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Caffeine-Induced Anxiogenesis: The Role of Adenosine, Benzodiazepine and Noradrenergic Receptors

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BALDWIN, H. A. AND S. E. FILE. *Caffeine-induced anxiogenesis: The role of adenosine, benzodiazepine and noradrenergic receptors*. PHARMACOL BIOCHEM BEHAV 32(1) 181–186, 1989.—The purpose of this study was to determine the mechanism by which caffeine increases anxiety. Rats were tested in the social interaction test of anxiety after administration of caffeine (20 or 40 mg/kg) alone or in combination with various compounds. In order to investigate the role of adenosine receptors, caffeine was given in combination with 2-chloroadenosine (0.1 and 1 mg/kg). To investigate the role of benzodiazepine receptors, chlordiazepoxide (5 mg/kg), a benzodiazepine antagonist, flumazenil (RO 15-1788, 1 and 10 mg/kg) and a triazolobenzodiazepine U-43,465 (32 mg/kg) were used. Finally, an α_2-receptor agonist, clonidine (0.1 and 0.025 mg/kg) and a β-adrenoceptor antagonist, DL-propranolol (5 mg/kg), were used to study the role of noradrenergic systems in the effects of caffeine. Caffeine (20 and 40 mg/kg) reduced the time spent in social interaction and this effect was antagonized by chlordiazepoxide, U-43,465 and DL-propranolol, but not by flumazenil, 2-chloroadenosine or clonidine. It was therefore concluded that the anxiogenic effect of caffeine was unlikely to be due to its effects at adenosine or benzodiazepine receptors. It is suggested that the reversal of caffeine's effects by chordiazepoxide may have been "functional," i.e., merely a cancellation of two opposite effects. It is discussed whether the reversal of caffeine's effects by propranolol and U-43,465 are functional, or reflect a noradrenergic site of action.

Caffeine Anxiety Adenosine Benzodiazepine Noradrenergic

IN healthy volunteers the methylxanthine caffeine increases anxiety, nervousness and tenseness (4, 23, 30), decreases fine motor coordination (23), but improves performance in various learning and cognitive function tests (13). Uhde *et al.* (30) found that 240–720 mg of caffeine significantly increased anxiety in normal healthy adults and at the highest dose produced panic attacks in some volunteers. Caffeine produced significantly greater increases in anxiety in patients suffering from panic disorder compared with healthy volunteers (7) and an unusually high number of patients suffering from panic disorder were found to have discontinued coffee consumption due to its unpleasant psychological effects (30). Caffeine has also been shown to be anxiogenic in rats (1, 11, 12, 25).

There is evidence that caffeine acts at several different sites in the central nervous system (20). In vitro caffeine binds to central adenosine receptors (Ki 50 μM) (3) and there is also evidence that it binds to benzodiazepine (BDZ) receptors (Ki 270 μM) (26, 27, 31). In addition, caffeine increases brain synthesis and release of noradrenaline (8).

In this study we have investigated the effect of caffeine in the rat, using the social interaction test of anxiety (10). In this test, benzodiazepines (BDZs) increase social interaction whilst anxiogenic compounds such as the β-carboline FG 7142, pentylenetetrazol (PTZ) and yohimbine reduce social interaction. The BDZ, chlordiazepoxide (CDP), antagonized the effect of FG 7142 (17) and PTZ (14) in this test, but was unable to antagonize the effect of yohimbine (24). The benzodiazepine antagonist, flumazenil (Ro 15-1788), only antagonized the effect of FG 7142 (16,17). It is thought that FG 7142 acts via BDZ binding sites whilst PTZ acts via the picrotoxin site on the GABA/BDZ receptor complex. The reduction of social interaction by yohimbine is reversed by clonidine (24), suggesting that its anxiogenic effects are mediated via α_2-receptors. Recently, a new class of BDZs, the triazolobenzodiazepines, have been developed. Two of these compounds, adinazolam and U-43,465, are able to antagonize the effects of FG 7142, PTZ and yohimbine in the social interaction test (19).

