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Δ^9-tetrahydrocannabinol and Pentobarbital as Discriminative Cues in the Mongolian Gerbil (*Meriones unguiculatus*)

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JÄRBE, T. U. C., J. O. JOHANSSON AND B. G. HENRIKSSON. *Δ^9-Tetrahydrocannabinol and pentobarbital as discriminative cues in the Mongolian gerbil* (Meriones unguiculatus). PHARMAC. BIOCHEM. BEHAV. 3(3) 403–410, 1975. – Male Mongolian gerbils were trained to escape electric shocks in a T-shaped maze contingent upon the presence or absence of certain drug effects (state-dependency; StD). The drug discriminative cues used were those of either Δ^9-tetrahydrocannabinol (THC) or pentobarbital (P-barb.) vis-à-vis the respective vehicles. Several doses of THC (0.5–16.0 mg/kg) were used and compared with P-barb. (20.0 mg/kg), a dose at which the most rapid drug discrimination occurs in the rat. When drug discrimination was established dose–time– and transfer characteristics for the training drugs were studied. Possible potentiation and antagonism was also examined in the pentobarbital trained gerbils. It was found that none of the THC doses were discriminated as rapidly as that of P-barb. Decreasing the amounts of training drug administered or increasing the injection-test intervals resulted in a decline of the number of drug associated choices. There was a maximum of 40% drug choices between THC and P-barb at the transfer tests. Mixtures of the two compounds increased the number of drug choices in an additive or even more than additive manner. Amphetamine (4.0 mg/kg) did not interact with the P-barb. induced choice responding. The analeptic drug, bemegride was found effective in antagonizing the P-barb. cued choice behavior.

Tetrahydrocannabinol Pentobarbital Drug discrimination (StD) Drug interaction Gerbils

THE stimulus or cue-properties of drugs have received much attention during recent years. Several classes of drugs have been studied but the conclusions so far drawn are mainly restricted to the rat [12].

The present study intended to examine some discriminable properties of Δ^9-tetrahydrocannabinol(Δ^9-THC) and pentobarbital (P-barb.) in the mongolian gerbil, *Meriones unguiculatus*. This was done in order to learn in what respects some of our previous results from work with the albino rat could be extended to another species.

Since the introduction of the gerbil for laboratory research [17] an increasing number of scientific publications have appeared pointing out characteristics peculiar to the gerbil as well as those shared with other laboratory rodents [9].

By drug discriminative control is meant that the ability of an organism to perform a certain task eg. turning differentially to the left or to the right side of a T-shaped maze in order to escape aversive stimulation, is dependent upon the presence or absence of certain drug effects (state-dependency, StD).

In the present study several doses of Δ^9-THC were used and compared with pentobarbital, 20.0 mg/kg, a dose at which the most rapid dissociation (StD) occurs in the rat [12]. When discriminative responding was well established dose–time– and transfer characteristics of the drugs were studied. The pentobarbital trained gerbils were challenged with bemegride in order to evaluate possible antagonism [11,20]. By simultaneously exposing the animal to both the training drug and another psychoactive agent we also examined if the cueing properties of a certain drug can be potentiated.

METHOD

Animals

Adult male gerbils *(meriones unguiculatus)*, weighing between 60–65 g, were used. They were individually housed during the experimental period with free access to food (Type 210, Anticimex AB) and tap water. Once a week supplies of cabbage were given to the animals. The

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gerbils were from our breeding colony and single housing was initiated three weeks prior to the experiments.

Apparatus

A T-shaped three-alley maze with a grid floor was used. To impel the animals the floor was electrified via a LeHigh-Valley shock generator (Type 113–2) set at output level 75. The closest distance to be traversed to escape the shocks was 78 cm.

Procedure

Prior to the discriminative training phase the gerbils were injected intraperitoneally (i.p.) with the appropriate vehicle for two days and placed on the grid floor (not activated) during five min. No exploration of the maze was allowed.

