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FURTHER OBSERVATIONS ON THE INTERACTIONS BETWEEN PHENOBARBITAL AND DIPHENYLHYDANTOIN DURING CHRONIC TREATMENT IN THE RAT

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Abstract—Levels of diphenylhydantoin (DPH) and phenobarbital (PB) in plasma and brain of rats after single or combined, acute or chronic treatment have been determined. The results obtained indicate (a) drug plasma levels are not always a reliable index of brain levels because DPH disappearance from plasma is frequently more rapid than from brain; (b) DPH administration enhances the plasma and brain levels of PB; (c) levels of DPH or PB in brain correlate with the protection against a maximal electroshock.

COMBINED treatment of convulsive disorders with phenobarbital (PB) and diphenylhydantoin (DPH) is commonly employed[1] but the mechanism underlying the therapeutic validity of this pharmacological association has never been clearly established. In many clinical studies PB and DPH plasma levels as well as urinary excretion of hydroxylated metabolites have been followed.[3–7] The results indicate that during chronic combined treatment with PB and DPH, PB may decrease DPH plasma levels and may increase the urinary excretion of hydroxylated DPH metabolites by an "inducing" effect on liver hydroxylating enzymes.[7–13,17] Also DPH may produce higher levels of plasma PB.[13,14]

These observations, however, do not give any information about possible modifications of the physiological availability of both drugs in the CNS which must be responsible for the clinical effects. In addition, even if we know that after a single dose there is a good relationship between plasma and brain levels, very little is known about this relationship during chronic combined treatment.[15]

In the present study we have investigated the relationship between plasma and brain levels of PB and DPH in various experimental conditions, i.e. acute or repeated treatment or both drugs given alone or in association, with the aim of reaching a better understanding of the physiological availability of PB and DPH in an experimental condition simulating the clinical situation.

MATERIALS AND METHODS

All experiments were carried out with female Sprague–Dawley rats (body wt. 140–160 g) kept in Meraklon cages at 21 ± 2° and constant humidity (60%).

Acute experiments. A group of 15 animals were treated with a single dose of 200 mg/kg of DPH suspended in methylcellulose, given orally. To a second group of 15 animals, DPH was administered 24 hr after five intraperitoneal injections of PB

(35 mg/kg every 12 hr). A third group of 15 animals was treated with PB only. A fourth group of 15 animals treated with methylcellulose was used as a control. Five animals from each group were sacrificed 4, 6 and 8 hr respectively after the administration of DPH or methylcellulose.

Subacute experiments. A group of 15 animals received 100 mg/kg by mouth of DPH suspended in methylcellulose every 12 hr for a total of seven times and saline solution i.p. for a total of five times. A second group of 15 animals received DPH, as in the first group, and PB (35 mg/kg i.p. every 12 hr) five times. A third group received PB, as the second group, and methylcellulose by mouth as in the DPH-treated groups. A fourth group of 15 methylcellulose-treated animals was used as a control group. Five animals from each group were sacrificed 4, 6 and 8 hr after the time of the last administration of DPH or methylcellulose.

Immediately before sacrifice the degree of protection against maximal electroshock was tested with Basile electroshock equipment for small animals (parameters: 100 mA, 0·2 p.W., 100 p/sec, shock duration: 0·4 sec). Animals were then sacrificed by decapitation and brain and blood samples were collected for drug determination.

DPH determinations were carried out on plasma and brain samples according to Morselli.[16] PB in plasma and brain was determined according to Svensmark and Kristensen with minor modifications.[17]

RESULTS

Acute experiments. In the rats which received DPH in a single dose of 200 mg/kg by the oral route there was a good correlation between plasma and brain levels with a peak around the sixth hour of 14 μg/ml or g (Fig. 1a). All animals were fully protected against maximal electroshock.

In the PB-pretreated rats DPH plasma levels were lower than in the DPH group by the 4th hour, and the plasma clearance rate was faster (Fig. 1b).

Whether this was due only to microsomal enzyme induction or also to an inhibition of DPH absorption, as recently suggested,[18] has not been verified.

However, the rate of entry of DPH into brain and its rate of removal did not appear to be significantly modified by PB pretreatment (Fig. 1b). In these animals the PB brain levels were practically the same as in the rats which received PB alone.

