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Effects of diazepam and flumazenil on food competition behavior in high- and low-aggression pigeons

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Abstract

The food competition interaction test performed with food-restricted pigeons with previously consolidated dominance is a useful tool for the study of offensive and defensive social aggression. In the present study, we examined the effect of GABA-A–benzodiazepine (BZD) receptor manipulation on aggression, emotion, feeder control, and eating behavior in high- and low-aggression female pigeons maintained at 80% of their normal weight and exposed to food competition interactions. The pigeons were divided into pairs by previously ranked high-aggression females (total time spent in aggression over 60 s/5 min; $n=6$ pairs) and low-aggression females (time spent in aggression less than 10 s/5 min; $n=6$ pairs). In Experiment 1, a pigeon in each pair of high- and low-aggression subjects were treated daily with an oral dose of diazepam (DZP, 0.6 mg/kg/0.3 ml) for 8 days. The other animal received the vehicle. On Day 8, food competition trials (10 min) were performed 30 min after treatments. In Experiment 2, pigeons were injected subcutaneously with flumazenil (FZL, 0.1 mg/kg/1 ml) or saline and exposed to a food competition trial 30 min after injections. In Experiment 3, one animal in each pair received DZP for 8 days. The other animal received the vehicle. On Day 8, the DZP-treated subjects were injected subcutaneously with FZL (0.1 mg/ kg/1 ml) 30 min before the oral dose of DZP. Trials were performed 30 min after DZP or vehicle administration. In Experiment 1, it was found that the DZP group of high-aggression pigeons showed lower scores of aggression ($P<.05$) and emotional responses ($P<.05$) than controls. The other group-scored behaviors were not affected. The DZP low-aggressions, however, showed scores of aggression eightfold higher than their controls ($P<.05$) but the other scored behaviors were not changed. In Experiment 2, FZL injection did not induce intrinsic effects on aggression either in the high- or in the low-aggression group. Experiment 3 showed that the emotional and aggressive responses to DZP were neutralized by FZL. This shows that GABA-A–BZD receptor mechanisms are implicated in the DZP responses in high- and low-aggression pigeons.

Keywords: Diazepam; Flumazenil; Aggressive behavior; Emotional behavior; Food competition; Pigeons

1. Introduction

Effective medical treatment for impulse aggression and impulse control disorders is needed. Basic studies have shown that depletion of brain 5-hydroxytryptamine (serotonin or 5-HT) consistently produces disinhibition and aggressive reactions in mammals (Soubrie, 1986). In humans, disinhibited impulse behaviors, such as murder, suicidal tendencies, and antisocial personality or substance abuse disorders, have been associated with signs of a deficiency in brain 5-HT (Soderpalm and Svensson, 1999). The understanding of the effects of drugs on aggressive behavior demands consideration of the biological variability, including genetic, previous experience, and interindividual differences (Liljequist and Engel, 1984). The food competition interaction test performed with food-restricted pigeons is a useful model for the study of offensive and defensive social aggression. Using this test, we have shown that the behavioral response of pigeons to brain 5-HT manipulations is related to their natural aggressiveness exhibited prior to the treatments. Either acute or chronic treatment with the 5-HT precursor 5-hydroxytryptophan (5-HTP) was found to block food competition aggression selectively in high-aggression pigeons, while 5-

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HT depletion by intracerebroventricular injection of 5,7-dihydroxytryptamine (DHT) increases aggression selectively in low-aggression subjects (Fachinelli et al., 1989, 1996; Ison et al., 1996).

Benzodiazepines (BZDs) have been reported to induce either anti- or proaggressive effects in animals and humans (Rodgers and Waters, 1984, 1985; Weisman et al., 1998). It has been suggested that some BZD (e.g., diazepam [DZP] and flunitrazepam) may exert proaggressive effects through GABA-ergic systems inhibiting 5-HT activity in the brain, thus causing disinhibition and aggression (Daderman and Lidberg, 1999). It might be that high- and low-aggression pigeons having different aggressive responses to brain 5-HT manipulations may also exhibit different responses to GABA-ergic manipulations. In fact, withdrawal from alprazolam has been shown to induce aggressive behavior only in mice with low pretreatment levels of aggression (Krsiak et al., 1998) and chlordiazepoxide reduced aggressive behavior in high-aggression mice (Weerts et al., 1992).

