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$GABA_A$ but not $GABA_B$ receptor stimulation induces antianxiety profile in rats

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Abstract

The effect of GABA receptor agonists and antagonists on anxiety behavior in rats in the elevated-plus-maze has been investigated. The increase in two parameters of %open arm entries (%OAE) and %time spent in the open arms (%OAT) and decrease in the %time spent in closed arm (%CAT) was considered as antianxiety effects. Intracerebroventricular (icv) injection of different doses of the $GABA_A$ receptor agonist muscimol (0.25, 0.5, and 1 μg/rat) increased %OAE and %OAT and decreased %CAT in rats dose-dependently. The higher response was obtained with 1 μg/rat of the drug. Neither icv (0.05, 0.1, and 0.2 μg/rat) nor intraperitoneal (ip) (1, 2, and 4 mg/kg) injection of the $GABA_B$ receptor agonist baclofen altered %OAE, %OAT, and %CAT. However, the $GABA_B$ receptor antagonist CGP35348 (5, 10, and 30 μg/rat icv) increased %OAE and %OAT and decreased %CAT in the animals. The response induced by injection of muscimol (0.5 μg/rat icv) or administration of CGP35348 (10 μg/rat icv) was reduced by icv (1, 2, and 4 μg/rat) or ip (0.25, 0.5, and 0.75 mg/kg) injection of the $GABA_A$ receptor antagonist bicuculline, except the effect of CGP35348 on %CAT which was not significantly altered by ip administration of bicuculline. Ip but not icv administration of bicuculline by itself reduced both %OAE and %OAT but did not alter %CAT. None of the drugs altered the locomotor activity of the animals. The current findings support our hypothesis that the anxiolytic effects of $GABA_B$ antagonist are mediated by autoreceptor blockade-induced release of endogenous GABA, which in turn activates postsynaptic $GABA_A$ receptors.

Keywords: GABA agonists and antagonists; Anxiety; Rats

1. Introduction

γ-Aminobutyric acid (GABA) is the main central inhibitory neurotransmitter (DeFeudis, 1977; Hõsli and Hõsli, 1978; Johnston, 1978), which is found in all areas of the human brain (Perry et al., 1971). GABA induces several pharmacological effects including sedation, analgesia, and anticonvulsant (DeFeudis, 1982; Sawynok and Labella, 1982). GABA receptors in the brain have been classified as $GABA_A$, $GABA_B$, and $GABA_C$ (Bowery et al., 1980; 1981; Drew et al., 1984; Hill and Bowery, 1981). $GABA_A$ receptors are directly associated with Cl^- channel, while $GABA_B$ receptors are coupled to Ca^{2+} or K^+ channels as second messenger systems (Bormann, 1988). The third class of GABA binding sites, $GABA_C$ sites, appears to be relatively simple ligand-gated Cl^- channels with a distinctive pharmacology in that they are not blocked by bicuculline (Enz and Cutting, 1998; Johnston, 1995).

Several central sites have been implicated in the modulation of fear or anxiety (Kopchia et al., 1992; Menard and Treit, 1996) and GABA receptor mechanisms may be involved in that modulation (Munro and Kokkinidis, 1997; Strzelczuk and Romaniuk, 1996). It has also been proposed that anxiogenic response during withdrawal of ethanol is due to a reduced GABA function, involving both $GABA_A$ and $GABA_B$ receptors (File et al., 1991). There are also reports indicating that $GABA_A$ receptor agonist muscimol induces anxiolytic effects (Drugan et al., 1986; Fridel and LeDoux, 1997). Endogenous GABA inhibit anxiety through $GABA_A$ receptors in the anterior basolateral amygdala (Sanders and Shekhar, 1995) and the central triggering mechanism for fear drive depends on the blockade of $GABA_A$-ergic transmission (Strzelczuk and Romaniuk,

