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Effects of Diazepam on Extinction Induced Aggression in Pigs

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ARNONE, M. AND R. DANTZER. *Effects of diazepam on extinction induced aggression in pigs.* PHARMAC. BIOCHEM. BEHAV. 13(1) 27–30, 1980.—Pigs were trained to press a panel with their snout to get food in an operant conditioning chamber. Aggressive behaviour which developed between two pigs submitted together to extinction was used as a baseline to study the effects of 1–2 mg/kg diazepam. When access to the response panel and feeding area was permitted, diazepam enhanced resistance to extinction and did not modify aggression. When access to the response panel and feeding area was not permitted, diazepam increased the severity of aggression observed between the animals. In both instances, plasma corticosteroid levels were depressed in diazepam-treated pigs. These results suggest that benzodiazepines do not act on frustration or aggressiveness per se, but rather strengthen the prevailing behavioural attitudes in the animals' repertoire at the time of test.

Pig Diazepam Extinction Aggression Frustration Plasma corticosteroids Response persistence

BENZODIAZEPINES release behaviour which has been suppressed by punishment or non-reinforcement but do not prevent the facilitating effects of aversive events on behaviour [4,5]. These behavioural changes may be regarded as manifestations of response persistence effects rather than interference with central motivation states such as anxiety or frustration. Benzodiazepine treated animals appear to persevere more than controls with their most likely behaviour on account of previous experiences and situations. For instance, subjects which had learnt to avoid electric shocks by jumping from one compartment to the other in a shuttle box, reacted to a fear signal by increasing their avoidance response rate. Diazepam does not inhibit this reaction but rather tends to strengthen it [6].

In the same manner, benzodiazepine derivatives do not prevent the invigorating effects of non-reinforcement such as the enhancement of response force during extinction [10] or the double alley frustration effect [12].

A better test of the response persistence effect interpretation would be to show that benzodiazepines induce qualitatively different effects according to the experimental situation characteristics in a same behavioural task. The omission of an anticipated reward induces aggressive behaviour in pairs of animals submitted together to food extinction procedures. In pigs, the intensity of aggression is critically dependent on the possibility of access to the response panel and the feeding area [7]. When a partition prevented the animals reaching the response panel and feeding area, aggression was more intense and pigs fought longer, but, when access was permitted, they appeared to spend more time pressing the panel and controlling the feeding area than fighting. Therefore the response persistence effect of diazepam would appear during aggressive behaviour in the former situation and during panel pressing in the later situation. The present experiments were designed to test this hypothesis in pigs which had learnt to press a panel with their snout to get food.

METHOD

Forty-eight cross-bred pigs 2 months old and weighing 16–20 kg at the beginning of the experiment were studied. Both females and castrated males were used and experimental pairs were formed without regard to sex since previous experiments had shown that there was no difference in aggressive behaviour of prepubertal pigs according to sex when the animals were submitted to paired social encounters [11].

The apparatus and experimental conditions have been described elsewhere [3,7]. All animals were placed on a 23 hr food deprivation schedule, with water available ad lib. The subjects were individually trained to press a panel with their snout in order to get about 5 g of a commercial granular feed as a reinforcement according to a continuous reinforcement schedule, i.e. one reinforcement for every panel press. Training all animals required two day sessions. On the third day, they received the first of daily 20-min sessions of continuous reinforcement. Following each session they were returned to their pens where they were given complementary feeding to make up for 4% of their body weight. On the eighth session, a single session of extinction was performed. This extinction session was run under two different conditions, using different groups of animals: (1) Two pigs placed together into the experimental cage ($n=16\times2$). No food was delivered when the animals pressed the panel. (2) Two pigs placed together into the experimental cage but with an opaque partition that prevented the animals from reaching the response panel and feeding area ($n=8\times2$).