It has previously been demonstrated that caffeine produces an anxiogenic-like response in the social interaction test, i.e., it reduces the time spent in social interaction (1, 11, 12). The following experiments were designed to determine the neurochemical site by which caffeine mediates its anxiogenic effects. In order to see if these effects of caffeine are mediated by its actions at adenosine receptors we have attempted to reverse them with 2-chloroadenosine (2-CA) (0.1 and 1 mg/kg), an adenosine receptor agonist.

TABLE 1

MOTOR ACTIVITY (MEAN ± SEM) FOR PAIRS OF RATS IN THE SOCIAL INTERACTION TEST (n=NUMBER OF PAIRS OF RATS)

Drug Treatment	n	Motor Activity
2 × control	8	970.9 ± 55.2
caffeine (40 mg/kg) and control	8	909.6 ± 39.1
2-CA (0.1 mg/kg) and control	7	974.1 ± 32.7
2-CA (1 mg/kg) and control	8	672.6 ± 42.7†
caffeine (40 mg/kg) and 2-CA (0.1 mg/kg)	7	916.4 ± 32.0
caffeine (40 mg/kg) and 2-CA (1 mg/kg)	8	877.1 ± 57.8
2 × control	8	466.9 ± 45.9
caffeine (20 mg/kg)	8	535.0 ± 20.6
caffeine (40 mg/kg) and control	8	487.2 ± 20.3
CDP (5 mg/kg) and control	7	332.6 ± 34.8*
caffeine (20 mg/kg) and CDP (5 mg/kg)	8	418.0 ± 55.5
caffeine (40 mg/kg) and CDP (5 mg/kg)	7	430.4 ± 31.3
2 × control	9	972.0 ± 16.6
caffeine (40 mg/kg) and control	8	1006.6 ± 21.4
flumazenil (1 mg/kg) and control	8	945.3 ± 31.6
flumazenil (10 mg/kg) and control	8	917.8 ± 36.8
caffeine (40 mg/kg) and flumazenil (1 mg/kg)	8	931.4 ± 45.4
caffeine (40 mg/kg) and flumazenil (10 mg/kg)	8	987.6 ± 54.9
2 × control	15	482.4 ± 21.1
caffeine (40 mg/kg) and control	15	478.3 ± 24.6
U-43,465 (32 mg/kg) and control	6	259.3 ± 31.8†
caffeine (40 mg/kg) and U-43,465 (32 mg/kg)	8	399.4 ± 32.2
2 × control	7	728.0 ± 44.6
caffeine (20 mg/kg) and control	8	744.5 ± 26.2
clonidine (0.01 mg/kg) and control	8	425.8 ± 31.1†
clonidine (0.025 mg/kg) and control	8	275.4 ± 32.9†
clonidine (0.01 mg/kg) and caffeine (20 mg/kg)	7	501.3 ± 23.9†
clonidine (0.025 mg/kg) and caffeine (20 mg/kg)	8	334.4 ± 26.8†
2 × control	9	555.9 ± 30.1
caffeine (40 mg/kg) and control	9	510.3 ± 30.7
propranolol (5 mg/kg) and control	7	610.6 ± 53.0
propranolol (10 mg/kg) and control	7	620.6 ± 27.2
caffeine (40 mg/kg) and propranolol (5 mg/kg)	7	612.6 ± 21.7
caffeine (40 mg/kg) and propranolol (10 mg/kg)	7	638.7 ± 28.2

Significantly different from controls: *$p<0.05$; †$p<0.01$.