When training the animals to respond differentially to the presence or absence of certain drug effects the following procedure was used. Half the number of gerbils in a group were injected i.p. with a drug (D) a specified interval prior to the training sessions while the other half received the vehicle (ND). During the training sessions of five trials each the animals were dropped on the activated grid floor of the T-shaped maze and were required to find the exit in order to escape the aversive stimulation. A trial was ended when the gerbil jumped off the grid and into the home box, which had been placed adjacent to the maze exit. A self-correcting procedure was used. The correct side (left or right) of the T-maze depended on the imposed state (D or ND). The left side of the maze was correct for half the drugged and half the nondrugged gerbils. The opposite side was appropriate for the remaining animals. Exit through the arm designated incorrect was prevented by a barrier not visible to the animal when making the choice (left or right turn). A choice was recorded when the gerbil had left the choice area, the 144 cm^2 junction of the three alleys, with its whole body excluding the tail. The imposed states, the only experimentally manipulated cues, alternated for five consecutive days a week. For example, at the first session a drugged animal found the exit at the left side of the maze; at the second session, when trained nondrugged, the exit for this animal was at the right side; at the third session (D) the exit was again found at the left side. Such a training desing was followed until drug discrimination appeared i.e. the direction of the first-trial turn (choice) was determined by the imposed state. Drug discrimination was considered completed when an animal reached the criterion of performing eight correct first-trial choices out of ten consecutive training sessions. If criterion performance was not established within forty sessions the gerbil was assigned the score 40 and removed. For those animals, except in Group 1, that had met the criterion test sessions were interposed in the regular training, mostly twice a week. At test days only one trial per gerbil was run and both goal-boxes were accessible in order to minimize the occurrence of new learning. Test trials were not run unless the first-trial choices from the preceding training sessions had been correct.

The gerbils were randomly assigned to each of the following drug conditions: Group 1 (n = 8): Δ^9-THC (0.5 mg/kg); Group 2 (n = 8): Δ^9-THC (2.0 mg/kg); Group 3 (n = 8): Δ^9-THC (8.0 mg/kg); Group 4 (n = 6): Δ^9-THC (12.0 mg/kg); Group 5 (n = 6): Δ^9-THC (16.0 mg/kg); and Group 6 (n = 8): Pentobarbital (20.0 mg/kg). The corresponding nondrug conditions consisted of an injection of the suspension (see below) for THC-treated animals whereas physiological saline was used for the barbiturate group.

Drugs

The tetrahydrocannabinol, (-)-trans-Δ^9-tetrahydrocannabinol, (generously supplied by Dr. O. J. Braenden, U.N. Office at Geneva) was assayed (by glc.) to be 95.4% pure (NIMH, U.S.A.). However, subsequent determinations (K. Leander, Stockholm) yielded a purity of 81%. The analyses were carried out as described elsewhere [1].) The calculation of the THC doses was based on the latter analysis. THC was suspended, as recommended by others [19], in a mixture containing propylene glycol (10%), polysorbate-80 (1%), and saline whereas pentobarbital (Nembutal®), bemegride (Megimide®), d-amphetamine sulfate and atropine sulfate were dissolved in physiological saline (0.9%) (brand of bemegride, Nicholas Labs. Ltd.: β-ethyl-β-methyl glutarimide). Only freshly prepared THC suspensions were used. All doses were calculated as salts on a weight basis of 60 g. Except for bemegride all injections were given in a constant volume of 0.2 ml per gerbil. The volume for bemegride varied between 0.25–0.50 ml per animal. The injection intervals were: THC, 30 min; pentobarbital, 10 min; bemegride, 5 min; amphetamine, 10 min; atropine, 10 min.

The administration of hashish-smoke to Groups 4 and 5 was analogous to the procedure described elsewhere [5]. The gerbils, maximum three at the time, were confined in an acrylic box and exposed to hashish-smoke for 3 × 3 min with 2 × 2 min of fresh air interposed. Approximately 2 g was used to fill the box with smoke for the three occasions for 3 min. Test trials were run 10 min after the last 3 min period of smoke inhalation. The THC content of the raw material was 3.2% (generously supplied by Dr. S. Agurell, Uppsala).

Data Analysis

Due to beterogeneity of variance (cf. Group 2) non-parametric statistics were used to analyse the acquisition scores of Groups 1–5. Kruskal-Wallis one-way analysis was used for overall significance, and Nemenyi's *a posteriori* test for pairwise comparisons for the proceeding evaluations. Error rate for the collection of comparisons was set at $p = 0.05$ meaning that the pairwise comparisons were performed with an error rate less than 0.05 [8]. However, the Student's *t*-statistic was used for the comparison between the THC- and pentobarbital treatments (Group 4 vs. Group 6) and the strength of the relation between the independent and dependent variables was assessed with Hays' omega^2, ω^2 [4]. Chi square analysis was used for comparisons of interest with respect to test trial data [18].

RESULTS

A graphical presentation of the acquisition data can be found in Fig. 1.