During the 20-min extinction session, an observer, unaware of the pharmacological treatment received by the animals, noted by means of a keyboard connected to an

TABLE 1

EFFECTS OF 1 OR 2 mg/kg DIAZEPAM ON THE TIME SPENT TO CONTROL THE FEEDING AREA, THE NUMBER OF TIMES THE RESPONSES PANEL WAS PRESSED AND THE DIFFERENT BEHAVIOURAL PATTERNS OBSERVED IN PIGS SUBMITTED BY PAIRS TO THE EXTINCTION PROCEDURE

	Control of the feeding area (sec)	Responses (number)	Pushing episodes (number)	Biting episodes (number)	Fighting frequency	Fighting duration (sec)	Total duration of aggressive episodes (sec)
Control (6 pairs)	122 ± 51	86 ± 13	27 ± 9	116 ± 62	67%	85 ± 34	449 ± 160
Diazepam (10 pairs)	411 ± 129	166 ± 25	50 ± 7	63 ± 13	60%	208 ± 168	476 ± 108
Comparison (Mann-Whitney U test)	$p=0.064$	$p<0.10$	$p>0.10$	$p>0.10$	/	$p>0.10$	$p>0.10$

The mean and the standard error of the mean are given for each variable, except for the fighting frequency which is the percent of pairs in which a fight occurred on the total number of pairs studied. The data on the time spent controlling the feeding area were obtained only in five control pairs and in eight diazepam-treated pairs.

event pen recorder, the following events: in group (1) only, the number of times the panel was pressed, the time spent to control the feeding area, and in both groups, the number of pushing and biting episodes between the two subjects and the occurrence and duration of fighting. All sessions took place in the morning, between 9:00 and 11:00 a.m. To assess the pigs' aversion to the experimental circumstances to which they were submitted, plasma corticosteroid levels were determined by a competitive protein binding method [18] in three pairs of animals within each experimental group. Before and 30 min after the completion of the experiment, 10 ml of blood was drawn from the vena cava. Plasma samples were obtained by centrifugation and frozen for biochemical assay. Blood collection was rapid enough to prevent a rise in the plasma corticosteroid levels as a consequence of the sampling procedure.

One or 2 mg/kg diazepam in commercially available vials (Valium, Roche) was injected intramuscularly 30 min before the extinction session. The control animals received an equivalent volume of the solvent (benzilic alcohol 31.4 mg in 2 ml distillated water).

Within each group, allocation of the treatment condition was random. The diazepam dosages were selected according to results of previous dose-effect relationships in pigs submitted to an approach-avoidance conflict [2].

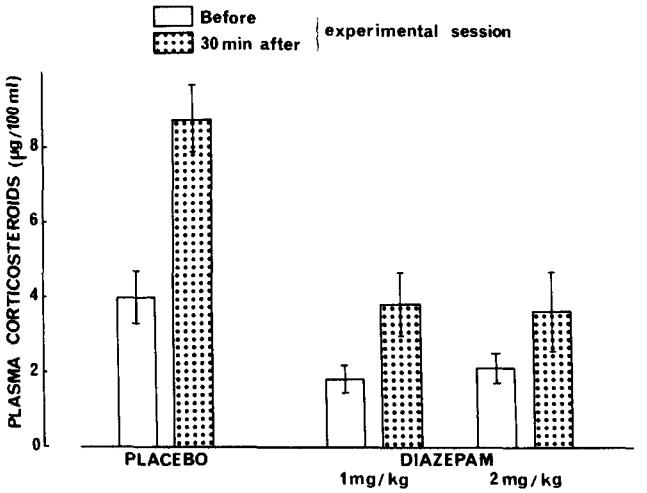

FIG. 1. Effects of diazepam on the mean plasma corticosteroid levels of pairs of pigs submitted to the extinction of an operant response for food (mean ± SEM). Plasma corticosteroids were measured in blood samples taken before (blank columns) and 30 min after the completion of the experiment (dotted columns). Placebo-treated pigs were injected with the solvent. Each experimental group included 3 pairs of pigs.

