Phenytoin Increases the Severity of Cortical Hemiplegia in Rats

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The effects of systemic phenytoin administration on the motor deficit resulting from a cortical lesion were studied in rats trained to walk coordinately on a narrow beam. The somatomotor cortex lesion was produced by an indwelling cannula through which saline or GABA were infused chronically via an osmotic minipump. Phenytoin (50 mg/kg i.p.) administered between days 3 and 5 after the intracortical catheter implantation produced a significant increase in the severity of the resulting hemiplegic syndrome. This DPH effect was more noticeable in those animals also receiving intracortical GABA infusions. The anticonvulsant at the dose used had no effect on motor performance when administered preoperatively or when given to the animals 14 days after surgical intervention when their hemiplegic syndrome had cleared. These findings suggest that phenytoin administration to brain-damaged individuals in the initial postlesion stage may be deleterious.

INTRODUCTION

Diphenylhydantoin (DPH) is among the first 50 drugs most frequently prescribed in the United States and is most commonly used to reduce seizure frequency including seizures associated with acute brain lesions. The mechanism by which DPH acts, however, is still uncertain.

Among the proposed mechanisms of action of DPH, the enhancement of GABA-mediated inhibition has been repeatedly suggested (for review, see ref. 19). The interactions between DPH and the GABA system have produced, however, conflicting results. Phenytoin administration increases the concentration of GABA in rat cerebral cortex and in mice brain, and increases the duration of GABA-mediated IPSPs in the crayfish stretch receptor neuron, decreases pyramidal responses to motor cortex stimulation, prolongs recurrent inhibition in cat pyramidal tract neurons, enhances postsynaptic inhibition in rat cuneate nucleus, in frog spinal cord and in isolated mouse spinal cord neurons. Phenytoin also has protective effects against picrotoxin-induced blockade of the GABA receptor, and against seizures induced by allylglycine, an inhibitor of GABA synthesis. Conversely, DPH has been reported to have no effect on GABA responses of frog spinal motoneurons, of rat and cat dorsal root ganglion neurons, or on rat hippocampal pyramidal cells. Furthermore, DPH has been reported to diminish the K+-induced release of GABA from rat cerebral cortex slices, without effect on the resting release of GABA, and has been reported to augment picrotoxin-induced seizure activity. Thus, the role of GABA in the effects of DPH is still a matter of controversy.

In previous reports we have shown that surface application of GABA to the somatomotor cortex of anesthetized cats produced a reversible inhibition of the negative component (N20) of the primary somatosensory evoked potential. Further, we have shown that intracortical GABA administration (via osmotic minipump) to the somatomotor area of unanesthetized young and aged rats produced a hemiplegic syndrome significantly more marked than the one produced by the same cannula-induced lesion but without intracortical GABA infusion. Thus, intracortical GABA infusion increases the functional deficit with-

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out changing the lesion size. This technique of intracortical GABA infusion permits the evaluation of the effect of phenytoin at different levels of hemiplegic severity without introducing the complication associated with varying lesion size.

MATERIALS AND METHODS

Subjects
Adult male F-344 rats weighing 240–270 g were used. They were housed in groups of 6 at constant temperature (21 °C) and maintained on a standard (12:12 h) light–dark cycle, so that the light cycle occurred during working hours. The animals had free access to food and water.

Training
One week after arrival, the animals were trained to walk on an elevated (45 cm above table level), narrow (2.5 cm) beam (2 m long) until they reached stable performance criteria (no slips or falls from the beam, no asymmetric positioning of the paws while walking, and no defects in paw placing on the surface of the wooden bar). Motor function on the beam was evaluated daily as described previously. Typically, after a motor cortex aspiration or chronic GABA infusion into the somatomotor region, the progression of the resultant motor deficit evolves from an animal totally unable to perform the task (score = 24), to animals who show falls, slips or defects in paw placing (score range = 8–20), to animals who walk without touching the lateral aspects of the bar (score = 0).