The aim of the present study was to examine the effects of subchronic DZP treatment (Experiment 1), the intrinsic activity of acute treatment with the selective BZD antagonist flumazenil (FZL) (Hunkeler et al., 1981) (Experiment 2), and the ability of FZL to antagonize the effect of DZP (Experiment 3) on the aggressive and emotional responses of food-restricted high- and low-aggression pigeons (*Columba livia*) exposed to food competition interactions.

2. Methods

2.1. Subjects

The subjects were feral female mature domestic pigeons (*C. livia*) weighing 350–400 g. They were captured in the University Campus and reared singly in $50 \times 40 \times 40$-cm cages that were visually isolated from one another for 30 days. The animal room was kept at constant temperature (24 ± 3 °C) and lighting (lights on from 0800 to 1900 h). Food (mixture of grains) and water were available ad libitum. On Day 31, pigeons were weighed (basal weight). Thereafter, the food provided was calculated to maintain subjects at 80% of basal weight. Water was provided ad libitum. All experimental treatments were approved by the Animal Welfare Committee of the National University of Cuyo Medical School.

2.2. Interaction cage

The interaction cage measured 2.0×2.0 (base) $\times$ 2.0 (height) m, which had four legs 35 cm high. The floor was covered with chaff. The inner walls were painted white. The front wall had a 1.35×0.41-m window provided with a dark glass allowing direct observation of animal behavior without visual disturbance. To induce fighting, a feeder was placed at the center of the arena. The feeder was a 25-cm high pyramid with a short lateral arm bearing a single 2.0-cm hole through which just one animal could freely eat a mixture of grains. Previous reports have shown that dominance for feeder control rapidly develops in pairs of food-restricted pigeons exposed to food competition interactions (Fachinelli et al., 1989). In order to familiarize the pigeons to the interaction cage, all animals were individually submitted to a daily 20-min exposure to the arena 15 days before ranking.

2.3. Ranking method

In order to estimate the spontaneous aggression levels of the captured birds, each of them was ranked daily in the interaction cage for six consecutive days in the presence of another bird, which was a different one for each day. In all cases, 5-min trials were performed from Monday to Friday and the pretrial and posttrial body weight of each animal was recorded (intratest body weight gain). The well-trained observers were 0.30 m away from the front of the interaction cage. The time (seconds) the animals exhibited aggressive behavior, emotional behavior (fear and anxiety scores), feeder control behavior, and eating behavior (the main motivators of aggression) was measured by an observer who was blind to the treatment conditions. Times were recorded on a PC computer using specially designed software. The specific behaviors recorded for each category are illustrated in Table 1.

Females showing a mean total time spent in offensive aggression over 60 s/5 min over the six test sessions were arbitrarily ranked as "high-aggression subjects" ($n = 12$). Pigeons with the shortest aggression time (less than 10 s/5 min) were ranked as "low-aggression subjects" ($n = 12$). A total of 12 pairs of similar offensive aggression scores were obtained.

2.3.1. Experiment 1: Subchronic DZP treatment

In preliminary dose–response experiments, we found that subchronic oral dose of 0.3 and 0.6 mg/kg DZP decreased emotional responses without impairing general performance in pigeons as did acute doses. Acute or subchronic larger doses (1 and 2 mg/kg) caused a sedative effect. In this experiment, one pigeon in each pair of high- ($n = 6$ pairs) and low-aggression subjects ($n = 6$ pairs) were treated daily with an oral dose of 0.6 mg/kg/0.3 ml DZP for 7 days. The other member of the pair received 0.3 ml of vehicle. On Day 8, the subchronic DZP-treated birds were provided as above with 0.6 mg/kg DZP. The control member received 0.3 ml of the vehicle. The food competition trials (10 min) were performed 30 min after treatments and the behaviors were recorded in a blind manner. Offensive and defensive aggression are expressed as follows: (a) total time spent/10 min in all forms of these behavior and (b) structure of the offensive aggressive behavior (times spent in each component of this behavior).