The Kruskal-Wallis analysis applied to the number of sessions required by the THC treated gerbils (Groups 1–5) until the beginning of a criterion of performing eight correct first-trial choices out of ten consecutive training sessions was significant ($H = 23.354$, $df = 4$; $p<0.001$). Further comparisons showed that Groups 3 (8.0 mg/kg), 4

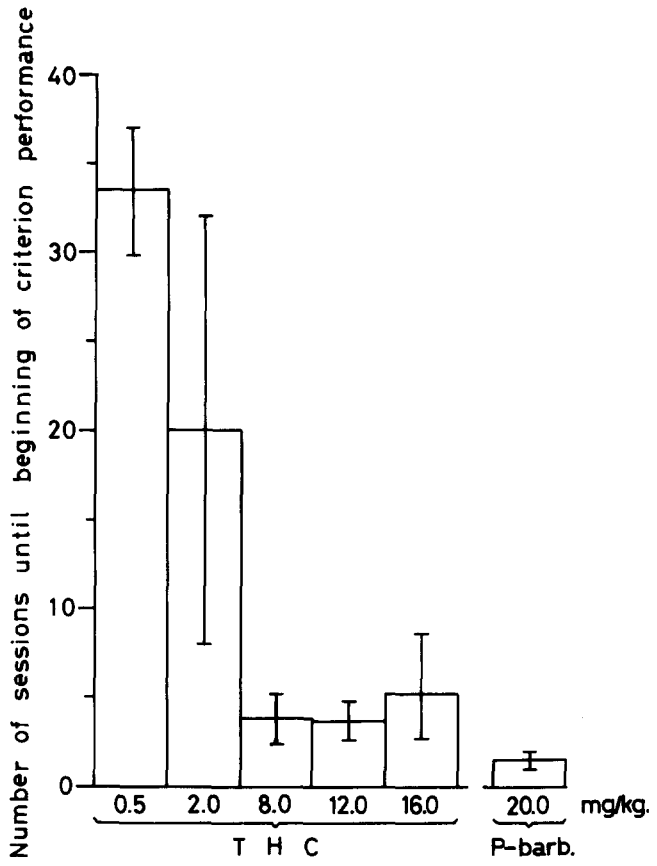

FIG. 1. Legend: Dose-related formation of drug discriminative response control.

Note: X-axis = intraperitoneal doses of Δ^9-tetrahydrocannabinol (THC, 0.5–16.0 mg/kg), given 30 min prior to the training sessions and an intraperitoneal dose of pentobarbital (P-barb., 20.0 mg/kg), given 10 min prior to the training sessions. Y-axis = average number of sessions and standard deviations until the beginning of criterion performance (8 correct first-trial choices out of 10 consecutive training sessions) by gerbils required to discriminate a drugged state from the vehicle state.

(12.0 mg/kg), and 5 (16.0 mg/kg) did not differ significantly between themselves but that either group was significantly different ($p<0.05$) from Groups 1 (0.5 mg/kg) and 2 (2.0 mg/kg). The difference between Groups 1 and 2 reached borderline significance ($0.10<p>0.05$). This suggests a dose-related formation of the THC induced discrimination. None of the THC doses were differentiated as fast as the 20.0 mg/kg pentobarbital dose (Group 4 vs. Group 6, $t = 3.16$, $df = 12$; $p<0.005$, one-tailed). The strength of the relation was estimated to be $\omega^2 \times 100 = 39\%$, suggesting a reasonable association.

Dose- and time characteristics of THC were studied in Groups 2 and 3; the number of observations were 2 X n where n = 6 for Group 2 since two gerbils did not reach the criterion within 40 sessions and n = 6 also for Group 3 due to the death of two animals. A decline in the number of drug associated choices was noted as a result of using one half, one fourth, and one sixteenth of the training doses. Increasing the injection-test interval (i.e., more than 30 min) also resulted in fewer drug choices. No significant difference was found due to shortening the interval to 15 min (cf. Table 1).

The dose- and time characteristics for the pentobarbital trained animals can be found in Table 2. These effects were clearly related to the amount drug given and the injection-test interval used.

When the THC trained gerbils (Groups 2 and 3) were tested with pentobarbital, 10.0 and 20.0 mg/kg, a maximum of 40% drug choices were noted with the highest dose of the barbiturate in both groups. This level is significantly different from the corresponding THC baselines ($p<0.001$, Chi square test). When atropine sulfate (10.0 mg/kg) was tested in Group 3 there was 20% drug choices. Yet, after being exposed to hashish-smoke and tested 10 min after the last inhalation period Groups 4 and 5 choose the non-drugged side of the maze at two occasions only. This is not significantly different from the corresponding D levels, 90.0 and 91,7% respectively ($p>0.05$, Chi square test, 2 X n observations per group).

