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EFFECT OF NIKETHAMIDE ON PENTOBARBITAL SLEEPING TIME IN VARIOUS ANIMAL SPECIES

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Abstract—1. Pretreatment of rats with nikethamide results in a shortened sleeping time on a standard dose of pentobarbital.

2. Three cold-blooded species, the chameleon, caiman and the frog, did not respond with a shortened sleeping time after treatment with nikethamide.

3. The chicken and guinea pig were refractory.

4. Two species did respond with a shortened sleeping time. One was the mouse, the other was the domestic rabbit.

INTRODUCTION

ADMINISTRATION of nikethamide (coramine) has been shown to produce several interesting intrahepatic phenomena in the rat. In the weanling rat a prompt enlargement of the liver occurred without accompanying indications of abnormality in the organ (Brazda & Coulson, 1948). A stabilization followed the initial increase in size, and further increase did not take place. Histologic sections of livers from treated animals revealed increased numbers of binucleate cells and of mitotic figures (Brazda & Coulson, 1948). Wilson & Leduc (1950), using mice rather than rats, also reported evidence of increased cellular multiplication. The mice were maintained through three generations on a diet containing nikethamide without developing signs of any hepatic abnormality. They observed also that adult mice placed on the diet showed an increased hepatic mitotic activity only rarely. Foster & Brazda (1958) reported a significantly increased rate of incorporation of P^{32} into the DNA of weanling rats treated with nikethamide. In a subsequent report Brazda & Baucum (1961) showed that both weanling and adults rats, following treatment with nikethamide, exhibited a sharp reduction in sleeping time in response to a standard dose of pentobarbital. Their data also showed that the activity of the enzymes which oxidize pentobarbital in liver microsomes was increased severalfold by pretreatment with nikethamide. Remmer (1962) has confirmed these effects.

These results, together with much additional data, led us to believe that the nikethamide effects might be of considerable use in understanding the phenomenon of growth. Support for this view could be given if it were proved that the identical

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type of hepatic response or reduced sleep response occurs in other, preferably unrelated, species of animals. This was the objective of the present work.

MATERIALS AND METHODS

Nikethamide may be administered to rats by being incorporated into a synthetic diet; it may be administered by stomach tube; it may be injected intraperitoneally. The three methods are equally efficacious in the elicitation of the usual effects. In the performance of the experiments that form the basis of this report nikethamide was administered intraperitoneally by injection, mostly for its ease.

It has been thoroughly established that the rat exhibits sex differences in terms of the magnitude of response to various stimuli such as that offered by nikethamide. The difference is slight at the age of weaning and becomes great in adulthood. Castration eliminates it.

Care, therefore, had been exercised in the past in having, as nearly as possible, equal numbers of males and females in experiments utilizing rats. However, in some of these experiments no attention was paid to this detail because of an inability to determine the sex of the animals involved or because of the difficult circumstances in the obtaining of a species.

The original report (Brazda & Baucum, 1960) regarding the effect of nikethamide on the sleeping time of rats following pentobarbital injection utilized a standard pretreatment procedure. It consisted of a 5 day period during which fourteen doses of a 0·2 M nikethamide solution, each 0·2 ml in volume, were given by stomach tube. Each such dose amounted to 7 mg of the drug. The animals were then allowed a 2 day "rest" period without the drug before being used in the sleeping time experiment, thus avoiding direct analeptic effects on the central nervous system. Subsequent work indicated that a single dose of nikethamide will produce the effect to a significant extent within 8–12 hr in the rat. However, for the sake of more ready comparison, the experiments here reported used the same 5 day procedure of pretreatment as that regularly used on the rats. The longer period of exposure to the drug seemed advisable since some of the species used were poikilothermic forms and had a lower rate of metabolism.

The nikethamide administered in rat experiments amounted to approx. 140 mg/kg of animal in each individual dose. This level of administration was retained in the present experiments except in species where such dosage was too toxic and productive of convulsions. Some reduction in dose was here necessary and is indicated beneath the appropriate table.

RESULTS

Seven species of animals were utilized in the study, in addition to the rat. Three were poikilothermic and, therefore, not closely related to the rat. A close relative, the mouse, another rodent, the guinea pig, and the domestic rabbit, a lagomorph, were also used. Finally the chicken, a representative of the class Aves, was also studied. The results are presented in Tables 1–8.

TABLE 1—THE RAT (*Rattus rattus norvegicus*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)	95% confidence limits
Control	9 (5♀, 4♂)	78–128	35 mg/kg	132 (45–232)	132 ± 49
Experimental	9 (5♀, 4♂)	85–108	35 mg/kg	47 (1–78)	47 ± 20

Pretreatment: 14 doses, 0·2 ml each, 0·2 M nikethamide, given over a span of 5 days.