Table 1
Structure of behaviors selected for recording

1. Offensive behavior (time spent in s/10 min)
–Pursuing
–Hooking
–Pecking to prevent opponent's access to food, or in areas far from food, after chasing
–Wing beating
–Aggressive vocalization close to feeder or in areas far from food, together with horizontal movements of head and body causing wing tremor in opponent or running away
–Threatening to cause running away in the opponent
2. Defensive behavior (time spent in s/10 min)
–Defensive fighting (mainly beating in response to an attack of the opponent)
3. Emotional behavior (time spent in s/10 min)
–Watching
–Wing tremor
–Fear vocalizations (short vocalizations accompanied by expiration and immobility after an aggressive attack of the opponent)
–Immobility in a corner to prevent an attack
–Attempts to leave the observation chamber (flying up to the top of the chamber)
4. Feeder control behavior (time spent in s/10 min)
–Eating attempts
–Pushing the opponent out of the feeder
–Wing covering the feeder
–Walking around the feeder
–Immobility close to the feeder
5. Eating behavior (time spent in s/10 min)
–Eating at the feeder
–Eating out of the feeder

The other behavioral categories are expressed as total time spent/10 min.

2.3.2. Experiment 2: Acute FZL treatment

In Experiment 2, the same six pairs of high-aggression pigeons and the six pairs of low-aggression pigeons were used. They were kept undisturbed and without any drug treatment for 7 days. On Day 8, the same animals that were treated with DZP in Experiment 1 were injected subcutaneously in the back of the neck with 1 ml of saline containing 0.1 mg/kg FZL. The other pigeons were injected with 1 ml of saline. Each pair of high- and low-aggression females was exposed to a food competition trial (10 min) 30 min after injection. The dose of FZL was selected based on earlier dose–response studies performed in pigeons (0.01–1 mg/kg) (Barret et al., 1986; Witkin and Barrett, 1985). The literature indicates that the interval between FZL injection and testing ranged from 10 to 60 min (Dalvi and Rogers, 1999).

2.3.3. Experiment 3: Acute FZL plus subchronic DZP treatment

After Experiment 2, animals were kept undisturbed for 7 days. From Days 8 to 15, the same animals that were treated with FZL in Experiment 2 were treated daily with an oral dose of DZP (0.6 mg/kg/0.3 ml). The controls received 0.3 ml of vehicle. On Day 15, the DZP-treated pigeons were injected subcutaneously with FZL (0.1 mg/kg/1 ml) 30 min before the oral dose of DZP. The controls were injected subcutaneously with saline 30 min before receiving the DZP vehicle. The literature indicates that the interinjection interval ranged from 0 to 30 min (Dalvi and Rogers, 1999). The food competition trials were performed 30 min after DZP or vehicle administration.

2.4. Statistics

Comparisons between the mean behavioral scores of experimental (drug-treated) animals and their controls in each experiment were analyzed with the Student's t test. Results are expressed as means ± S.E.M. throughout the text and figures.

3. Results

3.1. Experiment 1: Effects of subchronic DZP on food competition behavior

3.1.1. High-aggression pigeons

Fig. 1 shows that differences in the scores of total time spent in offensive aggression between DZP (0.6 mg/kg/day) and control (C) groups of high-aggression females were significant due to the lower scores of the DZP group ($P<.05$). Defensive aggression was almost absent in both groups. Analysis of the structure of aggressive behavior showed significantly lower scores of pecking (C: 35.8 ± 6.25; DZP: 10.8 ± 4.73, $P<.05$) and hooking (C: 34.9 ± 5.33; DZP: 4.6 ± 0.92; $P<.05$) in the DZP-treated group. The treatment also decreased the total time spent in emotional responses (Fig. 1) ($P<.05$) but did not change the scores of total time spent in feeder control behavior (C: 160.6 ± 25.3; DZP: 182.7 ± 32.4) and eating behavior (C: 383.7 ± 38.33; DZP 423.6 ± 45.8).

