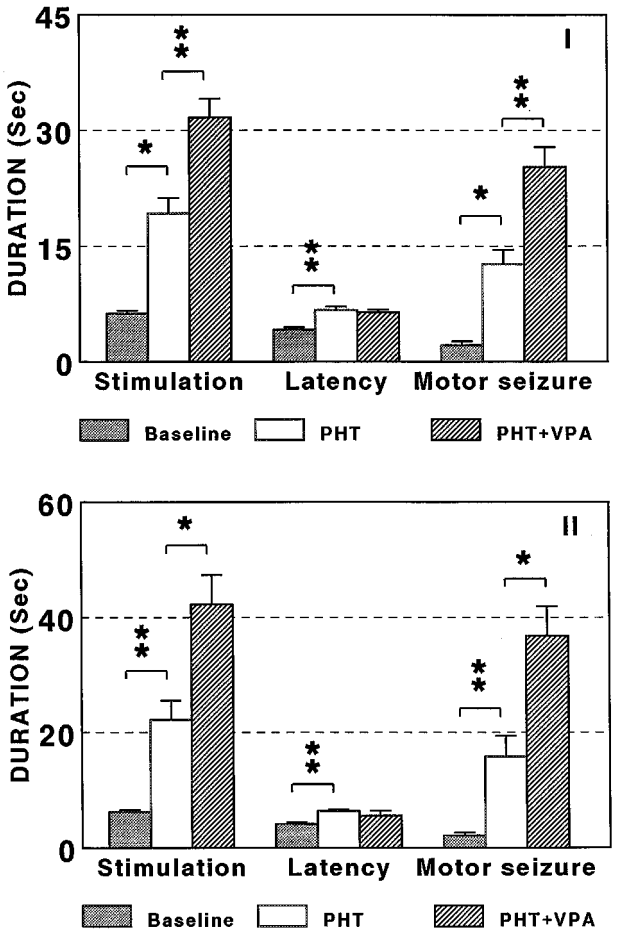

Figure 4 Effect of PHT and its interaction with VPA on total stimulation, seizure latency and motor seizure duration. The increase in the duration of the motor seizure suggests a synergistic effect between the two drugs. The effects were observed at 10 min (I) and 20 min (II) after administration of PHT. Data are expressed as means ± s.e.mean ($n=6$). Statistical significance at $^{*}P<0.05$ and $^{**}P<0.01$.

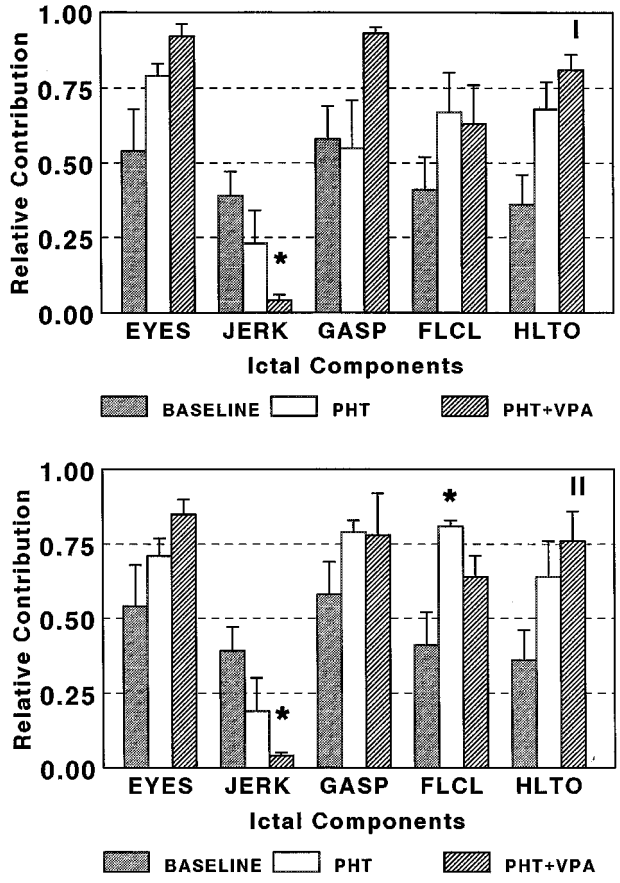

Figure 5 Effects of PHT and its interaction with VPA on the relative contribution of each ictal component to the seizure pattern. There is a specific suppression of JERK. The effects were observed at 10 min (I) and 20 min (II) after administration of PHT. Data are expressed as means ± s.e.mean ($n=6$). Statistical significance at $^{*}P<0.05$.