Sensory function testing was performed after completion of the motor task, and consisted of stimulation of vibrissae, trunk and dorsal and lateral surfaces of both extremities. Orientation to or withdrawal from the stimulus were considered to indicate normal sensory function.

Surgical procedures
After pretreatment with atropine methylbromide (6 mg/kg s.c.), the animals were anesthetized with ketamine (40 mg/kg i.p.) followed by pentobarbital (20 mg/kg i.p.). The scalp was shaved, cleaned and infiltrated with lidocaine hydrochloride (2%). The animals were then positioned in a stereotaxic apparatus. After opening the scalp, the bone sutures were visualized. A small (2 mm) trephine was then made over the hindlimb representation of the motor cortex, 2 mm posterior to bregma and 2 mm lateral to the midline. After opening the dura with a needle, a beveled cannula (PE60) to which an osmotic minipump (Alza 2001) was attached was inserted through the trephine for 2 mm tangentially to the skull at an angle of approximately −30° to the horizontal plane. The cannula was then attached to the skull with dental acrylic, using a bone screw to assure fixation. All implants were performed on the left side. The minipumps had been previously filled with either saline or GABA and inserted subcutaneously on the interscapular space. These devices are rated to deliver 1 μl/h for 7 days. The GABA-filled pumps were prepared to deliver 1 μl/h of a 100 μg/μl solution for 1 week. The wound was sutured and antibiotics were administered both locally and systemically.

Drug administration
Once the animals had achieved stable scores on the bar running task, they were divided in 3 groups of 12 animals each. After motor and sensory testing, they were given 0.1 ml/100 g of body weight, i.p., of one of 3 solutions (prepared beforehand by a colleague and randomly marked A, B, and C): phenytoin solvent (40% propylene glycol and 10% alcohol in distilled water, adjusted to pH 12), or phenytoin at 25 and 50 mg/ml concentrations. Animals were retested on the beam 30, 60 and 120 min after injection. The purpose of this presurgical control was two-fold. Firstly, it was intended to determine the dosage level of DPH which would not result in any ataxia or other alteration of motor performance in normal rats. Secondly, it was intended to determine the effects, if any, of the DPH solvent itself. The solvent did produce a relative ‘improvement’ in the scores of these normal rats which was significant at the P < 0.025 level. Since no significant changes in motor skills were observed with DPH we used the larger (50 mg/kg) concentration in the main experiment.

One week after this preliminary drug testing, the animals were subdivided in two groups: in one group saline-filled minipumps were implanted, and in another GABA-filled minipumps were used. These two groups were further subdivided; half of them received phenytoin solvent, and the other half received the anticonvulsant at a 50 mg/kg concentration. The response to drug administration was studied again on
Fig. 1. Effects of phenytoin (DPH) or its solvent (SOLV) on motor performance. Scores obtained before (Pre-surgery) and after surgical implantation of an intracortical cannula (Days 3–5 and Day 14). * P < 0.05, ** P < 0.005, *** P < 0.001.

Presurgery condition: no significant effects were found in animals treated with either DPH (25 or 50 mg/kg) or its solvent (n = 12/group). Days 3–5: Group A, animals implanted (on day 0) with an osmotic minipump delivering GABA (100 μg/μl/h for 7 days) and injected with DPH (50 mg/kg) on days 3–5. Scores obtained before and 30 min after drug administration; Group B, rats infused with saline-filled minipumps implanted at day 0 and injected with DPH solvent on days 3–5; Group C, rats implanted with intracortical cannulae identical to those of groups A and B but without drug infusion. DPH (50 mg/kg) administered on days 3–5; Group D, animals treated as group C (intracortical cannulae without infusion) and injected on days 3–5 with DPH solvent. Day 14: animals treated with DPH (50 mg/kg) 14 days after cannulae implantation. Group distribution as in Days 3–5 condition. No statistically significant effects were observed in these animals.

days 3, 4 and 5 postsurgery. Motor and sensory functions were evaluated before and 30 min after injection. The experimenter was unaware of either the minipumps' content or the identity of the drugs used.