3.1.2. Low-aggression pigeons

Differences in the scores of total time spent in offensive aggression between the DZP and control groups of low-aggression females were also significant ($P<.05$). However, this was due to the higher scores of aggression in the DZP group. Fig. 1 shows that the control group of low-aggression females displayed very low scores of total offensive aggression; DZP treatment increased these scores by eightfold. Defensive aggression was not affected by DZP. Analysis of the structure of offensive behavior showed a significant increase in the scores of pecking (C: 1.8 ± 0.36, DZP: 12.3 ± 3.0; $P<.05$) and wing beating (C: 1.8 ± 0.45; DZP: 10.7 ± 2.82; $P<.05$). The total time spent in emotional behavior was very low in the control group of submissives; DZP caused only a trend toward lower emotional responses (Fig. 1). The other scored behaviors (feeder control: C: 180.4 ± 38.2; DZP: 132.5 ± 42.5; eating behavior: C: 280.7 ± 42.3; DZP: 225.6 ± 36.5) were not changed by the treatment.

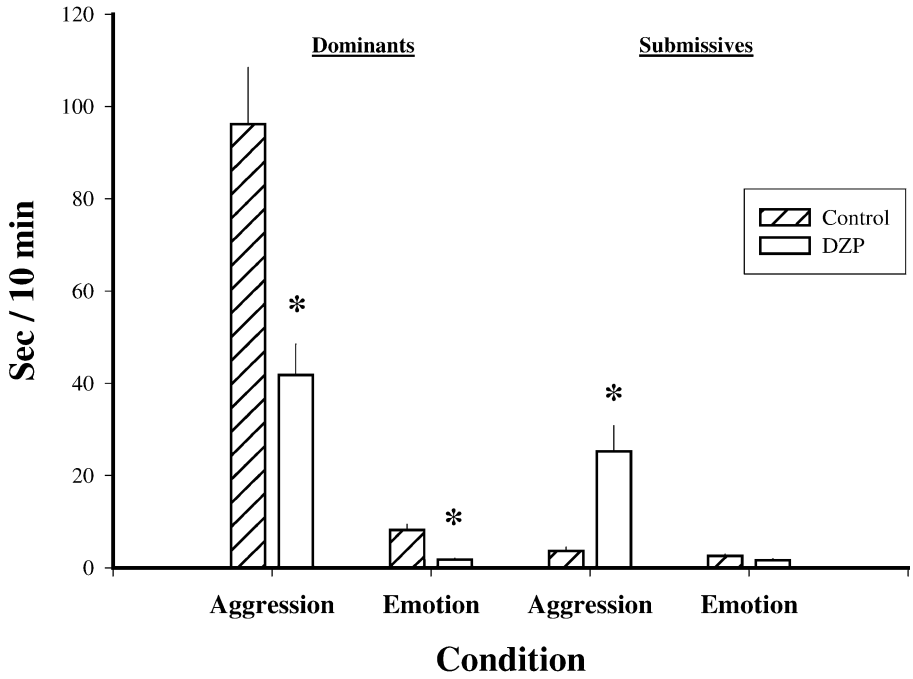

Fig. 1. Effect of DZP treatment (0.6 mg/kg) on the total time spent by high- and low-aggression pigeons in offensive aggression and emotional behavior (Experiment 1). C = control pigeons; DZP = diazepam-treated pigeons (means ± S.E.M.). * $P < .05$ vs. the control mean.

3.2. Experiment 2: Effects of acute FZL treatment on food competition behavior

3.2.1. High-aggression pigeons

The subcutaneous FZL injection (0.1 mg/kg) did not change the total time spent in offensive aggression in high-aggression pigeons compared to their controls (C: 96.16 ± 12.8; FZL 82.27 ± 9.7). The structure of the aggressive behavior revealed similar scores of hooking and a trend toward decreased scores of pursuing, pecking and threatening (results not shown). Both groups displayed very low scores of defensive aggression (C: 0.66 ± 0.6; FZL: 2.0 ± 0.7). The total time spent in emotional behavior (C: 3.56 ± 0.7; FZL 11.83 ± 1.0), feeder control behavior (C: 129.4 ± 23.6; FZL: 142.16 ± 30.2), and eating behavior (C: 310.4 ± 46.3; FZL: 285.0 ± 38.3) was not changed by FZL.