In the barbiturate trained gerbils there was no or little drug transfer when substituting amphetamine (4.0 mg/kg) or Δ^9-THC (2.0 and 8.0 mg/kg) for pentobarbital as can be seen from Fig. 2. This figure also shows that combinations of THC and pentobarbital (10.0 and 15.0 mg/kg) resulted in more drug choices than what was noted when these compounds and doses were administered singly. No increase of pentobarbital choices appeared with amphetamine.

Combinations of pentobarbital (20.0 mg/kg) and bemegride (20.0 and 40.0 mg/kg), on the other hand, reduced the number of pentobarbital associated choices in a dose related fashion. The results are shown in Fig. 3. At a separate test (not shown in the figure) the antagonistic property of bemegride, 20.0 mg/kg, against pentobarbital, 15.0 mg/kg, was evaluated. The barbiturate was given 10 min and the antagonist was given 5 min prior to the test trial. It was found that all animals choose the nondrug associated arm of the maze.

DISCUSSION

This study has shown that the formation of drug discrimination in gerbils is related to the amount THC given. The lower the training dose given the slower the appearance of the discriminative response control. For rats this has also been shown with pentobarbital, alcohol, anticholinergics, phencyclidine, and Δ^8-THC as well as Δ^9-THC [7, 12, 13]. The strongest dissociation in the rat have occurred with depressant drugs like barbiturates, alcohol, and benzodiazepines [12] and Δ^9-THC (unpublished).

In the present study, however, the formation of discriminative response control was faster with pentobarbital than for any of the THC doses studied. This species difference may perhaps be related to the impression that rats initially are more susceptible to the depressant properties of the THC syndrome than our gerbils. This may in turn mean that the species difference results from a shift in the dose-response curve. Nevertheless, it is clear that if compared on a milligram per kilogram basis the rat acquires the discriminative task faster than the gerbil.

Fewer drug responses were noted as a result of increasing the injection-test interval or by testing successively lower doses of both pentobarbital and Δ^9-THC. The cue properties of pentobarbital are comparably transient and are

TABLE 1

DOSE CHARACTERISTICS (PART 1) AND TIME CHARACTERISTICS (PART 2) FOR Δ^9-TETRAHYDROCANNABINOL (THC)

Drug	Dose (mg/kg)	Time After Injection (min)	No. of Gerbils	No. of Trials	% First-Trial Choices Into THC Correct Arm
PART 1					
Suspension (ND)	–	30	6	78	10.3‡
THC (D)	2.0	30	6	77	89.6§
THC	1.0	30	6	12	83.3§
THC	0.5	30	6	12	58.3‡§
THC	0.125	30	6	12	50.0‡§
Suspension (ND)	–	30	6	71	7.0‡
THC (D)	8.0	30	6	74	95.9§
THC	4.0	30	6	12	75.0*§
THC	2.0	30	6	12	66.7*§
THC	0.5	30	6	12	41.7‡§
PART 2					
Suspension (ND)	–	30	6	78	10.3‡
THC (D)	2.0	30	6	77	89.6§
THC	2.0	15	6	12	75.0§
THC	2.0	120	6	12	58.3‡§
THC	2.0	240	6	12	8.3‡
Suspension (ND)	–	30	6	71	7.0‡
THC (D)	8.0	30	6	74	95.9§
THC	8.0	15	6	12	83.3§
THC	8.0	120	6	12	66.7†§
THC	8.0	240	6	12	66.7†§

D: drug training condition
ND: non-drug training condition
*Probability of difference from corresponding THC score being due to chance; $p<0.05$. Chi-square test.
†Probability of difference from corresponding THC score being due to chance; $p<0.01$. Chi-square test.
‡Probability of difference from corresponding THC score being due to chance; $p<0.001$. Chi-square test.
§Probability of difference from corresponding suspension score being due to chance; $p<0.001$. Chi-square test.

markedly dose related. No drug choices were noted one hour post injection and only a few drug choices appeared when testing half the training dose. In contrast, THC treated gerbils (2.0 and 8.0 mg/kg) disclosed drug choices after much longer injection-test intervals and at doses lower than the training doses. As for the rat, injections of THC can be substituted with hashish-smoke also in the gerbil. This means that route of administration is of minor importance in the maintenance of the drug discriminative control [6] as long as the drug affects the central nervous system [12].

