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Reduction of Frequency of Seizures by Carbamazepine During Cobalt Experimental Epilepsy in the Rat

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CRAIG, C. R. AND B. K. COLASANTI. *Reduction of frequency of seizures by carbamazepine during cobalt experimental epilepsy in the rat.* PHARMACOL BIOCHEM BEHAV **41**(4) 813–816, 1992.—Adult female Sprague-Dawley rats rendered epileptic by bilateral cerebral implantation of cobalt wire were simultaneously prepared with permanent cortical and temporalis muscle electrodes for continuous recording of electroencephalogram and electromyogram activities. Carbamazepine (50 or 100 mg/kg) was administered intraperitoneally twice daily days 8–12 after cobalt implantation. The high dose of carbamazepine was effective in decreasing seizure incidence each day of its administration, but the lower dose was effective in decreasing seizure incidence only for 6 h following its administration on days 8 and 9. These results provide additional evidence that cobalt epilepsy in the rat is a valid model for the study of seizure disorders.

Cobalt epilepsy Rat ECoG Carbamazepine

THE application of cobalt to the brain of a variety of species results in the development of a chronic seizure state that has been utilized as an experimental model of epilepsy. Of the several species examined, cobalt epilepsy in the rat has been the most widely studied. The seizure state induced in the rat by the cerebral cortical application of cobalt exhibits several features desirable in a model of epilepsy. In addition to decreased seizure thresholds to a variety of convulsant agents (9) and electroencephalographic (EEG) spiking (2), the regular appearance of generalized motor convulsions is a prominent feature of cobalt epilepsy (3). The time course for these generalized motor convulsions makes this a particularly interesting model to study. Very few seizures are recorded for 5–6 days following application. This is followed by a period of about 1 week that is characterized by a large number of motor convulsions (10). At approximately 2 weeks following application of cobalt, the number of seizures is markedly diminished, probably reflecting a calcification of the cobalt lesion (13).

Cobalt epilepsy in the rat shares many properties with human epilepsy, such as lowered thresholds to chemical convulsants, EEG spiking, and intermittent and spontaneous convulsions. The purpose of this study was to determine the effect of carbamazepine on the frequency of seizures over several days of treatment in the cobalt-epileptic rat. Carbamazepine is a widely employed antiepileptic agent that has utility in several types of human epilepsy but has not been evaluated in this model.

METHOD

Adult, female Sprague-Dawley rats were used in all experiments. Prior to surgery, rats were anesthetized by the subcutaneous administration of 0.6 ml Innovar® (a commercial preparation containing 0.05 mg fentanyl and 25 mg droperidol per ml). A hole was drilled in the skull two mm to the left and the right of the midline at the bregma and 1- to 2-mm lengths of cobalt wire, 1 mm in diameter, were inserted directly into both left and right cortices. The openings in the skull were covered with small pieces of Gelfoam® (an absorbable gelatin sponge). For bipolar recording of the electrocorticogram (ECoG), stainless steel screws were engaged in the skull over frontal and parietal cortices 4 mm anterior and 3 mm posterior to the bregma and 3 mm lateral to the midline. Stainless steel wires were sutured into the temporalis muscles for bipolar recording of the electromyogram (EMG). All electrodes were soldered to a seven-pin Amphenol connector positioned over the skull with acrylic dental cement.

Groups of five rats were prepared for each experiment. Following completion of operative procedures, rats were placed in individual recording cages where they were con-

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nected to a cable for recording ECoG and EMG activity. The ECoG and EMG activities were recorded on Grass model 7 polygraphs. The recordings were conducted continuously from days 1-14 at the slow chart speed of 25 mm/min. Rats had free access to food and water and were unrestrained and freely moving during the entire experimental period. Lights were regulated by a timer with a dark period 8 p.m. to 8 a.m.

Carbamazepine was administered intraperitoneally as a suspension in 10% polyethylene glycol 400 (PEG) in a volume of 0.4 ml/100 g twice per day for 4 days commencing 8 days after cobalt implantation. Other rats received a similar volume of the PEG vehicle. This dosage regimen was evaluated for evidence of neurotoxicity using the positional sense test (17). In this test, a hind leg of the rat is lowered from the edge of a laboratory bench. If the animal cannot quickly lift the leg to its original position, it is considered to have a neurological deficit. Neurological deficits are also indicated by ataxia, abnormal body posture, and events such as zigzag gait.

Generalized motor seizures were quantified on the basis of the ECoG and EMG tracings. Electrographic criterion was the occurrence of repetitive, bilaterally hypersynchronous spiking in all cortical leads. This was accompanied behaviorally by clonic and tonic movements of forelimbs and facial muscles; sometimes, the hind limb musculature was also affected with loss of righting.

Statistical comparisons between results for carbamazepine, vehicle, and untreated cobalt-epileptic rats were made using Student's *t*-test.