3.2.2. Low-aggression pigeons

The FZL group of low-aggression subjects showed a trend toward higher scores of total time spent in offensive aggression than controls (C: 6.09 ± 1.2; FZL: 12.92 ± 2.6). Analysis of the structure of offensive aggression revealed a trend toward increased scores of all offensive behavior components (results not shown). Both groups reached similar scores of defensive aggression (C: 1.86 ± 0.4; FZL: 2.58 ± 0.4). The control group in this experiment showed very low scores of total time spent in emotional behavior; FZL injection slightly increased the scores of emotional responses (C: 1.96 ± 0.3; FZL 6.38 ± 2.7). The scores of time spent in eating behavior (C: 250.33 ± 40.5; FZL: 316.33 ± 52.4) and feeder control behavior (C: 250.3 ± 52.5; FZL: 150.45 ± 36.4) were not affected by FZL.

3.3. Experiment 3: Effect of acute FZL on food competition behavior of pigeons treated with subchronic DZP

3.3.1. High-aggression pigeons

When a subcutaneous injection of FZL (0.1/kg) was given 30 min before the seventh daily dose of DZP (0.6 mg/kg), the drugged and control high-aggression subjects showed similar scores of total time spent in offensive aggression (C: 83.52 ± 11.1; FZL: 72.95 ± 10.6). Defensive behavior was almost absent in both groups. Emotional responses (C: 8.93 ± 2.3; FZL: 6.32 ± 1.7), feeder control behavior (C: 140.7 ± 42.5; FZL + DZP: 180.8 ± 38.2), and eating behavior (C: 356.3 ± 57.5; FZL + DZP 402.3 ± 62.4) were not affected by the treatment.

3.3.2. Low-aggression pigeons

FZL + DZP low-aggression pigeons and their controls displayed similar scores of total time spent in offensive aggression (C: 7.63 ± 1.9; FZL: 5.90 ± 0.9) and emotional behavior (C: 3.82 ± 0.4; FZL: 3.20 ± 0.3). This was so also for defensive aggression (C: 1.17 ± 0.28; FZL + DZP: 0.65 ± 0.2), feeder control behavior (C: 180.3 ± 36.2; FZL + DZP 215.4 ± 29.1), and eating behavior (C: 272.3 ± 48.2; FZL + DZP: 305.2 ± 50.0).

4. Discussion

The untreated high- and low-aggression pigeons used as controls showed similar behavioral scores in Experiment 1, 2, and 3. This suggests that the behavior of the treated pigeons was the direct result of the treatments and not the indirect result of behavioral modifications of their controls.

In Experiment 1, it was found that subchronic DZP (0.6 mg/kg/day) clearly decreased both offensive aggression and emotional responses in the food-restricted high-aggression pigeons exposed to food competition interactions. Since the other scored behaviors—i.e., time spent in feeder control behavior and eating behavior, which are the main motivation to aggression in food-restricted animals—were not affected, the results cannot be attributed, to a decrease of general activity by a sedative effect of DZP. In the low-aggression pigeons, subchronic DZP selectively stimulated offensive aggression without affecting, despite some trends, the spontaneous low scores of emotional responses as well as the other scored behaviors.

The antianxiety and anticonflict properties of many BZD in birds (Barret et al., 1986; Kleven and Kok, 1999) and mammals (Lee and Rodgers, 1991; Liljequist and Engel, 1984) are well known. Pigeons have been proposed as a suitable preclinical animal model for anxiolytic BZDs (Kleven and Kok, 1999). The DZP-induced lowering of emotional responses in high-aggression pigeons would therefore be an expression of anxiety-like behavior. We speculate that the lack of a significant decrease in emotional response in low-aggressions after DZP treatment might be attributed to their initially low emotional levels.