Fourteen days after minipump implantation, all animals were given phenytoin (50 mg/kg i.p.) and tested as previously described.

Histology
The animals were sacrificed with a barbiturate overdose and perfused intracardially with a cold buffered formaldehyde solution (10%). After perfusion, the rats were positioned on a stereotaxic apparatus, and after removal of the cannula fixture, two insect pins were inserted in symmetrical positions (2 mm from the midline) over bregma. After bone and dura were removed, the implantation site lesion was photographed and measured. The brain was then removed for further histological analysis (Cresyl violet staining). Minipumps were opened to verify for complete emptying of the reservoir.

Data evaluation
The data were analyzed using an ANOVA test for repeated measures.

RESULTS
All animals reached performance criteria (a stable score on at least 3 consecutive days) after 2 weeks of training. The training was given every other day during the first week and daily thereafter.

Presurgery testing
As shown in Fig. 1, neither dose of phenytoin (25 or 50 mg/kg) produced any significant changes in motor performance. In contrast, DPH solvent decreased the motor deficit score.

Minipump implantation
In approximately half of the animals the extracranial end of the cannula was found to be detached from the minipump. In our previous work the severity and course of recovery of animals with saline injection into motor cortex did not differ from those who had simple aspiration of motor cortex. Since the animals found to have detached cannulas had the same severity and time course of recovery as we have found in those with saline injections, it can be assumed that the cannula disconnection occurred shortly after surgery and that no intracortical solution was delivered. Thus we can consider these animals to have only a cannula induced motor cortex lesion. Accordingly, 4 groups of animals were designated as: Group A, GABA-filled minipump with DPH treatment; Group B, saline-filled minipump with solvent treatment; Group C, motor cortex lesion with DPH treatment; and Group D, motor cortex lesion with solvent treatment.

The motor deficits observed in Group A (GABA-filled minipump with phenytoin treatment) in the first 3 days after implantation were similar to the ones reported previously. Administration of DPH between days 3 and 5 after surgery in this group produced a transient but significant (F₁,₅ = 73.42, P < 0.001) en-
Fig. 2. Dorsal view of the superficial extent of the lesions in animals from all 4 experimental groups. Two insect pins positioned at 2 mm from the midline, bilaterally, mark the bregma suture. Scale in mm.
hancement of the deficit (see Fig. 1). These deficits consisted in an increased incidence of slips and falls, limping and defective paw placing. All these deficits were contralateral to the implanted site for all 3 days of systemic drug administration.

Animals with saline-filled minipumps (Group B) showed motor deficits in the first 3 days after implantation similar to those previously reported\(^\text{7}\): administration of phenytoin solvent to these animals did not produce significant changes in motor performance.

In the animals treated with phenytoin but without GABA infusion (Group C), a significant \((F_{1,5} = 24.04, P < 0.005)\) increase in the severity of the lateralized motor deficits was observed (see Fig. 1). These abnormalities were less severe than the ones observed in group A (who had received GABA infusions) and consisted of slips and falls, limping and abnormal positioning of the contralateral hindlimb.

In Group D (motor cortex lesion and phenytoin solvent treatment), the systemic administration of the DPH solvent actually resulted in a decreased motor deficit score. It will be noted that the DPH solvent also ‘improved’ performance even presurgery. Although the cause of this effect is uncertain, it does suggest that the presence of the solvent in the DPH groups (A and C) actually may have attenuated the hemiplegia enhancing effects of diphenylhydantoin itself.

In all groups, the observed deficits were primarily motor deficits, and no persistent sensory abnormalities were detected.

