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Ethyl Alcohol on Escape From Electrical Periaqueductal Gray Stimulation in Rats

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BOVIER, Ph., C. L. BROEKKAMP AND K. G. LLOYD. *Ethyl alcohol on escape from electrical periaqueductal gray stimulation in rats.* PHARMACOL BIOCHEM BEHAV 21(3) 353–356, 1984.—In a procedure to measure the escape latency and threshold for aversive electrical stimulation of the Peri-Aqueductal Gray (PAG) the effect of ethyl alcohol was tested in order to validate aversive PAG stimulation further as a model for testing potential anxiolytics. Doses of 0.2–1.6 g/kg were given orally in an ascending sequence and were found to increase escape latency and threshold. A second dose of 0.8 g/kg had no effect, suggesting development of tolerance. Spontaneous activity in the test environment was not changed by alcohol. These results indicate specific effects of alcohol on aversive stimulation and reinforce the suggestion to use this phenomenon as a model for human anxiety.

Anxiety model Aversive brain stimulation Ethyl alcohol Peri-aqueductal gray

ELECTRICAL stimulation of dorsal parts of the periaqueductal gray (PAG) induces symptoms of fear or pain in rats [6, 21, 29] and feelings of fear and anxiety in man [17]. Rats will quickly learn to avoid stimulation of this area enabling quantitative evaluation of the punishing effect of this brain stimulation. Chlordiazepoxide diminishes the effect of dorsal PAG stimulation. This observation led to the suggestion that the PAG is a site of action for benzodiazepines [5,26]. It was reported that meprobamate, pentobarbital, diazepam and lorazepam also reduce the escape response [2, 3, 18, 19, 20] and that morphine is active only at high cataleptogenic doses [13,27]. It is therefore attractive to use aversive PAG stimulation as a model for anxiety and test new potential anxiolytics in this model [2]. As a further evaluation of the model we tested ethyl alcohol. Alcohol has consistent anxiolytic-like properties in existing animal models [4, 7, 8, 10, 30] and reduces anxiety in man under many but not all circumstances [24].

METHOD

Eleven male rats from Charles River France (CD-COBS; 180–220 g) were stereotaxically implanted with a bipolar twisted stainless steel electrode (Plastic Products Company; MS 303/2, total diameter 0.7 mm) aimed at the dorsal periaqueductal gray. Sodium pentobarbital 50 mg/kg IP was used for anaesthesia and the implantation co-ordinates were A=+0.9, L=0.5 and D=+4.5 mm with the interaural point as zero and skull position such that the virtual line connecting the interaural point and the incisor bar made an angle of 5° below the horizontal. After surgery the animals were housed singly in macrolon cages (20×30×15 cm).

Seven days after surgery training commenced for escape from PAG stimulation. A rat was connected for electrical stimulation via a lead allowing free movements and rotation and placed in the experimental environment. This consisted of a rectangular box (20.5×48×21.5 cm) with a grid floor and a 3.5 cm high barrier dividing the cage in half. The animal could be observed from above.

For standard testing of the latencies of escape for different current intensities the trials started with 5 min of habituation to the cage. The rat was stimulated with pulse trains (1 train/sec, 300 msec train duration, 15 pulses per train, 0.2 msec pulse duration, negative voltage via constant current unit) irrespective of its position in the cage and stimulation was maintained until the rat escaped to the other side of the cage or until a maximum stimulation time of 60 seconds had elapsed. Five to seven training sessions were required to shape the escape response into a smooth passage over the barrier either way. Training sessions also served to find the threshold currents for each animal and to habituate the animals to the following procedure for testing alcohol effects: An ascending sequence of current intensities was tested with 20 μA differences. Each intensity was presented three times with intervals of 30 sec between similar intensities and 60 seconds between different intensities. The lowest current intensity tested is always the one which fails to elicit escape within a mean latency of 55 sec or more. This lowest intensity was known per animal from the training period. The highest intensity tested provoked escape within 15 seconds. Generally, an animal was tested at four to seven different intensities. During the first 9 interstimulation intervals behavior of the rat was recorded into categories of freezing, grooming, exploration or traverses to the other side of the cage.