RESULTS

On the basis of preliminary results in our laboratory and published results (18), it was decided to use 50 and 100 mg/kg each twice daily as the dose of carbamazepine. The higher dose produced some evidence of toxicity, particularly during the second and third day of treatment. It appeared that tolerance to neurotoxicity was present by day 4. There was no indication that the vehicle contributed to the toxicity.

Figure 1 illustrates the frequency of seizures in untreated rats rendered epileptic by insertion of cobalt into both left and right cerebral cortices. This pattern is similar to that previously reported. It can be seen that relatively few seizures occur during the first week after cobalt implantation. The number of seizures is then relatively constant for about 5 or 6 days. After about 14 days, the incidence of seizures is markedly reduced.

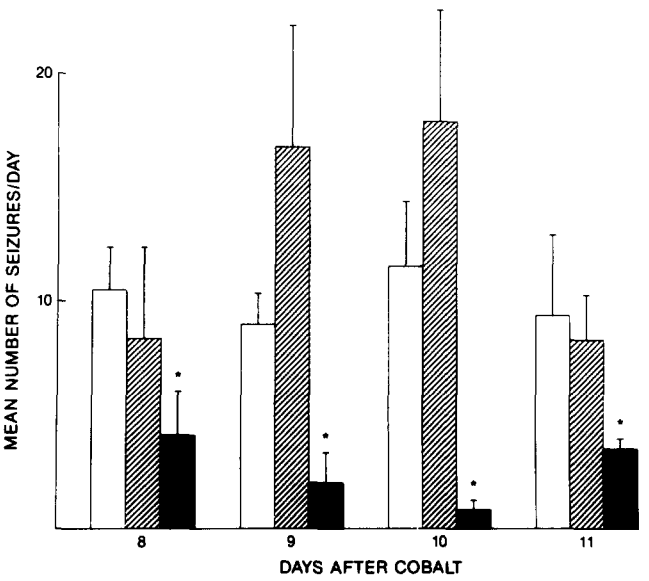

FIG. 2. Number of generalized seizures (± SE) on days 8-11 following cobalt implantation in both left and right cerebral cortices. (□), PEG vehicle; (▨), carbamazepine 50 mg/kg, administered twice daily IP; (■), carbamazepine 100 mg/kg, administered twice daily IP. *Different from vehicle control ($p < 0.05$).

Following carbamazepine administration (Fig. 2), no significant alteration in seizure frequency was observed at the dose of 50 mg/kg twice daily, but the dose of 100 mg/kg twice daily produced a significant reduction in the incidence of both ECoG and behavioral seizures each day it was administered.

To more clearly evaluate the effectiveness of carbamaze-

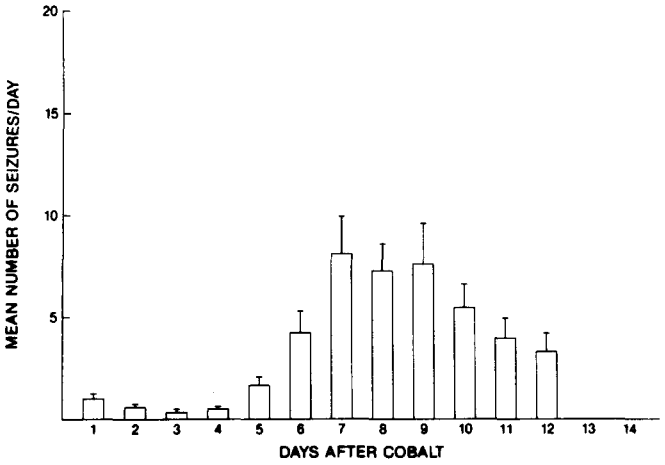

FIG. 1. Time course for the appearance of generalized seizures in rats in which cobalt metal was applied to both left and right cerebral cortices ($n = 12$). The number of generalized seizures (± SE) for each day are indicated.

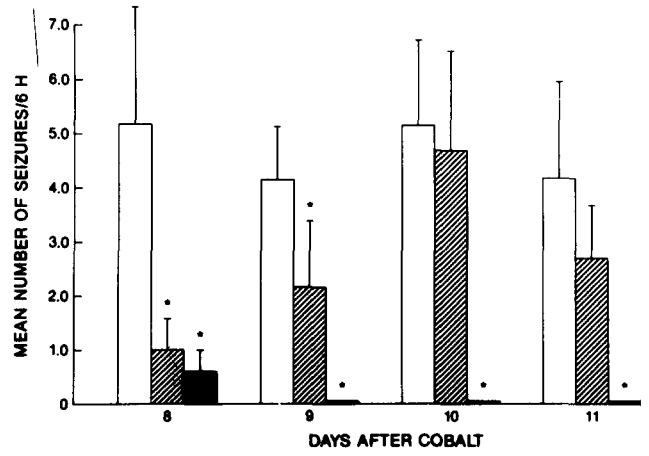

FIG. 3. Number of generalized seizures (± SE) on days 8-11 for each 6-h period following administration of: (□), PEG; (▨), carbamazepine 50 mg/kg, twice daily; or (■), carbamazepine 100 mg/kg, twice daily. *Significantly different from vehicle control ($p < 0.05$).

pine, the number of seizures that occurred in the first 6 h following drug or vehicle was ascertained. Figure 3 illustrates that the lower dose of carbamazepine reduces seizure incidence for each 6-h period during the first 2 days of administration; however, there was no reduction during the last 2 days.