**Postrecovery testing**

Fourteen days after surgery, and when the animals had recovered from their motor deficits, phenytoin (50 mg/kg i.p.) was administered. As seen in Fig. 1, no significant effects were produced by the drug. However, some of the animals had a transient generalized ataxia after drug administration without any sign of lateralization.

**Histology**

All lesions were localized in a comparable area — in reference to the bregma marks — corresponding to the hindlimb representation of the somatomotor cortex\(^\text{11,14,23}\). The structural lesions were of similar extent in all groups. In length, width and depth they measured \(2.7 \times 2.2 \times 1.8\) mm in group A, \(3.2 \times 2.3 \times 1.9\) mm in group B, \(2.4 \times 1.9 \times 1.7\) mm in group C and \(2.5 \times 2.1 \times 1.9\) mm in group D. None of these lesion dimensions were significantly different between groups (see Fig. 2).

**DISCUSSION**

All groups of animals in this study had structural lesions of the somatomotor cortex induced by the cannula which were comparable in size. However, the group of animals which received GABA through the cannula had a significantly more dense hemiplegia than the other 3 groups that did not have GABA infusions. The 3 non-GABA infused animal groups had a less severe hemiplegia which was not statistically different in Groups B, C and D. This finding replicates our previous observation\(^\text{7}\) on the potentiation of hemiplegia by cortical GABA infusions.

The purpose of the present experiments was to determine whether DPH administration would increase the functional deficit produced by an acute cortical lesion as might be anticipated if DPH potentiated the inhibitory effects of GABA. In the two groups receiving DPH (A and C) a statistically significant worsening of the functional deficit was observed. This DPH effect was reversible, however, since the animals returned to their prephenytoin motor performance score by the next day. When GABA was infused (Group A) with increase in the severity of the hemiplegia (but not changing the size of the lesion), the deleterious DPH effect on motor performance was more noticeable (see Fig. 1). It should be emphasized, however, that the larger increment in the DPH-enhanced motor deficit in the GABA treated group (A) compared to the non-GABA treated group (C) does not necessarily reflect a synergistic interaction between GABA and DPH. The larger increment in the motor deficit score in Group A could be due in part to the non-linear characteristics of the scoring system used. For this reason, the present experiment does not provide unequivocal evidence about GABA involvement in the reversible exacerbation of the hemiplegic syndrome produced by DPH.

Nevertheless, the results suggest that DPH reversibly increases the functional deficit produced by cortical damage in the early stages of the lesion. The less severe the deficit, the less noticeable are the drug effects, and in a very mildly impaired animal, the DPH
effect may not be detected. The advantage of the present technique of GABA infusion is that it can generate a more marked functional deficit without the necessity of creating larger lesions. It is of particular interest to note that on day 14, when the motor deficit score of our animals had returned to presurgical levels, no effect of DPH was detected, even in the GABA-treated animals. In contrast, we have reported that systemic administration of haloperidol, a dopaminergic blocker, on day 14 after surgery caused a re-emergence of the hemiplegic syndrome in animals who had GABA infused in a procedure identical to the Group A animals of the present study. Thus, those mechanisms involved in the expression of the effects of injury in the first week after cortical damage were refractory to DPH 2 weeks later but still capable of being activated by haloperidol, indicating some specificity of the DPH effect.

It has been reported that cerebral ischemia in various brain regions results in increased intracerebral GABA levels. We can reasonably assume that the cerebral lesions made by the cannula in all our animal groups also caused an elevation of endogenous GABA. Whether or not the potentiation of the hemiplegia by DPH is related to intracerebral GABA levels cannot be ascertained in the present experiment. However, the fact that GABA infusions increase the severity of the hemiplegia raises the interesting possibility that the deleterious DPH effects on acutely brain-injured animals might be mediated by GABA or GABA-like inhibitory neurotransmitters. Whether or not the effects of DPH are GABA-mediated, the present results may be of clinical importance as they suggest that some prudence be exercised in the administration of DPH in the initial days following an acute brain injury.

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REFERENCES