With regards to the protection against maximal electroshock in this group, all of the rats which received DPH were fully protected. In the group which received PB alone, full protection was present only at 4 hr from methylcellulose administration i.e. 36 hr from the last PB administration.

At 6 hr there was protection against tonic but not clonic phase. At 8 hr 80 per cent of the animals had tonic and 100 per cent had clonic seizures (Table 1).

Subacute experiments

Repeated administration of DPH (100 mg/kg by mouth for seven doses) led to a picture very similar to that obtained with PB pretreated rats in the acute experiments.

There was a faster plasma clearance of the drug, probably due to autoinduction,[19] while the brain levels were higher and were constant throughout all the length of time considered (Fig. 1c).

In the combined treatment group DPH levels were significantly lower for plasma

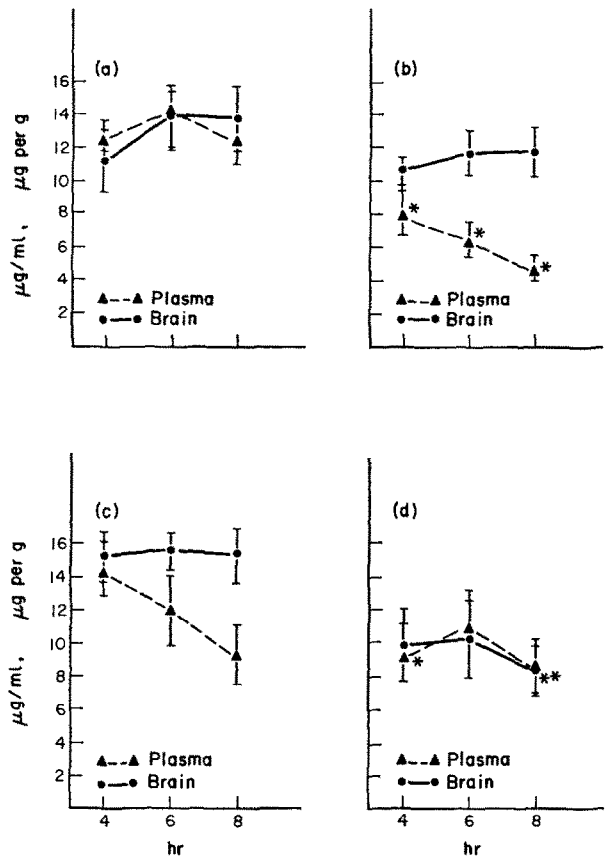

FIG. 1. DPH brain (μg/g) and plasma (μg/ml) after acute or repeated administration, with or without PB combined treatment.

(a) Brain and plasma levels of DPH after a single administration (200 mg/kg p.o.).

(b) Effect of PB pretreatment on brain and plasma levels of DPH after a single administration (200 mg/kg p.o.) PB was injected i.p. (35 mg/kg) at 9 a.m. and 6 p.m. of day 1, 2 and at 9 a.m. of day 3. DPH was administered 24 hr later. * $P < 0{\cdot}05$ in respect to plasma levels of (a).

(c) Brain and plasma levels of DPH after repeated treatment (100 mg/kg p.o. every 12 hr for 3 days and at 9 a.m. of day 4).

(d) Brain and plasma levels of DPH after repeated combined treatment with DPH and PB. DPH was administered at the dose of 100 mg/kg p.o. at 9 a.m. and 6 p.m. of day 1, 2, 3 and at 9 a.m. of day 4. PB was administered at the dose of 35 mg/kg i.p. at 9 a.m. and 6 p.m. of day 1, 2 and at 9 a.m. of day 3.

* $P < 0{\cdot}05$ in respect to brain and plasma levels of (c).

** $P < 0{\cdot}025$ in respect to brain levels of (c).

($P < 0{\cdot}05$) and brain ($P < 0{\cdot}05$) at the fourth hour and for brain at the eighth hour ($P < 0{\cdot}025$) (Fig. 1d).

In both groups there was full protection against maximal electroshock despite the low level of brain DPH at the 8th hour. The PB plasma ($P < 0{\cdot}02$) and brain ($P < 0{\cdot}05$) levels were significantly higher than in group which received PB alone (Fig. 2).