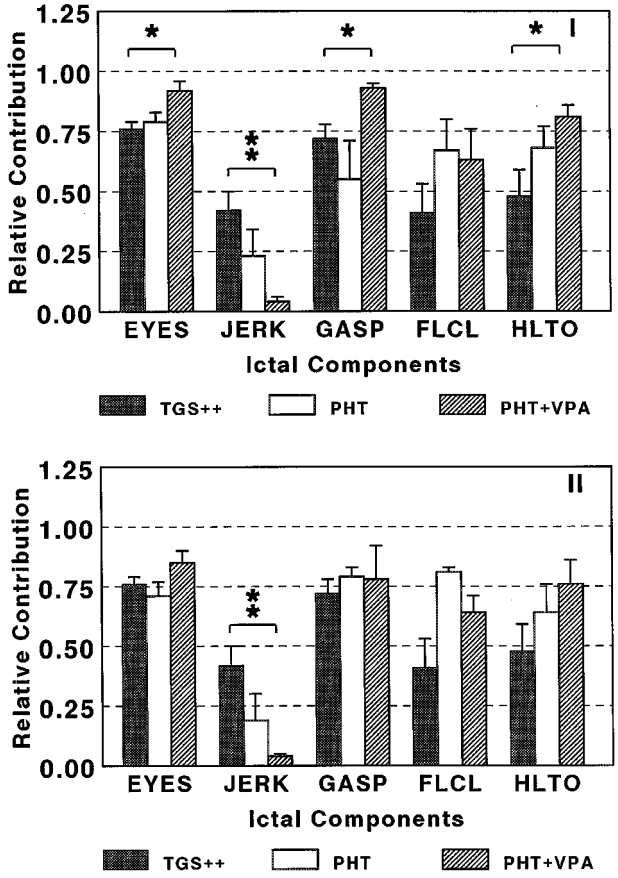

Figure 6 The effects of PHT and its interaction with VPA is compared to the effect of current intensity on the relative contribution of each ictal component to the seizure pattern. The suppression of JERK seems to be caused by a pharmacodynamic interaction. The effects were observed at 10 min (I) and 20 min (II) after administration of PHT. EYES, GASP and HLTO in group C are apparently higher because of stimulation with higher current intensity in that group. Data are expressed as means ± s.e.mean ($n=6$). Significance at $^{*}P<0.05$ and $^{**}P<0.01$.

latency until the first motor sign of seizure activity was only slightly increased after PHT administration and drug combination had no additional effect on this parameter.

Since motor seizure was prolonged upon drug administration, it might be expected that the total duration of the constituent ictal components was also proportionally prolonged. In fact, this was true for most but not all ictal components when the anti-epileptic drugs were administered. By normalizing differences in seizure duration, a specific suppression of JERK was found upon administration of pHT and VPA (Figure 5). This effect demonstrated additional evidence for a synergistic interaction between the two drugs. Moreover, no significant changes were observed in seizure pattern following the administration of either VPA or PHT alone. Intriguingly, forelimb clonus activity was increased 20 min after administration of PHT, though it was not changed in the first measurement (10 min after administration). We cannot explain this result, since it is correlated neither with the concentration of PHT nor with current intensity.