As to aggressive responses, the DZP-induced inhibition of offensive aggression in high-aggression pigeons conform to previous literature on the antiaggressive effects of some BZD in rats and mice exposed to a variety of aggression models (Abe et al., 1998; Rodgers and Waters, 1985; Skolnick et al., 1985). Proaggressive effects of DZP have already been described in a number of behavioral paradigms. In low-aggression pigeons, DZP was capable of significantly increasing two main components of offensive aggression, i.e., the proaggressive effect of subchronic DZP in the low-aggression pigeons is in agreement with a previous report showing that chlordiazepoxide-induced increases in maternal aggression is higher in spontaneously low-aggression female rats than in females with higher baseline levels of maternal aggression (Mos et al., 1987). In addition, differences at the GABA-A–BZD receptor complex have been suggested for high- and low-aggression lines of mice (Weerts et al., 1992). To generalize that a spontaneous low level of aggression is a condition to expect proaggressive effects of BZD treatment remains to be elucidated through studies in other animal and human models of aggression. In fact, studies on the effects of DZP on extinction-induced aggression in pigs have suggested that BZD do not act on aggressiveness per se, but rather strengthen the prevailing behavioral attitudes in the animals' repertoire at the time of test (Arnone and Dantzer, 1980).

Dose ranges used for FZL in mammals are 5–50 mg/kg, and findings ranging from anxiogenesis (File et al., 1982; File and Pellow, 1984; File and Hitchcott, 1990; Lee and Rodgers, 1991; Pokk and Zharkovsky, 1997; Weisman et al., 1998), to no effects (Dalvi and Rogers, 1999), to anxiolysis (Kapczinski et al., 1994) have been reported. These highly variable intrinsic effects of FZL could be related to a partial agonist-like effect of FZL at the BZD site of the GABA-A receptor (File and Pellow, 1984), DZP-insensitive BZD sites on GABA-A receptors (Acri et al., 1997; Sieghart, 1995), or involvement of non-GABA-ergic actions at least at high doses (Dalvi and Rogers, 1999). In previous work from this laboratory, a 25-mg/kg sc dose of FZL was found to cause antiaggressive effects in high-aggression pigeons and proaggressive effects in low-aggression pigeons (unpublished experiments). Since this was the case for DZP in Experiment 1, it might be that at this dose FZL may have partial agonist-like effects at the GABA-A–BZD receptor also in pigeons.

Dose ranges for FZL are 0.01–1 mg/kg (Barret et al., 1986; Witkin and Barrett, 1985). It has been shown that in the pigeon, the behavioral effects of low doses of FZL (0.01–0.1 mg/kg) would be related to BZD receptor mechanisms, but other systems appear to be involved in the effects of higher doses (Witkin and Barrett, 1985). In the pigeon, these doses of FZL produced intrinsic effects on key-peck responses maintained by food and punishing alternately under one component of a multiple schedule by the presentation of electric shock (Witkin and Barrett, 1985). In Experiment 2, however, the intrinsic effects of 0.1 mg/kg FZL on food competition behavior were not found. With the dose used in the present experiments, FZL failed to affect aggression and emotional behavior in both high- and low-aggression pigeons.

In Experiment 3, the combined treatment with FZL and DZP was found to neutralize the anxiolytic effect of DZP in high-aggression pigeons. As to offensive aggressive responses, 0.1 mg/kg FZL neutralized the antiaggressive effects of DZP in high-aggression pigeons and the proaggressive effects of DZP in low-aggression pigeons. In both cases, the neutral profile of 0.1 mg/kg FZL indicates that its interaction with DZP is not due to opposing intrinsic action on aggression. Therefore, the effects of DZP + FZL on emotional and aggressive responses would be related to GABA-A–BZD receptor mechanisms in both high- and low-aggression pigeons.

In food-restricted high-aggression pigeons, the acute and chronic treatment with the 5-HT precursor 5-HTP has been shown to block the aggressive reaction induced by food competition (Fachinelli et al., 1989, 1996). This treatment, however, was not effective in modifying aggression in low-aggression pigeons (Fachinelli et al., 1989). Conversely, 5-HT denervation by 5,7-DHT increased total aggression exclusively in low-aggression pigeons (Ison et al., 1996).

This suggests that differences in 5-HT function might occur between high- and low-aggression pigeons. It has been suggested that BZD may exert proaggressive effects through GABA-ergic systems inhibiting 5-HT activity in the brain (Blanchard et al., 1998; Daderman and Lidberg, 1999; Soderpalm and Svensson, 1999). Whether differences in 5-HT function between high- and low-aggression pigeons are linked to differences in their aggressive response to DZP remains to be investigated.

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