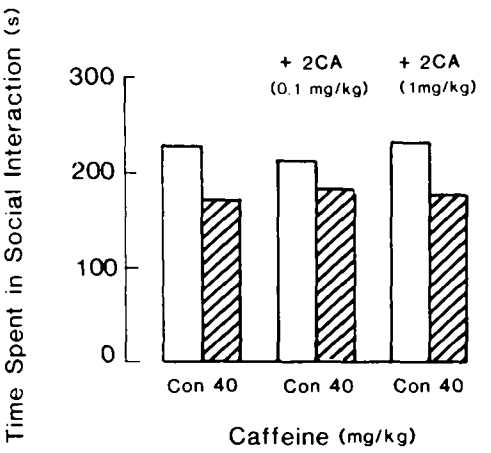

FIG. 1. Mean time (sec) spent in active social interaction by pairs of rats after administration of caffeine (40 mg/kg) and 2-chloroadenosine (0.1 and 1 mg/kg). Means have been adjusted after ANCOVA, see text for statistical details.

These doses were chosen on the basis of a previous study where caffeine antagonized the effects of 2-CA on motor activity and play fighting in juvenile rats (21). To investigate the role of benzodiazepine receptors, we have used chlordiazepoxide (CDP) (5 mg/kg), a benzodiazepine antagonist, flumazenil (1 and 10 mg/kg) and the triazoloBDZ, U-43,465 (32 mg/kg) in combination with caffeine. Finally, we have examined the role of noradrenergic systems by studying the effect of caffeine in combination with an α_2-receptor agonist, clonidine (0.01 and 0.025 mg/kg) and a β-adrenoceptor antagonist, DL-propranolol (5 and 10 mg/kg).

METHOD

Animals

Male hooded Lister rats (Olac Ltd., Bicester, U.K.) weighing 180–300 g were housed in a room with an 11-hr light:13-hr dark cycle and allowed free access to food and water.

Drug Treatment

The drug treatments are shown in Table 1. All injections were applied intraperitoneally (IP) in a volume of 2 ml/kg. Each rat received two injections, and all drugs were given 30 minutes before testing except for flumazenil which was given 20 min before the test.

Caffeine (Sigma), 2-chloroadenosine (Sigma), CDP (Roche Products Ltd.), clonidine (Sigma), DL-propranolol (Sigma) and U-43,465 [8-chloro-1-(2-(dimethylamino)ethyl)-6-phenyl-4H-s-triazolo(4,3-a)(1,4)benzodiazepine (UpJohn)] were all dissolved in distilled water. Flumazenil (RO 15-1788) (Hoffmann-La Roche) was suspended in distilled water using two drops of Tween 20 followed by sonication in an ultrasonic water bath for 10 min. Controls received vehicle only.

Apparatus

The test arena was dimly-lit (56.5 radiometric lux) wooden box, 60×60×35 cm. Motor activity was measured

