THE PROPHYLACTIC EFFECT OF DIPHENYLHIDANTOIN IN THE KINDLING EFFECT ON REPTILES (*GALLOTIA GALLOTI*)

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Abstract—1. Lizards *Gallotia galloti* received daily 3 mg/kg body wt of diphenylhidantoin (DPH) over a period of 15 days and at the same time the animals were kindled.

2. The progression of the kindling effect was evaluated by counting the number of spontaneous epileptiform potentials, the duration of afterdischarges and the duration of electroencephalographic spontaneous seizures.

3. The diphenylhidantoin treated group, relative to controls presented: (a) significant reduction in the duration of afterdischarges and spontaneous electroencephalographic seizures; and (b) increased frequency of the spontaneous epileptiform potentials.

INTRODUCTION

The descent of the threshold for electrical elicitation of convulsive seizures was first described by Delgado and Sevillano (1961) and later Goddard *et al.* (1969) named the phenomenon as the Kindling effect.

The Kindling effect has been described mostly in mammals. However, it has been demonstrated that in reptiles kindling could be easily achieved, producing in these animals a variety of epileptic manifestations. This, added to the simplicity of the reptilian brain, make the use of reptiles a good choice for studying experimental epilepsy (Rial and Gonzalez, 1978).

On the other hand, the pharmacological prophylaxis of epilepsy is a growing topic, especially in the prevention of the increasing number of posttraumatic epilepsies produced after head concussion in automobile accident, (Majkowsky, 1977). In this respect, kindling is ideally suited for pharmacological screening of prophlactic antiepileptic drugs (Wada, 1977), and in particular reptilian kindling has been used in conjunction with diphenylhidantoin (DPH) to prevent the development of motor stimulus-bound convulsions (Rial and Gonzalez, 1977).

The aim of this paper is to study the prophylaxis of electrographic kindling in the reptilian brain using a well known drug, DPH, to assess the validity of the reptilian model.

MATERIAL AND METHODS

Twenty adult lizards of the *Gallotia galloti galloti* species were used. The animals were captured from their natural habitat and maintained in the laboratory at 22°C with food and water *ad libitum*.

Two groups of 10 animals were used. The experimental group received an aqueous solution of sodium diphenylhidantoin through an oral cannula at a dose of 3 mg/kg body wt every 24 hr. The treatment began 24 hr before the first electrical stimulation and lasted for 15 days, the drug being administered each day 30 min before stimulation and recording. After ceasing the drug administration 15 additional sessions were made. The control group received tap water in the same schedule.

For stimulation and recording, three stainless steel, 75 μ diameter, Teflon coated (except in the cut tip) electrodes were implanted under penthobarbital anaesthesia in each animal. Two electrodes were placed bilaterally on the surface of the dorsal telencephalic cortex and a third one, used as a reference electrode, was placed on the telencephalic interhemispheric line. One week was allowed for surgery recuperation.

Daily recording–stimulation sessions began with 30 sec of EEG recording 2 sec of 400 μA, 50 hz square wave electrical pulses of 1 msec duration were applied always to the same hemisphere, usually the left. After the electrical stimulation, at least 10 min of EEG recording was made from each animal with a Nihon-Kohden polygraph. A filter cutting frequency above 30 hz and a time constant of 0.1 sec were used for recording.

As a measure of the kindling progression, the number of isolated, rapid high voltage spikes (HVS) present in the EEG from the first 10 min of afterstimulation recording were counted. Also, when they appeared in the same record, the duration in seconds of afterdischarges (AD) or spontaneous electrographic spikes (SES) were measured.

To compare the differences between (a) control and experimental groups, and (b) before and after pharmacological treatment, the Wilcoxon and/or the Student's *t*-test were used comparing whole groups or blocks of convenient numbers of trials. For graphic representation of the results, the mean and SEM have been calculated.