Nature of the observed effects

The suppressive effect of the drug combination of JERK might be caused by other factors than the pharmacological action since higher current intensity was applied following drug administration. Previous studies have shown that differences in current intensity in electroshock tests affect the anticonvulsant potency of a drug (Toman *et al*., 1946; Piredda *et al*, 1985; Schmutz *et al*., 1988; Löscher *et al*., 1991). Therefore, evaluation of the effect of current intensity on seizure pattern was determined by a supra-threshold stimulation. Supra-threshold stimulation did not cause any specific change in the contribution of the ictal components to the total seizure pattern. Moreover, the values of the relative contribution of JERK to the seizure pattern following supra-threshold stimulation did not differ from baseline conditions. These results suggest that the change in seizure pattern following drug combination was due to a pharmacological effect.

Our data also suggest that the acute interaction between PHT and VPA is of pharmacodynamic rather than pharmacokinetic nature. Neither total nor free plasma concentrations of PHT were significantly altered by the continuous infusion of VPA. The slight decrease in the extent of protein binding cannot explain the potentiation of the effects observed in the behavioural analysis (Klockowski & Levy, 1987; Modi & Veng-Pedersen, 1994). Furthermore, the results are in agreement with previous observations on the pharmacodynamic nature of interaction between VPA and PHT (Chez *et al*., 1994).

With regard to the mechanisms underlying of the synergistic interaction between PHT and VPA, it is known that both drugs act by altering properties of voltage-dependent sodium channels (Rogawski & Porter, 1990; Macdonald, 1991b; Davis *et al*., 1994; DeLorenzo, 1995). There is some evidence that VPA and PHT bind on different sites of the sodium channels (reviewed by Macdonald, 1991a). A possible hypothesis would be that of enhancement of the binding of PHT to its binding sites, similarly to what is observed with GABA-ergic drugs, for which facilitation of the pharmacological activity of a certain agonist is detected after allosteric binding of a second drug (Brouilett *et al*., 1991; Gale, 1992a).

We have also shown that a drug may affect different seizure components selectivity. It is known that motor seizure expression is different in various animal models. Main categories of seizures were found to depend on specific subcortical structures and pathways (Gale, 1992b). The selective suppression of JERK suggests that paroxysms may be induced by a specific pathway, other than the one involved in the execution of tonic-clonic activity.

The synergistic pharmacological interaction between PHT and VPA may have clinical relevance. However, in clinical practice, route, duration and regimen of administration differ considerably from the conditions of our study. In addition, plasma protein binding of the drugs in humans is much higher than in rats (Löscher & Nau, 1993; Cloyd, 1991; Brodie, 1992). During chronic treatment this means that plasma concentrations may actually fall due to the displacement of the bound fraction and rapid clearance of the excess free drug (Nation *et al*., 1990; Morrow, 1991). Nevertheless, our results suggest that the pharmacodynamic interaction of these drugs on seizure pattern and thresholds may have a role in the therapy in humans too.

References

AVANZINI, G. (1995). Animal models relevant to human epilepsies. *Ital. J. Neurol. Sci.,* **16,** 5–8.

BERENBAUM, M.C. (1989). What is synergy? *Pharmacol. Rev.,* **41,** 93–141.

BOURGEOIS, B.F. (1986). Antiepileptic drug combinations and experimental background: the case of phenobarbital and phenytoin. *Naunyn Schmiedebergs Arch. Pharmacol.,* **333,** 406–411.

BRODIE, M.J. (1992). Drug interactions in epilepsy. *Epilepsia,* **33,** (suppl 1), S13–S22.

BRODIE, M.J. & DICHTER, M.A. (1997). Established antiepileptic drugs. *Seizure,* **6,** 159–174.

BROUILLET, E., CHAVOIX, C., BOTTLAENDER, M., KHALILI-VARASTEH, M., HANTRAYE, P., FOURNIER, D., DODD, R.H. & MAZIERE, M. (1991). In vivo bidirectional modulatory effect of benzodiazepine receptor ligands on GABAergic transmission evaluated by positron emission tomography in non-human primates. *Brain Res.,* **557,** 167–176.