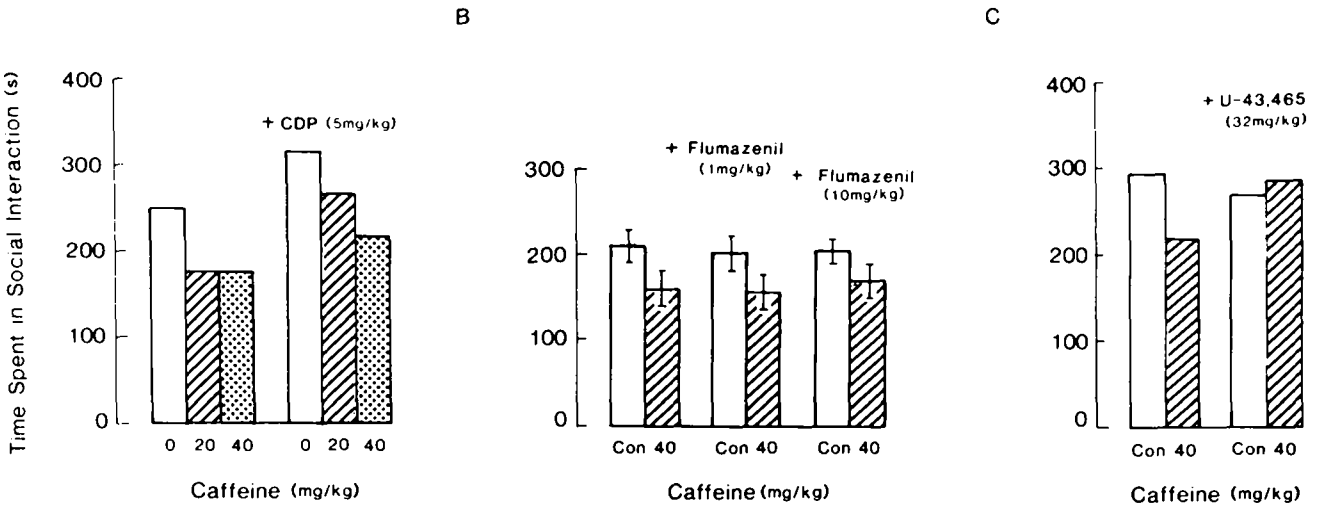

FIG. 2. Mean time (sec) spent in active social interaction by pairs of rats after administration of caffeine (20 or 40 mg/kg) in combination with drugs acting at the BDZ receptors: Left—CDP (5 mg/kg); middle—flumazenil (1 and 10 mg/kg); right—U-43,465 (32 mg/kg). For the CDP and U-43,465 experiments, means have been adjusted for ANCOVA. For the flumazenil experiment values are actual means (±SEM). See text for statistical details.

by the breaking of infrared photobeams positioned 4.5 cm from the floor. A camera was mounted vertically over the box and the rats were observed on a monitor in an adjacent room. A video recording of each trial was made to permit rescoring. The time spent in social interaction was scored using keyboards linked to a Control Universal microcomputer.

Procedure

The rats were allocated to pairs on the basis of weight (±10 g). Both members of a pair always received the same drug treatment. Two days before testing, pairs of rats were placed into the test arena for 7.5 min, and the day before testing rats spent 7.5 min in the arena alone. Thus, the rats were familiar with both the apparatus and their partner.

On the test day, pairs of rats were placed into the arena for 7.5 min. The duration of the following behaviours were scored by an independent observer who was blind to the drug treatment: sniffing, following, grooming, mounting, boxing, wrestling, kicking or pushing the partner. Passive body contact (when the rats were sitting or lying in contact with each other, but without interacting) was scored separately. At the end of each trial the box was thoroughly wiped clean with a damp cloth. Rats were tested in an order randomized for drug treatment between 08.00 and 13.00 hr.

In this test anxiolytic drugs increase, and anxiogenic drugs decrease, the time spent in social interaction independent of any effect on motor activity (9,10).

Statistical Analyses

For the social interaction test, time spent in social interaction and motor activity was analyzed using analysis of variance (ANOVA). If there was a significant change in motor activity, analysis of covariance (ANCOVA) was used, with social interaction as the dependent variable and motor activity as the covariate, to see whether any change in social interaction was independent of the change in motor activity. These were followed, where appropriate, by Duncan's Multiple Range post hoc tests.

RESULTS

Effects of Caffeine Alone

In all 6 experiments caffeine significantly decreased time spent in active social interaction shown by a significant caffeine factor (ANCOVA $p<0.05$). Since, in the first experiment (with CDP), both doses of caffeine (20 and 40 mg/kg) were equally effective (see Fig. 1), only one dose of caffeine was used for the rest of the study.

In 5 of the experiments caffeine (20 or 40 mg/kg) had no significant effect on the motor activity of the pairs of rats. In the U-43,465 experiment there was a significant caffeine factor [ANOVA, $F(1,40)=5.6$, $p<0.05$], however post hoc analysis showed that caffeine (40 mg/kg) had not significantly reduced motor activity compared with controls.