RESULTS

Extinction with Access to the Response Panel and Feeding Area

Four pairs of animals were treated with 1 mg/kg diazepam and six other pairs with 2 mg/kg, while 6 pairs were used as controls. A comparison of the results obtained in pigs treated with 1 mg/kg diazepam to those obtained in pigs treated with 2 mg/kg by means of the Mann-Whitney U test revealed no difference in the variables studied. Both groups of results were therefore regrouped and compared to those obtained in controls (Table 1). Diazepam-treated pigs spent more time controlling the feeding area and pressed the panel at a higher rate than controls. The occurrence and duration of aggressive interactions did not differ according to the treatment.

Plasma corticosteroid levels were significantly depressed by diazepam treatment, $F(2,30)=14.96$, $p<0.001$ (Fig. 1). The extinction procedure activated the pituitary-adrenal axis which was indicated by the increase of the plasma corticosteroid levels at the end of the extinction session, $F(2,30)=20.42$, $p<0.001$. However the increase tended to be less pronounced in diazepam-treated pigs than in the controls, $F(2,30)=2.62$, $p<0.10$.

Extinction Without Access to the Response Panel and Feeding area

Four pairs of pigs were treated with 2 mg/kg diazepam and four other pairs served as controls. Diazepam-treated pigs spent twice as much time than controls exchanging blows and fighting (Table 2).

Plasma corticosteroid levels tended to increase in pigs submitted to the extinction procedure, $F(1,20)=3.44$, $p<0.10$. Diazepam depressed the plasma corticosteroid

TABLE 2

EFFECTS OF 2 mg/kg DIAZEPAM ON AGGRESSION IN PIGS SUBMITTED TO THE EXTINCTION PROCEDURE WITH A PARTITION PREVENTING THE SUBJECTS FROM REACHING THE FOOD BOWL AND FEEDING AREA

	Pushing episodes (number)	Biting episodes (number)	Fighting frequency	Fighting duration (sec)	Total duration of aggressive episodes (sec)
Controls (4 pairs)	24 ± 16	49 ± 24	75%	342 ± 42	530 ± 85
Diazepam (4 pairs)	25 ± 11	86 ± 32	75%	681 ± 61	1138 ± 22
Comparison (Mann-Whitney U test)	$p>0.10$	$p>0.10$	/	$p<0.05$	$p<0.05$

The mean and the standard error of the mean are given for each variable, except for the fighting frequency which is the percent of pairs in which a fight occurred on the total number of pairs studied.

levels, $F(1,20)=6.76$, $p<0.025$, but did not prevent their rise under extinction conditions, $F(1,20)=0$, (Fig. 2).

DISCUSSION

When two pigs having previously learnt to get food by pushing a panel with their snout are submitted together to extinction conditions they display instances of aggressive behaviour and a rise of plasma corticosteroids. The intensity of the aggressive interactions depends upon the characteristics of the experimental situation, with more severe fighting occurring when access to the response panel and feeding area is prevented [7]. Using this procedure, it is therefore possible to assess the effects of a pharmacological treatment on behavioural patterns of different likehood as a consequence of a common motivational state, i.e. frustration.

When access to the response panel and feeding area was permitted, attempts to control the feeding area and to press the panel were the predominant activities. Under these conditions, diazepam increased both the number of panel presses and the time spent controlling the feeding area, without modifying aggressive behaviour. As a matter of fact, benzodiazepines are known to enhance resistance to extinction (cf. for a general review [4,12]). For instance, oxazepam produced large increases in the rate of non-reinforced responding of rats trained to press a lever to obtain sweetened milk [16] and chlordiazepoxide restored behaviour attenuated by non reinforcement in food withdrawal procedures [17]. Similarly diazepam injected to pigs which had been previously trained to perform operant responses for food according to a continuous reinforcement schedule, increased the number of times the panel was pressed under extinction conditions [3]. The effects of diazepam observed in the present experiment might therefore be viewed on first analysis as just another case of a specific property of this class of drugs, i.e. a reduction of the behavioural responses to frustrative non-reward [12].