Although THC has been classified as a depressant drug

TABLE 2

DOSE CHARACTERISTICS (PART 1) AND TIME CHARACTERISTICS (PART 2) FOR PENTOBARBITAL (P-BARB.)

Drug	Dose (mg/kg)	Time After Injection (min)	No. of Gerbils	No. of Trials	% First-Trial Choices Into P-barb. Correct Arm
PART 1					
Saline (ND)	–	10	8	160	0.0*
P-barb. (D)	20.0	10	8	160	98.1†
P-barb.	15.0	10	8	16	50.0*†
P-barb.	10.0	10	8	16	18.8*†
PART 2					
Saline (ND)	–	10	8	160	0.0*
P-barb. (D)	20.0	10	8	160	98.1†
P-barb.	20.0	5	8	16	81.3†
P-barb.	20.0	30	8	16	37.5*†
P-barb.	20.0	60	8	16	0.0*

D: drug training condition
ND: non-drug training condition
*Probability of difference from corresponding P-barb. score being due to chance; $p<0.001$. Chi-square test.
†Probability of difference from corresponding saline score being due to chance; $p<0.001$. Chi-square test.

[10], we found that the cue properties of the THC doses tested were not interchangeable with those of pentobarbital as shown in the substitution tests. Yet, when the gerbils were treated with combinations of THC and pentobarbital the effects were augmented as defined by the increase in the number of pentobarbital associated choices. This conclusion is further reinforced by the observation that the effects were dose-related. Due to unsatisfactory base lines similar experiments were not performed with the THC trained gerbils. In another experiment, where pigeons have been trained to discriminate the effects of an injection of THC from the vehicle, an augmenting effect has been noted with a THC–pentobarbital combination (unpublished).

The amphetamine dose of 4 mg/kg did not enhance or attenuate the effect of pentobarbital as a discriminative cue. Several studies have presented evidence in favor of an additive phenomenon when amphetamine and barbiturates are administered together [3, 14, 16], but it is clear that the drug interaction may result in both antagonism and potention. This depends on the parameters studied which has been nicely illustrated by Branch [2].

In accordance with results presented by Overton [11] we also found that bemegride antagonized in a dose-related manner the discriminative properties of pentobarbital. Yet, in one instance [15], pentobarbital (15.0 mg/kg) produced discrimination was not antagonized after challenge with bemegride (5.0–15.0 mg/kg). According to Zapata-Ortiz *et al.* [20] the effects of bemegride vanish rapidly and comparably high doses are needed to oppose the depressant properties of pentobarbital. The reason for the discrepancy between the above mentioned results may therefore be understood with reference to the time- and dose parameters used. Although not explicitly stated, it is possible that Rosecrans *et al.* [15] have used a too long injection-test interval (15 min?) together with rather low doses of bemegride.

A general conclusion from these studies is that drug discrimination with THC and pentobarbital in the gerbil is similar in most respects to that reported for the rat. However, it seems that THC produces strong dissociative effects in the rat whereas moderate dissociation is found for the gerbil.

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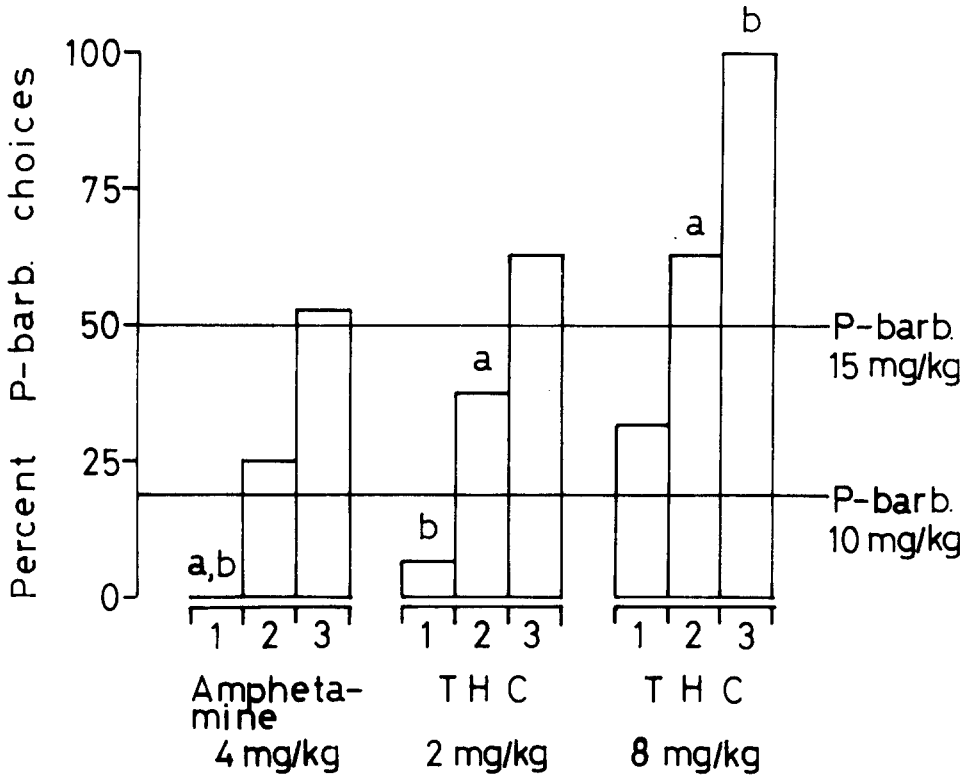