TABLE 1. EFFECT OF DIPHENYLHYDANTOIN AND PHENOBARBITAL GIVEN ALONE OR IN ASSOCIATION ON MAXIMAL ELECTROSHOCK AT VARIOUS TIMES AFTER ADMINISTRATION

Treatment	(mg/kg)	% of animals showing convulsions		Tonic phase (sec ± S.E.)		Clonic phase (sec ± S.E.)	
		6 hr	8 hr	6 hr	8 hr	6 hr	8 hr
Methylcellulose	—	100	100	11 ± 0·3	11 ± 0·7	12 ± 0·4	11 ± 0·5
DPH	200	0	0	absent	absent	absent	absent
PB (°) + methyl-cellulose	35 × 5	100	100	absent	10 ± 0·2	17 ± 1·2	12 ± 0·3
DPH + PB	200 35 × 5	0	0	absent	absent	absent	absent

(°) In this group 6 and 8 hr are considered from the methylcellulose administration, i.e. 30 and 32 hr from the last PB administration.

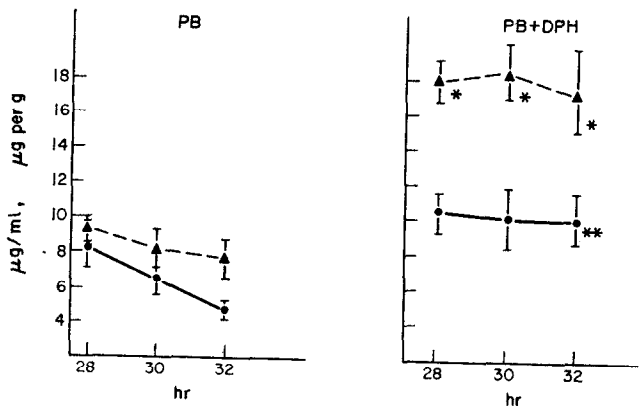

FIG. 2. Effect of DPH on PB plasma (µg/ml) and brain (µg/g) levels after repeated administrations.
PB = drug was administered i.p. at the dose of 35 mg/kg at 9 a.m. and 6 p.m. of day 1 and 2 and at 9 a.m. of day 3.
PB + DPH = PB was administered as in previous group, and DPH (100 mg/kg p.o.) at 9 a.m. and 6 p.m. of day 1, 2, 3 and at 9 a.m. of day 4.
* $P < 0{\cdot}02$ in respect to plasma levels of PB group.
** $P < 0{\cdot}05$ in respect to brain levels of PB group.
▲— — —▲ plasma ●———● brain

DISCUSSION

The question of the relationship between plasma and brain levels during a chronic combined treatment with PB and DPH still presents a problem. Most of the experimental data available on DPH and PB and on their metabolic interactions have been obtained in acute experiments.[20,21] In many instances, the two anticonvulsant drugs are not given in combination at the same time, as in clinical practice, but one drug precedes the other at a relatively long interval.[22,23]

Our results show that the determination of drug plasma levels as an index of tissue levels is valid if it is performed at the proper time. In fact, for PB the parallelism between plasma and brain levels seems to be longlasting, but for DPH this may be true only for a limited length of time, as in the case of chronic DPH administration. The longlasting levels of DPH in the brain are probably due to a higher binding to tissue components.[24,25] This could also explain why in the PB induced animals a faster clearance is evident only in the plasma compartment. Furthermore, after chronic combined treatment, PB plasma and brain levels were much higher than after PB alone, while a single administration of DPH did not cause any variation in PB levels.

The present findings confirm our previous observation in children,[13,14] where the addition of DPH led to a substantial rise in plasma PB levels. Whether this rise in PB brain and plasma levels induced by DPH is due to a competitive inhibition of PB metabolism or to an inhibition of renal excretion remains to be determined.

A good correlation was also found between drug brain levels and protection against maximal electroshock if we consider the total level of both drugs, while this correlation is not always present if we consider the drug levels separately. In fact, in the case of the DPH-PB chronic group, the brain levels of DPH do not justify the full protection achieved.

Simultaneous control in animals of plasma and brain levels after repeated treatment with drugs commonly given together in humans may provide valuable information not only on the metabolic interactions, but also on the possible modifications of the physiological availability of both drugs, which in some instances cannot be seen in acute experiments.

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