CHEZ, M.G., BOURGEOIS, B.F., PIPPENGER, C.E. & KNOWLES, W.D. (1994). Pharmacodynamic interactions between phenytoin and valproate: individual and combined antiepileptic and neurotoxic actions in mice. *Clin. Neuropharmacol.,* **17,** 32–37.

CHIOU, W.L. (1989). The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part I). *Clin. Pharmacokinet.,* **17,** 175–199.

CLOYD, J. (1991). Pharmacokinetic pitfalls of present antiepileptic medications. *Epilepsia,* **32** (suppl 5), S53–S65.

DANHOF, M., VOSKUYL, R.A. & HOOGERKAMP, A. (1992). Pharmacokinetic-pharmacodynamic modelling with anticonvulsants in animal models and in humans. In *The in vivo study of drug action*. eds. van Boxtel, C.J., Holford, N.H.G. & Danhof, M. 179–201. Amsterdam: Elsevier Science Publishers B.V.

DAVIS, R., PETERS, D.H. & MCTAVISH, D. (1994). Valproic acid. A reappraisal of its pharmacological properties and clinical efficacy in epilepsy. *Drugs,* **47,** 332–372.

DELLA PASCHOA, O.E., KRUK, M.R., HAMSTRA, R., VOSKUYL, R.A. & DANHOF, M. (1997). Seizure patterns in kindling and cortical stimulation models of experimental epilepsy. *Brain Res.,* **770,** 221–227.

DELLA PASCHOA, O.E., MANDEMA, J.W., VOSKUYL, R.A. & DANHOF, M. (1998). Pharmacokinetic-pharmacodynamic modelling of the anticonvulsant and EEG effects of phenytoin in rats. *J. Pharmacol. Exp. Ther.,* **284,** 460–466.

DELORENZO, R.J. (1995). Phenytoin : mechanisms of action. In *Antiepileptic drugs*, 4th edn. eds. Levy, R.H., Mattson, R.H. & Meldrum, B.S. 271–282. New York: Raven Press.

DUROZARD, D. & BAVAREL, G. (1987). Gas chromatographic method for the measurement of sodium valproate utilization by kidney tubules. *J. Chromatogr.,* **414,** 460–464.

EADIE, M.J. (1991). Formation of active metabolites of anticonvulsant drugs. A review of their pharmacokinetic and therapeutic significance. *Clin. Pharmacokinet.,* **21,** 27–41.

FRENCH, J. (1994). The long term therapeutic management of epilepsy. *Ann. Intern. Med.,* **120,** 411–422.

GALE, K. (1988). Progression and generalization of seizure discharge: anatomical and neurochemical substrates. *Epilepsia,* **29,** S15–S34.

GALE, K. (1992a). Role of GABA in the genesis of chemoconvulsant seizures. *Toxicol. Lett.,* 64–65, Spec No: 417–428.

GALE, K. (1992b). Subcortical structures and pathways involved in convulsive seizure generation. *J. Clin. Neurophysiol.,* **9,** 264–277.

GIBALDI, M. & PERRIER, D. (1982). Nonlinear pharmacokinetics. In *Pharmacokinetics*, 271–318. (Anonymous) New York: Marcel Dekker Inc.

GUMBLETON, M., OIE, S. & VEROTTA, D. (1994). Pharmacokinetic-pharmacodynamic (PK-PD) modelling in non-steady-state studies and arterio-venous drug concentration differences. *Br. J. Clin. Pharmacol.,* **38,** 389–400.

HOOGERKAMP, A., VIS, P.W., DANHOF, M. & VOSKUYL, R.A. (1994). Characterization of the pharmacodynamics of several antiepileptic drugs in a direct cortical stimulation model of anticonvulsant effect in the rat. *J. Pharmacol. Exp. Ther.,* **269,** 521–528.

KIMURA, M., TANAKA, N., KIMURA, Y., MIYAKE, K., KITAURA, T. & FUKUCHI, H. (1992). Pharmacokinetic interaction of zonisamide in rats. Effect of other antiepileptics on zonisamide. *J. Pharmacobiodyn.,* **15,** 631–639.