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RESULTS

In Fig. 1 the variation in numbers of HVS relative to trial numbers is shown. The arrow in trial number
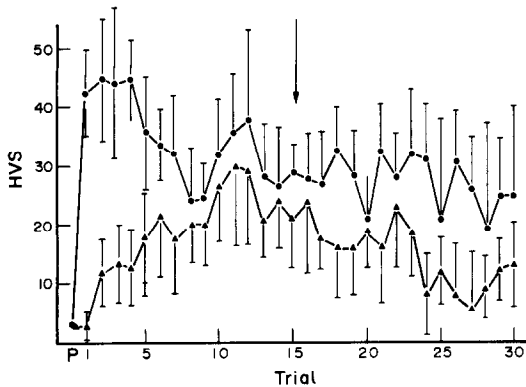
Fig. 1. Time course of the number of HVS in controls (triangles) and DPH treated animals (circles) recorded in 10 min after stimulation, relative to trial number. The arrow shows the end of the DPH treatment. *P*, value obtained prior to the first stimulation; *N* = 10.

15 represents the end of the treatment with DPH and *P* is the number of HVS prior to the first stimulation. The circles correspond to the DPH animals and the triangles to the controls. In the DPH group there was a large increase in the number of HVS shown immediately after the first stimulation, whereas in the controls the increase, though evident, was moderate. In the DPH group after the first rise, the number of HVS showed a slow decay. The suspension of the DPH treatment produced no noticeable change in this parameter, but the statistics showed a significant difference ($P \leqslant 0.01$) when the block of figures before and after DPH suspension were compared. The difference between control and DPH animals was also significant ($P \leqslant 0.01$).

In Fig. 2 the mean duration in seconds of the self sustained manifestations have been represented with

the same conventions as in Fig. 1. It can be seen that the controls spent a lot of time in electrographic seizures, which was greatly different to the DPH treated animals ($P \leqslant 0.001$). It is also evident that when the DPH treatment was suspended the time spent in electrographic seizures abruptly rose to levels similar to those of controls, but this occurred only in the first 7–8 sessions after DPH suspension; from day 24 no differences were found with the values prior to the DPH suspension.

DISCUSSION

The effects of different antiepileptic drugs are extremely species dependent. The effect of DPH in man is known, however, in rabbits Tanaka (1972) found no effects, whereas Wada *et al.* (1976) in baboons and cats and Ito *et al.* (1977) in cats found evident action of this drug in preventing kindling, provided that minimal plasma levels were reached. In the same *Gallotia galloti* Rial and Gonzalez (1977) found DPH effective for prevention of motor responses. These results, in conjunction with those presented here, reveal a high sensitivity of the lizards to DPH. Peterson *et al.* (1981) found that the progress in kindling effect was determined in each animal by the total accumulated time of afterdischarge. In this aspect DPH has been very active in suppressing kindling in lizards.

The frequency of HVS has also been used as an index of the kindling progression (Morrel and Tsuru, 1976). In respect to the high frequency of these spikes in the drug treated animals, the results agree with those of Edmond and Stark (1974) and Bustamante *et al.* (1980). It has been assumed that this effect is produced through reduction of the posttetanic potentiation caused directly by DPH (Esplin, 1957; Raines and Sandaet, 1967) which especially affects the long polysynaptic pathways. This means that DPH acted as an inhibitor on these long pathways, blocking the spreading of the selfsustained activity. From this point of view, the increased number of HVS could be considered as demonstrative of aborted AD and SES.

In conclusion, the reptilian model used seems to be valuable in assessing prophylactic drugs, showing great similarity with the action on other experimental animals and man.

REFERENCES

Bustamante L., Lueders H., Pippenger C. and Goldensohn E. S. (1980) The effects of phenitoin in the penicillin induced spikes focus. *Electroenceph. clin. Neurophysiol.* **48**, 90–97.

Delgado J. M. R. and Sevillano M. (1961) Evolution of repeated hippocampal seizures in the cat. *Neurophysiology* **13**, 772–733.