TABLE 2—THE MOUSE (*Mus musculus*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)	95% confidence limits
Control	12 (all ♀)	21–28	90 mg/kg	174 (50–420)*	174 ± 74
Experimental	12 (all ♀)	18–28	90 mg/kg	127 (93–179)	127 ± 17

* One animal slept much longer than did the one above it in the series.
Pretreatment: As above, under Table 1: The rat.

TABLE 3—THE DOMESTIC RABBIT (*Oryctolagus cuniculus*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)	95% confidence limits
Control	6 (5♀, 1♂)	1800–2500	30 mg/kg	116 (69–191)	116 ± 49
Experimental	7 (5♀, 2♂)	1730–2390; one at 1270	30 mg/kg	38 (0–48)	38 ± 16

Pretreatment: 14 intraperitoneal doses, 2·0 ml each, 2·0 M nikethamide, given over a span of 5 days. Each such dose offered thirty times as much nikethamide as did the dose given to rats; the ratio is the same as that of the animal weights, 1800–2400 g of rabbit to 60–80 g of rat.

TABLE 4—THE GUINEA PIG (*Cavia porcellus*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)	95% confidence limits
Control	8 (4♀, 4♂)	174–280	25 mg/kg	205 (157–376)	205 ± 64
Experimental	12 (6♀, 6♂)	178–278	25 mg/kg	176 (82–348)	176 ± 53

Pretreatment: As with the rats (see Table 1).

TABLE 5—THE CHICKEN (*Gallus domesticus*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)
Control	18 (12♀, 6♂)	66–80	50 mg/kg	144 (0–475)
Experimental	23 (10♀, 13♂)	49–87	50 mg/kg	146 (0–456)

Pretreatment: As with the rats (see Table 1).

TABLE 6—THE CHAMELEON (*Anolis carolinensis*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)*
Control	12	1·8–4·4	50 mg/kg	102 (37–270)
Experimental	24	2·0–6·3	50 mg/kg	100 (50–257)

* One animal in each group died after pentobarbital treatment.

Pretreatment: The experimental animals received ten doses of nikethamide over a 5 day span of time instead of 14. Each dose contained 1 mg of nikethamide/7 g body weight. This corresponds to a 7 mg dose per 49 g body wt. in weanling rats.

TABLE 7—THE CAIMAN (*Caiman latirostris*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)
Control	9	32–53	10 mg/kg	692 (133–1560)
Experimental	9	34–57	10 mg/kg	999* (255–1306)*

* One of the experimental animals died 4 days after pentobarbital without awakening. The average and range are based on the eight surviving animals.

Pretreatment: Nine doses, each 0·2 ml of 0·2 M nikethamide, were given over the usual 5 day span.

TABLE 8—THE FROG (*Rana pipiens*)

	No. of animals	Wt. (g) (range)	Pentobarbital dose	Avg. sleeping time (min) (range)*
Control	18	40–54	35 mg/kg	94 (0–297)
Experimental	41	41–66	35 mg/kg	109 (0–285)

* Two animals of the control group failed to sleep after pentobarbital, as did five animals in the experimental group. Three animals in the experimental group died after the barbiturate and were eliminated from the calculations.

Pretreatment: Ten doses, each 6 mg of nikethamide, spread over 5 days, were administered to each experimental animal. This was based on exploratory trials using frogs weighing an average of 42 g or about $\frac{6}{7}$ of a 49 g weight of a weanling rat.

DISCUSSION

The data presented in the tables indicate that a nikethamide-induced shortening of sleeping time after pentobarbital administration is neither a universal phenomenon nor a single species effect. None of the poikilothermic species used as samples in this study showed such an effect. The same is true of the chicken.

Only in the class Mammalia were found species, beside the rat, which exhibited the shortened sleeping time effect. It was most pronounced in the rat. Oddly enough, the rabbit responded to about the same extent to the nikethamide pretreatment as did the rat, whereas the mouse, a more close relative of the rat, did so

much less dramatically. The work of Wilson & Leduc (1950), based on different parameters but bearing on the same general hepatic effect of nikethamide, showed unequivocally the sensitivity of the mouse liver to nikethamide. There can be no doubt that the mouse must be included with the rat and the rabbit as hepato-responsive to nikethamide.

The guinea pig showed no effect of nikethamide in these experiments.

It had been part of the experimental plan to study other orders of the class Mammalia if all of the Rodentiae and Lagomorphae samples exhibited the phenomenon under scrutiny. However, in view of the results with the guinea pig, and of the difficulty in conducting experiments with large numbers of the bigger mammalian species, such an exploration was not pursued.

In summary, then, it seems probable that nikethamide is able to cause an enhancement of the enzymatic activity responsible for metabolizing a barbiturate, such as pentobarbital, only in some species of animals. At present we have shown this for the rat and rabbit, but nikethamide has no such effect in the mouse, guinea pig, chicken, chameleon, caiman and frog.

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