2-Chloroadenosine

2-CA (0.1 and 1 mg/kg) had no significant effect on social interaction and nor did it reverse the effects of caffeine, i.e., there was no significant caffeine × 2-CA interaction (see Fig. 1).

2-CA (1 mg/kg) significantly reduced motor activity [ANOVA, $F(2,40)=9.1$, $p<0.001$, followed by Duncan's tests: $p<0.01$]. There was a significant interaction factor for caffeine and 2-CA [ANOVA, $F(2,40)=5.6$, $p<0.01$], showing that caffeine (40 mg/kg) had significantly antagonized the decrease in motor activity produced by 2-CA (1 mg/kg) (see Table 1).

CDP

CDP (5 mg/kg) produced a significant increase in time spent in active social interaction [ANCOVA, $F(1,39)=8.7$,

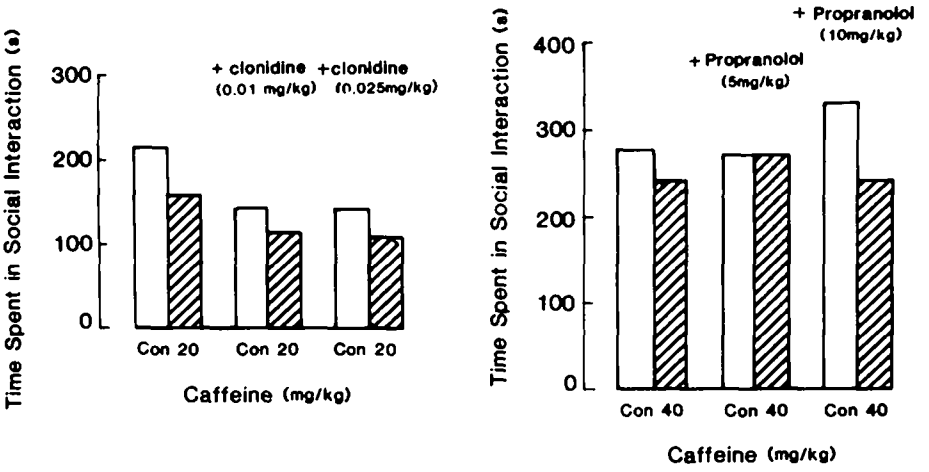

FIG. 3. Mean time (sec) spent in active social interaction by pairs of rats after administration of caffeine (40 mg/kg) in combination with drugs acting at noradrenergic receptors: Left—clonidine (0.01 and 0.025 mg/kg); right—DL-propranolol (5 and 10 mg/kg). Means have been adjusted after ANCOVA, see text for statistical details.

$p<0.01$]. CDP (5 mg/kg) significantly reversed the decrease in social interaction produced by the low dose of caffeine (20 mg/kg) (Duncan's tests: $p<0.05$) (see Fig. 2). However, there was no significant caffeine × CDP interaction suggesting that the effects of caffeine and CDP were simply additive.

CDP (5 mg/kg) produced a significant decrease in motor activity compared with controls [ANOVA, $F(1,40)=11.3$, $p<0.01$, followed by Duncan's tests: $p<0.05$] (see Table 1). There was no caffeine × CDP interaction for motor activity.

Flumazenil

Flumazenil had no significant effect on time spent in social interaction or motor activity. The caffeine × RO 15-1788 interactions were not significant (see Fig. 2 and Table 1).

U-43,465

U-43,465 (32 mg/kg) had no significant effect on social interaction. However there was a significant caffeine × U-43,465 interaction [ANCOVA, $F(1,39)=4.9$, $p<0.05$], showing that U-43,465 was able to significantly reverse the decrease in social interaction caused by caffeine (40 mg/kg) (see Fig. 2).