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TABLE 1

EFFECT OF ALCOHOL ON ESCAPE LATENCY AND THRESHOLD CURRENT FOR PAG STIMULATION

Dose of alcohol pretreatment in g/kg PO	Mean control escape threshold current in μA (n=11) ± s.e.m. T	Mean change in escape latency in sec ± s.e.m. at T+20 μA	T+40 μA	T+60 μA	Mean change in escape threshold in μA ± s.e.m.
0	95 ± 9	+ 3 ± 3	+ 3 ± 2	+ 2 ± 5	+ 2 ± 3
0.2	95 ± 9	+ 16 ± 4†	+ 9 ± 2‡	+ 6 ± 2*	+ 11 ± 3†
0.4	97 ± 9	+ 16 ± 3‡	+ 12 ± 3‡	+ 4.9 ± 0.6‡	+ 13 ± 5*
0.8	113 ± 10	+ 18 ± 3‡	+ 16 ± 3‡	+ 9 ± 2‡	+ 26 ± 5†
1.6	115 ± 9	+ 16 ± 3‡	+ 21 ± 5†	+ 11 ± 5	+ 26 ± 8†
0.8	115 ± 9	+ 1 ± 4	+ 7 ± 4	+ 5 ± 2*	+ 7 ± 5

*$p<0.05$; †$p<0.01$; ‡$p<0.001$; with paired two-tailed t-test.

Following training sessions one dose of ethyl alcohol was tested each week. On one day a standard test was run without treatment, on the following day the test was performed 30 minutes after oral treatment with alcohol or distilled water. In six weeks we tested 0–0.2–0.4–0.8–1.6 and 0.8 g/kg ethanol in this order. For alcohol treatment 100% ethyl alcohol was diluted with distilled water in order to give an intubation of a volume of 5 ml/kg.

Alcohol effects were quantified on several parameters: (1) a change in escape threshold with respect to the test on the day preceding the treatment. The escape threshold (but not the lowest current tested!) is the current which elicits escape with a latency between 40 and 55 seconds. (2) A change in escape latency with respect to the test on the day preceding the treatment at the three lowest current intensities provoking an escape response on the test preceding the treatment. (3) The number of interstimulus intervals with one or more explorative bouts. (4) The number of interstimulus intervals with one or more spontaneous traverses. At the end of these experiments the electrode locations were verified by sectioning the brain in 30 μm slices and staining with methylene blue.

Electrode implantations were in the dorso-lateral part of the peri-aqueductal gray as exemplified previously [2]. Electrode tip positions for each rat were determined and expressed in stereotaxic coordinates by comparing brain sections, stained with methylene blue, with the sections depicted in the atlas of König and Klippel [15]. The mean± standard deviation of these co-ordinates for the whole group of rats was A=+1.2±0.3, L=0.4±0.3 and D=+0.3±0.4.

RESULTS

At all doses tested alcohol increases the threshold and latencies for escape for PAG stimulation. Results are summarized in Table 1. Vehicle treatment did not have a significant effect. The increments in summed escape latency and in escape threshold induced by the doses in the ascending sequence of 0.2–1.6 g/kg are statistically significant both with respect to the tests on days preceding the treatments as well as with respect to the change induced by placebo. When, under alcohol treatment, the escape occurs at a higher intensity and/or after a longer latency it is performed rapidly with the usual efficiency and is unlike the undirected escape attempts and occasional jumps of naive animals. Freezing occurs both under control conditions as well as after alcohol treatment at onset of stimulation until the escape response.

The second treatment with 0.8 g/kg alcohol after completion of the ascending dosing series did not induce such clear-cut increase in escape latency or threshold. Such tolerance development seems also reflected in the lack of increasing effects with increasing doses.

Other activity in the experimental box during stimulation intervals were not changed by any of the doses of alcohol (Table 2). If anything, there is a tendency for an increased number of intervals with a spontaneous traverse.