FIG. 2. Legend: Discrimination trials with amphetamine and Δ^9-THC in pentobarbital trained gerbils.

Note: The bars designated No. 1 represent the results from transfer trials when the test drugs (amphetamine and THC) were given solely. Bars 2 and 3 represent the effects of combinations of the separate test drugs with pentobarbital, 10.0 and 15.0 mg/kg respectively. The horizontal lines represent the number of pentobarbital choices when doses of 10.0 and 15.0 mg/kg of the drug were given solely. Amphetamine was injected i.p. 15 min, THC 30 min, and pentobarbital 10 min prior to the test trials. Bars designated 1 are based on 16 observations each and bars designated 2 and 3 are based on 8 observations each.

[a]Probability of difference from P-barb. score (10.0 mg/kg) being due to chance; $p<0.001$. Chi-square test.
[b]Probability of difference from P-barb. score (15.0 mg/kg) being due to chance; $p<0.001$. Chi-square test.

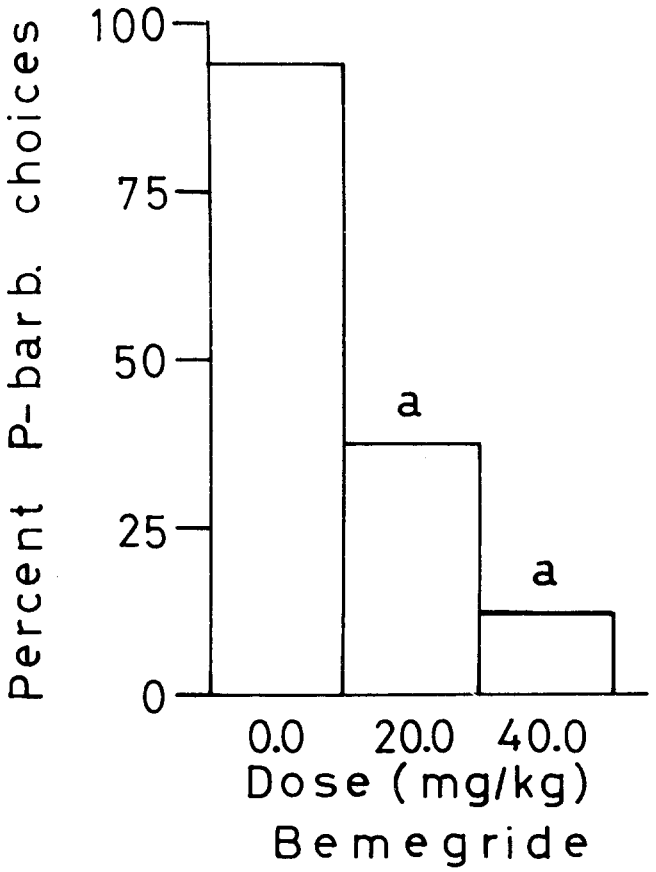

FIG. 3. Legend: Antagonism of pentobarbital cued behavior by bemegride.

Note: The training drug, P-barb. (20.0 mg/kg), was given 10 min and the various doses of bemegride (Megimide®) were given 5 min prior to the test. The 0.0 mg/kg dose consisted of an i.p. injection of 0.5 ml per gerbil of physiological saline. The number of observations per test dose were 2 × n = 16.

[a]Probability of difference from P-barb. score, 20.0 mg/kg (D), being due to chance; $p<0.001$. Chi-square test.

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