U-43,465 (32 mg/kg) produced a significant decrease in motor activity compared with controls [ANOVA, $F(1,40)=27.7$, $p<0.0001$, followed by Duncan's tests: $p<0.01$]. There was a significant caffeine × U-43,465 interaction [ANOVA, $F(1,40)=6.3$, $p<0.05$] showing that caffeine (40 mg/kg) significantly antagonized the decrease in motor activity produced by U-43,465 (see Table 1).

Clonidine

Clonidine (0.1 and 0.025 mg/kg) significantly reduced social interaction compared with controls [ANCOVA, $F(2,39)=3.5$, $p<0.05$, followed by Duncan's tests: $p<0.01$ for both doses]. There was no caffeine × clonidine interaction.

Clonidine (0.01 and 0.025 mg/kg) significantly decreased motor activity compared with controls [ANOVA, $F(2,40)=97.2$, $p<0.0001$, followed by Duncan's tests: $p<0.01$ for both doses]. There was no caffeine × clonidine interaction for motor activity.

DL-Propranolol

The higher dose of propranolol (10 mg/kg) significantly increased social interaction compared with controls (Duncan's: $p<0.05$), whilst the lower dose (5 mg/kg) had no effect. The decrease in social interaction produced by caffeine (40 mg/kg) was antagonized by the lower dose of propranolol (5 mg/kg), indicated by a significant caffeine × propranolol interaction [ANCOVA, $F(2,39)=4.4$, $p<0.05$]. However, the higher dose of propranolol (10 mg/kg) did not antagonize this effect (see Fig. 3).

Propranolol (5 and 10 mg/kg) had a significant effect on motor activity [ANOVA, $F(2,40)=4.7$, $p<0.05$], but post hoc comparisons showed that the motor activity scores for groups treated with propranolol were not significantly different from controls (see Table 1).

DISCUSSION

In this study, we have further investigated the effects of caffeine in the social interaction test of anxiety. In accordance with previous findings, caffeine consistently decreased the time spent in active social interaction in this test independently of any effect on motor activity (1, 11, 12). In this test, drugs that increase anxiety in man, such as FG 7142, PTZ and yohimbine, produce decreases in social interaction (16, 17, 19, 24). Thus, caffeine exhibits an anxiogenic profile in the social interaction test. This is consistent with several reports that caffeine increases levels of anxiety in man (4, 23, 31). The aim of this study was to attempt to determine the critical site(s) of action responsible for this anxiogenesis. In order to do this, pairs of rats were tested after administration of caffeine in combination with various other centrally acting compounds.

Caffeine is known to bind to central adenosine receptors (3) and it has been suggested that caffeine's stimulant prop-

erties are mediated by its antagonist activity at adenosine receptors (29). In order to see whether caffeine's anxiogenic properties are due to an interaction with these receptors, we investigated the effect of an adenosine receptor agonist, 2-chloroadenosine (2-CA). 2-CA (0.1 and 1 mg/kg) had no effect on social interaction alone and did not antagonize the decrease in social interaction produced by caffeine (40 mg/kg). However, 2-chloroadenosine (1 mg/kg) significantly reduced motor activity in this test and this was completely reversed by caffeine (40 mg/kg). Thus, although there is no direct evidence that 2-CA has central effects, caffeine antagonized its effects on motor activity and has previously been demonstrated to reverse its effects on play fighting in juvenile rats (21). These interactions suggest that 2-CA achieves sufficient quantities in the brain to compete with caffeine for adenosine receptors. Thus, our results on social interaction suggest that the anxiogenic properties of caffeine are not mediated at adenosine receptors.