DISCUSSION

Escape behavior from PAG stimulation is reduced by ethyl alcohol at doses of 0.2–1.6 g/kg given in an ascending sequence. In a study by M. Olds [19] an acute dose of 1.5 g/kg was tested on escape for PAG stimulation as well as on approach of hypothalamic stimulation. This dose was found to block both escape and approach behavior and therefore did not seem to have specific blocking effects on aversive stimulation. In our study we used the spontaneous activity in the experimental cage as an index for possible nonspecific depressant effects on behavior. Although more sensitive methods might have demonstrated some diminution of motor activity it appears that 0.2–1.6 g/kg ethanol, given to animals in an ascending sequence, has no gross nonspecific depressant effect on behavior and that therefore the effect on escape for PAG stimulation is actually related to escape such as a diminution of fear, the perception of the aversive stimulus or a change in the selection mechanisms for a defense response [1]. An effect by alcohol on the learned response by interfering with the motor organisation of escape is also ruled out as the escapes which did occur at higher intensities were similar to the escapes of experienced animals and unlike the responses of animals with motor interference as observed previously after haloperidol [2].

The ascending series of doses all produced approximately the same effect. There was hardly any increase in the effect. As observed in our previous experiments the ceiling for any drug effect in our procedure is much higher than the threshold increments seen with alcohol; therefore, a ceiling effect cannot explain this phenomenon. Also, the dose of 0.8 g/kg was tested a second time in the same animals and no effect was measured this second time. Although some in-

TABLE 2

EFFECT OF ALCOHOL ON EXPLORATION AND TRAVERSES† DURING INTERVALS BETWEEN PAG STIMULATION

	Number of intervals with exploration ± s.e.m.	Number of intervals with traverse ± s.e.m.
reference test*	4.8 ± 0.7	1.4 ± 0.4
placebo treatment	4.8 ± 0.9	2.6 ± 0.6
reference test	4.8 ± 0.7	1.3 ± 0.4
alcohol 0.2 g/kg	6.3 ± 0.7	2.6 ± 0.5
reference test	6.5 ± 0.8	3.3 ± 0.9
alcohol 0.4 g/kg	7.2 ± 0.9	4.4 ± 0.9
reference test	6.3 ± 0.9	2.6 ± 0.7
alcohol 0.8 g/kg	6.6 ± 0.8	4.6 ± 0.7
reference test	7.1 ± 0.8	3.9 ± 0.8
alcohol 1.6 g/kg	7.3 ± 0.9	4.7 ± 0.9
reference test	5.8 ± 0.8	2.7 ± 0.6
alcohol 0.8 g/kg	7.6 ± 0.4	4.4 ± 0.6

*The reference test is the test on the day preceding an experimental pretreatment with alcohol or placebo.

†A traverse over the barrier dividing the box in halves.

crease in the control escape threshold current occurred from the third to the fourth week of testing, this cannot easily be an explanation for a decreasing alcohol sensitivity between the fourth and the sixth week of testing. Therefore some form of tolerance for alcohol apparently occurred but it would require more experiments to establish the mechanism for the tolerance in this experimental situation [11,32].

As mentioned in the introduction the inhibiting effect of ethyl alcohol on escape reinforces the suggestion to use aversive PAG stimulation as a model for human anxiety and as a test method for detecting new anxiolytic drugs [2,26]. Many drugs applied to combat anxiety are shown to reduce escape from aversive stimulation. Diazepam [2,19], chlordiazepoxide [3, 19, 26], lorazepam [3], meprobamate [20] and sodium pentobarbital [18,22] all reduce escape from aversive brain stimulation. In contrast, neuroleptic drugs shorten escape latency or produce clear interference with the motor organisation of the escape response [2, 18, 20, 25]. An important advantage of PAG aversive stimulation is also the lack of escape reducing effects of drugs interfering with serotonergic transmission [14,26]. In conflict procedures parachlorophenylalanine and serotonin receptor blockers have disinhibiting effects [9, 12, 28], although these drugs lack anxiolytic effects in man [23,31]. Morato di Carvalho *et al.* [16] demonstrated that, when aversive PAG stimulation is used instead of foot shock, the conflict test does not indicate disinhibiting effects of antiserotonin drugs. Using PAG stimulation in a simple escape procedure appears therefore to be preferable over existing conflict tests for routine testing of new compounds.

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