KLOCKOWSKI, P.M. & LEVY, G. (1987). Kinetics of drug action in disease states. XXI. Relationship between drug infusion rate and dose required to produce a pharmacologic effect. *J. Pharm. Sci.,* **76,** 516–520.

KUPFERBERG, H.J. (1992). Strategies for identifying and developing new anticonvulsant drugs. *Pharm. Weekbl. Sci.,* **14,** 132–138.

LEVIEL, V., FAYADA, C., GUIBERT, B., CHAMINADE, M., MACHEK, G., MALLET, J. & BIGUET, N.F. (1990). Short- and long-term alterations of gene expression in limbic structures by repeated electroconvulsive-induced seizures. *J. Neurochem.,* **54,** 899–904.

LOLIN, Y.I., RATNARAJ, N., HJELM, M. & PATSALOS, P.N. (1994). Antiepileptic drug pharmacokinetics and neuropharmacokinetics in individual rats by repetitive withdrawal of blood and cerebrospinal fluid: phenytoin. *Epilepsy Res.,* **19,** 99–110.

LÖSCHER, W., FASSBENDER, C.P. & NOLTING, B. (1991). The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. *Epilepsy Res.,* **8,** 79–94.

LÖSCHER, W. & NAU, H. (1983). Distribution of valproic acid and its metabolites in various brain areas of dogs and rats after acute and prolonged treatment. *J. Pharmacol. Exp. Ther.,* **226,** 845–854.

LÖSCHER, W. & NOLTING, B. (1991). The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. IV. Protective indices. *Epilepsy Res.,* **9,** 1–10.

LÖSCHER, W. & SCHMIDT, D. (1988). Which animal model should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res.,* **2,** 145–181.

MACDONALD, R.L. (1991a). Antiepileptic drug actions on neurotransmitter receptors and ion channels. In *Neurotransmitters and Epilepsy*, eds. Fisher, R.S. & Coyle, J.T. 231–245. New York: Wiley-Liss, Inc.,

MACDONALD, R.L. (1991b). Anticonvulsant drugs, ion channels and neurotransmitter receptors. *Curr. Opin. Neurol, Neurosurg.,* **4,** 576–581.

MANDEMA, J.W., SANSOM, L.N., DIOS-VIEITEZ, M.C., HOLLANDER-JANSEN, M. & DANHOF, M. (1991). Pharmacokinetic-pharmacodynamic modeling of the electroencephalographic effects of benzodiazepines. Correlation with receptor binding and anticonvulsant activity. *J. Pharmacol. Exp. Ther.,* **257,** 472–478.

MILLER, D.R. (1994). Intravenous infusion anaesthesia and delivery devices. *Can. J. Anaesth.,* **41,** 639–651.

MODI, N.B. & VENG-PEDERSEN, P. (1994). Application of a variable direction hysteresis minimization approach in describing the central nervous system pharmacodynamic effects of alfentanil in rabbits. *J. Pharm. Sci.,* **83,** 351–356.

MORROW, J. (1991). The pharmocokinetics of the individual antiepileptic drugs. In *A practical approach to epilepsy*, ed. Dam, M. 87–104. Chichester: Pergamon Press Inc.

NATION, R.L., EVANS, A.M. & MILNE, R.W. (1990). Pharmacokinetic drug interactions with phenytoin (Part I). *Clin. Pharmacokinet.,* **18,** 37–60.

PERUCCA, E. (1982). Pharmacokinetic interactions with antiepileptic drugs. *Clin. Pharmacokinet.,* **7,** 57–84.

PERUCCA, E. (1996). The new generation of antiepileptic drugs: advantages and disadvantages. *Br. J. Clin. Pharmacol.,* **42,** 531–543.

PIMENTEL, J. (1977). Therapeutic strategies in epilepsy. *Acta. Med. Port.,* 10, 573–579.

PIREDDA, S.G., WOODHEAD, J.H. & SWINYARD, E.A. (1985). Effect of stimulus intensity on the profile of anticonvulsant activity of phenytoin, ethosuximide and valproate. *J. Pharmacol. Exp. Ther.,* **232,** 741–745.