When access to the response panel and feeding area was not permitted, aggressive interactions became the predominant activities. Under these conditions, diazepam increased the severity of aggression observed between the two animals. Since aggression in this situation is believed to be elicited by frustrative non reward, these results are not suggestive of a reduced effect of non reward in diazepam-

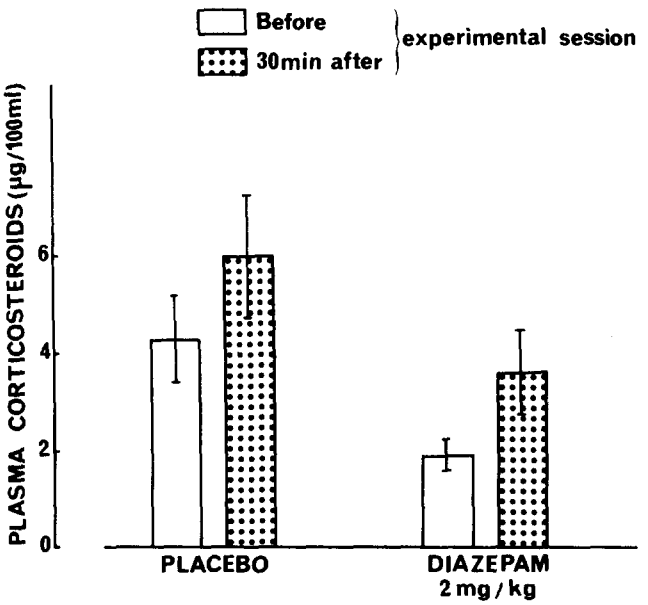

FIG. 2. Effects of diazepam on the mean plasma corticosteroid levels of pigs submitted to the extinction procedure by blocking access to the response panel and feeding area (mean ± SEM). Plasma corticosteroids were measured in blood samples taken before (blank columns) and 30 min after the completion of the experiment (dotted columns). Each experimental group included 3 pairs of pigs.

treated animals. Effects of diazepam on aggressiveness do not account either for the observed effects: benzodiazepine derivatives are classically described as anti-aggressive drugs [19], inhibiting both defensive-aggressive behaviour and attack behaviour in animal aggression tests. However attention has recently been drawn to the possibility of increased aggression in benzodiazepine-treated animals depending on the behavioural baseline, the species, the dosage used and length of time for which the drug treatment was given [8]. Although initially believed to be restricted to chronic treatment conditions, this effect has been found under acute treatment conditions. For instance, low doses of chlordiazepoxide increased aggression in pairs of rats submitted

to a food extinction procedure [17]. In the same vein, we have observed that acute treatment with 1–2 mg/kg diazepam enhanced aggression in pigs submitted to a paired encounter test (unpublished results). However such an effect cannot account for the present results since it should manifest itself also on aggression exhibited by pigs in Group 1, which is not the case.

The rise in plasma corticosteroids observed in the present experiments illustrates the aversive value of the extinction procedure in pigs [7]. This rise was less marked in diazepam-treated pigs which had also lower baseline plasma levels of corticosteroids. The lower corticosteroid levels observed in diazepam-treated pigs cannot be ascribed to a decreased reaction of animals to handling associated with venous puncture since we have checked that corticosteroid levels measured in plasma samples obtained this way did not differ from those measured in chronically catheterized pigs [9]. Such results extend further the antistress effects of benzodiazepines which had been demonstrated mainly in situation involving electric shock [1, 6, 14, 15].

In conclusion, the present experiments suggest that benzodiazepines do not affect behaviour by such general effects as enhanced resistance to extinction or changes in aggressiveness. These drugs might more easily be described as inducing a response persistence syndrome, the expression of which depends upon the respective probabilities of the behavioural patterns occurring in the experimental situation, on account of previous experience and environmental stimuli [3,4].

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