During the entire study, interictal focal spike discharges and other EEG evidence of interictal epileptogenic activity previously reported (3) was present and was not prevented by carbamazepine.

Unlike results in another report (1), rats treated with PEG only did not exhibit any anticonvulsant effects when compared with animals not receiving the vehicle.

DISCUSSION

One measure of the validity of experimental models of epilepsy is whether or not clinically useful antiepileptic drugs are able to decrease the frequency and severity of the seizure episodes. Based on the present study and previous investigations, most clinically useful antiepileptic drugs are able to reduce the frequency of seizures in the cobalt-epileptic rat. In many cases, however, the dose of drug necessary for a significant anticonvulsant effect in the cobalt-epileptic rat is higher than the dose necessary to demonstrate anticonvulsant properties in other assay systems and is higher than the dose in human epilepsy.

There may be various reasons for this. The type of epilepsy produced in the rat by cerebral implantation of cobalt is a severe seizure disorder, particularly if cobalt is placed bilaterally. Although the number of generalized convulsions may be modest, the EEG abnormalities (epileptic spiking) are marked and persistent. For our purposes, we quantified only generalized seizures that have a prominent EEG component and made no attempt to quantify other manifestations of a seizure state. By evaluating the effects of a drug only on generalized seizures, it is possible that we underestimated its effectiveness. However, it is clear that carbamazepine, even at the high dose, was not able to normalize EEG tracing. At later time periods, it is probable that brain levels of carbamazepine are too low to provide anticonvulsant effects. In the female rat, carbamazepine has a reported $T_{1/2}$ of 111 min after a single 25-mg/kg dose; this declines to 60 min following repeated doses for 7 days (8). The inability to detect any significant anticonvulsant effect at 6 h during the last 2 days at 50 mg/kg may be a consequence of a lower $T_{1/2}$ due to induction of hepatic microsomal drug-metabolizing enzymes.

Carbamazepine is very effective against maximal electroshock seizures in mice and rats and is, likewise, a very useful antiepileptic compound in the human. It is also reasonably effective against many other seizure models. It is active against kindling seizures in rats (12), as well as seizures in the genetically epileptic-prone (GEP) rat (6,14).

One of the aims of the present study was to determine if the cobalt-epileptic rat was a suitable model in which to evaluate potentially useful anticonvulsant compounds. It is now almost 40 years since the generally employed anticonvulsant assays (maximal electroshock seizure test and subcutaneous pentylenetetrazol seizure test) in mice were developed and standardized (16). The use of these assays resulted in the development of several antiepileptic agents and the discovery of endless others that for one reason or another were dropped along the line. There is a belief that not all drugs useful in human epilepsy will be detected using these two assays. A case in point is valproic acid, a very useful antiepileptic drug in humans but possessing little effectiveness in routine screening procedures (17).

The overall effectiveness of carbamazepine against seizures in the cobalt-epileptic rat is similar to that of phenytoin (4). Both are effective in decreasing the incidence of seizures although at only relatively high doses. Neither is particularly effective in altering epileptic spiking activity in cobalt epilepsy. Both drugs display similar patterns against routine screening assays: effective against maximal electroshock seizures and ineffective against pentylenetetrazol seizures (17). Both also display similar clinical profiles in human epilepsy (18).

Most antiepileptic drugs that have been evaluated against the seizures in cobalt epilepsy in the rat are effective, although at relatively high dose levels (2,4,5,7,15). The reasons for a high dose being required may well relate to the severity of the seizures in this model. Pharmacokinetic factors may also come into play since seizures may be attenuated when drug brain levels are high and any therapeutic effect may well disappear as brain levels decrease. The fact that anticonvulsant drugs are effective in decreasing seizure incidence indicates that cobalt epilepsy in the rat is a useful model for the further study of seizure disorders.

It has not been established if a single experimental model of epilepsy is superior to others in all respects or even if one model is better for the evaluation of anticonvulsant drugs. As can be imagined, there is strong support for a variety of models ranging from alumina epilepsy in the monkey (11) to GEP rats (6). Loscher and Schmidt (12) in a recent extensive review provided evidence to support the use of amygdala kindling in rats as a reliable screening method for finding drugs useful in focal epilepsy in humans.

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