POLLACK, G.M. & SHEN, D.D. (1985). A timed intravenous pentylenetetrazol infusion seizure model for quantitating the anticonvulsant effect of valproic acid in the rat. *J. Pharmacol. Methods,* **13,** 135–146.

RACINE, R.J. (1972). Modification of seizure activity by electrical stimulation: I. After-discharge threshold. *Electroencephalogr. Clin. Neurophysiol.,* **32,** 269–279.

REIFE, R.A. (1998). Assessing pharmacokinetic and pharmacodynamic interactions in clinical trials of antiepileptic drugs. *Adv. Neurol.,* **76,** 95–103.

REITER, P.D., HOGUE, S.L. & PHELPS, S.J. (1992). Ability of three pharmacokinetic equations to predict steady-state serum theophylline concentrations in pediatric patients. *Ther. Drug Monit.,* **14,** 354–359.

RIVA, R., ALBANI, F., CONTIN, M. & BARUZZI, A. (1996). Pharmacokinetic interactions between antiepileptic drugs. Clinical considerations. *Clin. Pharmacokinet., 31,* 470–493.

ROGAWSKI, M.A. & PORTER, R.J. (1990). Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol. Rev.,* **42,** 223–289.

SABERS, A. & GRAM, L. (1996). Drug treatment of epilepsy in the 1990s. Achievements and new developments. *Drugs,* **52,** 483–493.

SAMORISKI, G.M. & APPLEGATE, C.D. (1997). Repeated generalized seizures induce time-dependent changes in the behavioral seizure response independent of continued seizure induction. *J. Neurosci.,* **17,** 5581–5590.

SCHMIDT, D. (1996). Modern management of epilepsy: Rational polytherapy. *Baillieres Clin. Neurol.,* **5,** 757–763.

SCHMUTZ, M., KLEBS, K. & BALTZER, V. (1988). Inhibition or enhancement of kindling evolution by antiepileptics. *J. Neural Transm.,* **72,** 245–257.

SNEAD, O.C. (1992). Pharmacological models of generalized absence seizures in rodents. *J. Neural Transm. Suppl.,* **35,** 7–19.

SWINYARD, E.A. (1969). Laboratory evaluation of antiepileptic drugs. Review of laboratory methods. *Epilepsia,* **10,** 107–119.

TOMAN, J.E.P., SWINYARD, E.A. & GOODMAN, L.S. (1946). Properties of maximal seizures and their alteration by anticonvulsant drugs and other agents. *J. Neurophysiol.,* **9,** 231–240.

TUK, B., DANHOF, M. & MANDEMA, J.W. (1997). The impact of arteriovenous concentration differences of pharmacodynamic parameter estimates. *J. Pharmacokin. Biopharm.,* **25,** 39–62.

TUK, B., HERBEN, V.M.M., MANDEMA, J.W. & DANHOF, M. (1998). Relevance of arteriovenous concentration differences in pharmacokinetic-pharmacodynamic modeling of midazolam. *J. Pharmacol. Exp. Ther.,* **284,** 202–207.

VAN DER VLUGT, M.J., KRUK, M.R., VAN ERP, A.M. & GEUZE, R.H. (1992). CAMERA: a system for fast and reliable acquisition of multiple ethological records. *Behav. Res. Meth.,* **24,** 147–149.

VELISEK, L., VELISKOVA, J. & MOSHE, S.L. (1995). Developmental seizure models. *Ital. J. Neurol. Sci.,* **16,** 127–133.

VOSKUYL, R.A., DINGEMANSE, J. & DANHOF, M. (1989). Determination of the threshold for convulsions by direct cortical stimulation. *Epilepsy Res.,* **3,** 120–129.

VOSKUYL, R.A., HOOGERKAMP, A. & DANHOF, M. (1992). Properties of the convulsive threshold determined by direct cortical stimulation in rats. *Epilepsy Res.,* **12,** 111–120.

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