Edmonds H. L. and Stark L. G. (1974) Penicillin induced epileptogenic foci. II: The anticonvulsant and neuropharmacological effects of SC 13504 in the cat. *Neuropharmacology* **13**, 269–277.

Esplin D. V. (1957) Effect of diphenylhidantoin on synaptic transmission in the cat spinal cord and stellate ganglion. *J. Pharmac. exp. Ther.* **120**, 301–323.

Goddard G. V., McIntyre D. C. and Leech C. K. (1969) A permanent change in brain function resulting from daily electrical stimulation. *Exp. Neurol.* **25**, 295–330.
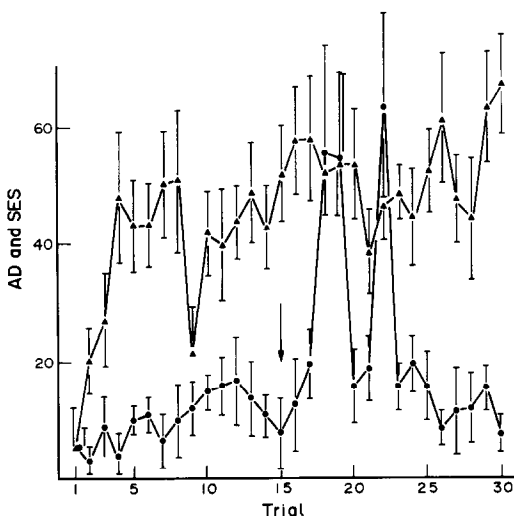
Fig. 2. Mean duration of AD + SES in sec for controls (triangles) and DPH treated animals (circles) recorded in 10 min after stimulation, respective to trial number. The arrow shows the end of the DPH treatment. *N* = 10.

Ito T., Hori M., Yoshida K. and Shimizu M. (1977) Effect of anticonvulsants on cortical seizure in cats. *Epilepsia* **18**, 63–71.

Majkowsky J. (Ed) (1977) *Posttraumatic Epilepsy and Pharmacological Prophilaxis*. Polish chapter of the I.L.A.E., Warzsawa.

Peterson S. L., Alberson T. E., Stark L. G. , Joy R. M. and Gordon L. S. (1981) Cumulative afterdischarge as the principal factor in the acquisition of kindled seizures. *Electroenceph. clin. Neurophysiol.* **51**, 192–200.

Raines A. and Sandaet F. G. (1967) An effect of diphenylhidantoin on posttetanic hyperpolarization of intramedullary nerve terminals. *J. Pharmac. exp. Ther.* **156**, 591–597.

Rial R. V. and Gonzalez J. (1977) The effect of diphenylhidantoin in the prevention of threshold descents in the electroshock on the reptilian trelencephalon. In *Posttraumatic Epilepsy and Pharmacological Prophylaxis* (Edited by Majkowsky J.), Chapter 28, pp. 203–208.

Rial R. V. and Gonzalez J. (1978) Kindling effect in the reptilian brain: motor and electrographic manifestations. *Epilepsia* **19**, 581–589.

Tanaka A. (1972) Progressive changes of behavioral and electrographic responses to daily amigdaloid stimulation in rabbits. *Fukuoka Acta Med.* **63**, 152–164.

Tuttle R. S. and Preston J. B. (1963) The effect of diphenylhydantoin (dilantin) on segmental and supra-segmental facilitation and inhibition of segmental neurons in the cat. *J. Pharmac. exp. Ther.* **141**, 84–91.

Wada J. A. (1977) Pharmacological prophylaxis in Kindling model of epilepsy. In *Posttraumatic Epilepsy and Pharmacological Prophylaxis* (Edited by Majkowsky J.), Chapter 23, pp. 154–166.

Wada J. A., Osawa T., Sato M., Wake A., Corcoran M. E. and Troupin A. S. (1976) Acute anticonvulsant effects of diphenylhidantoin, phenobarbital and carbamazepine: a combined electroclinical and serum level study in amigdaloid kindled baboons. *Epilepsia* **17**, 77–88.