Caffeine is known to have some affinity for BDZ receptors and it is posible that its ability to increase anxiety may be linked to this. We therefore investigated the effect of administration of the BDZ, chlordiazepoxide, in combination with caffeine. CDP (5 mg/kg) significantly reversed the decrease in social interaction produced by the lower dose of caffeine (20 mg/kg), whilst only partially reversing the effects of the higher dose of caffeine (40 mg/kg). However CDP (5 mg/kg), alone, significantly increased social interaction, therefore the reversal could have been merely "functional," i.e., a cancellation by two drugs with opposing behavioural effects. Therefore, to further investigate the role of the BDZ receptor we investigated the effect of the BDZ antagonist, flumazenil, during caffeine-induced anxiogenesis. Surprisingly, flumazenil (1 and 10 mg/kg) had no effect on social interaction alone, although it has previously been found to be anxiogenic at the higher dose (15). Neither dose of flumazenil was able to reverse the anxiogenic effect produced by caffeine (40 mg/kg). This finding suggests that the increased anxiety produced by caffeine is not produced by its ability to bind BDZ receptors, and therefore CDP's partial reversal of this effect may have merely been a "functional" reversal.

The triazoloBDZ, U-43,465 (32 mg/kg), had no effect on social interaction alone but significantly antagonized the reduction in social interaction produced by caffeine (40 mg/kg). As well as antagonizing the anxiogenic effects of compounds acting at the GABA/BDZ receptor complex, U-43,465 has been shown to antagonize the anxiogenic effects of the α_2-adrenoceptor antagonist yohimbine (19). Although U-43,465 had no effect on social interaction alone in this experiment, a lower dose (16 mg/kg) has been shown to be anxiolytic in the social interaction test (18). Therefore, it is possible that the ability of U-43,465 to antagonize the anxiogenic effects of caffeine and yohimbine is due to its anxiolytic properties, i.e., these were only "functional" reversals. Yohimbine markedly increases noradrenaline release and it has been suggested that this is the mechanism by which yohimbine increases anxiety in humans (5,6). There is some evidence that caffeine increases brain synthesis and release of noradrenaline (8). There is evidence that alprazolam, another triazolobenzodiazepine, has α_2-adrenergic receptor agonist activity (9) and has some effects at β-adrenergic receptors (28), in addition to their actions at BDZ receptors. This may also be the case for other triazolobenzodiazepines such as U-43,465. Thus, the ability of U-43,465 to antagonize caffeine may be due to these effects of noradrenergic receptors.

To further investigate the possibility that caffeine increases anxiety by an effect on noradrenergic function, the α_2 agonist, clonidine and the β-blocker, DL-propranolol were used. Whilst reducing social interaction alone, clonidine (0.01 and 0.025 mg/kg) did not reverse the anxiogenic effect of caffeine. However, we recently showed that the α_2-blocker yohimbine could reverse the effects of caffeine in this test (2). The results obtained with the two doses of propranolol (5 and 10 mg/kg) were conflicting. The lower dose of propranolol (5 mg/kg) had no effect in this test alone, but antagonized the decrease in social interaction produced by caffeine (40 mg/kg). However, the higher dose of propranolol (10 mg/kg) significantly increased social interaction and did not reverse the decrease produced by caffeine. Previous experiments showed that propranolol had no effect in the social interaction test (11). Thus, the anxiolytic effect of the higher dose of propranolol (10 mg/kg) is difficult to interpret. However, the fact that the lower dose of propranolol (5 mg/kg) did not increase social interaction alone, suggests that this was not merely a "functional" reversal. Thus, the results with the lower dose of propranolol and our previous findings with yohimbine (2) suggest a role for noradrenergic systems in caffeine's ability to increase anxiety. Interestingly, the discriminative stimulus produced by caffeine can be antagonized with propranolol and yohimbine but not with clonidine (22).

In conclusion, the results with 2-CA and flumazenil suggest that the anxiogenic effects of caffeine are not mediated by its actions at adenosine or BDZ receptors. Propranolol and U-43,465 were able to antagonize the effects of caffeine to some extent. The possibility that these were merely "functional" reversals cannot be ruled out completely. However, in conjunction with the ability of yohimbine to reverse caffeine, these data suggest a role for noradrenergic systems in caffeine-induced anxiogenesis.

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