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1996). Furthermore, it has been proposed that peripheral administration of baclofen (Andrew and File, 1993a; Hinderer, 1990) or muscimol (Dalvi and Rodgers, 1996) induce anxiolytic effects. Other investigators showed that peripheral injection of baclofen and $GABA_A$ receptor antagonist bicuculline induced anxiogenic effect, while $GABA_B$ receptor antagonist CGP35348 was inactive (Dalvi and Rodgers, 1996) and the injection of baclofen into amygdala elicited no response (Sanders and Shekhar, 1995). In the present study, the effects of intracerebroventricular (icv) or intraperitoneal (ip) injection of $GABA_A$ and $GABA_B$ receptor agonists or antagonists on anxiety have been investigated.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 150–200 g, obtained from the Institute of Pasteur Laboratories (Tehran, Iran) were used. They were housed in groups of seven in plastic cages ($54 \times 38 \times 24.5$) at 22–24°C on a 12-h light/dark cycle with lights on from 7.00h to 19.00h. Food and water were freely available except during the experiments. All experiments were performed between 1300 and 1600 h.

2.2. Chronic guide cannula implantation

Rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and Rompun (4 mg/kg), and placed in a Kopf stereotaxic instrument. The stainless steel guide cannula (23 gauge) was implanted in the right lateral cerebral ventricle using the rat atlas of Paxinos and Watson (1986) at the following coordinates: AP = −0.8 mm, ML = +1.6 mm (both with respect to bregma), and V = 3.5 mm from the dura. It was then fixed to the skull with steel screws and dental cement. Each cannula was kept patent with a steel obturator. The drug solutions were injected over a period of 1 min, by means of an internal cannula (30 gauge) connected by polyethylene tubing to a 25-μl Hamilton syringe and the injection cannula was left in place for an additional 1 min before being slowly withdrawn.

2.3. Behavioral test

2.3.1. Elevated plus-maze

The methods were basically the same as those used in previous experiments (Treit et al., 1998). The elevated plus-maze was a wooden, cross-shaped maze, consisting of four arms arranged in the shape of a plus sign. Two of the arms have no side or end walls (open arms; 50×10 cm). The other two arms have side walls and end walls, but are open on the top (closed arms; $50 \times 10 \times 40$ cm). Where the four arms intersect, there is a square platform of 10×10 cm. The maze was elevated to a height of 50 cm. Rats were placed in a wooden test arena ($60 \times 60 \times 35$ cm) for 5 min prior to maze testing, in order to elevate total arm entries on the maze (Pellow and File, 1986).

Five days after implantation, the effects of icv or ip administration of drugs were tested in the elevated plus-maze. The rats were individually placed in the center of the maze facing a closed arm and allowed 5 min of free exploration. During this test period the observer measured the number of entries into open arms, the number of entries into closed arms, and the total time spent in the open arms and total time spent in the closed arms. Entry was defined as all four paws in the arm. The following measures were taken: (a) %OAE (the ratio of entries into open arms to total entries × 100); (b) %OAT (the ratio of times spent in the open arms to total times spent in any arms × 100); (c) %CAT (the ratio of times spent in the closed arms to total times spent in any arms × 100). Significant increases in %OAE and %OAT and decreases in %CAT are conventional indices of anxiety reduction in this test (e.g. Pellow et al., 1985; Treit et al., 1998). The total number of entries into both open and closed arms was taken to indicate the locomotor activity (Appenrodt et al., 1998; Bowen et al., 1996; Espejo, 1997; Skutella et al., 1998).

2.4. Drug treatments

The drugs used were baclofen, CGP35348 (Ciba-Geigy, Switzerland), bicuculline, and muscimol (Sigma, England). The drugs were dissolved in saline except for bicuculline which was dissolved in a drop of glacial acetic and then was diluted with saline. All the drugs were administered icv (in a volume of 2.5 μl/rat) or ip (in a volume of 10 ml/kg). Icv and ip injection were made 5 and 30 min before testing, respectively.

Animals in Experiment 1: four groups of rats received saline or different doses of muscimol (0.25, 0.5, and 1 μg/rat icv).

Animals in Experiment 2: 11 groups of rats received either icv injection of saline, different doses of baclofen (0.05, 0.1, and 0.2 μg/rat), and CGP35348 (5, 10, and 30 μg/rat) or ip injection of baclofen (1, 2, and 4 mg/kg).

Animals in Experiment 3: 12 groups of animals received icv injection of saline, bicuculline (1, 2, and 4 μg/rat), and bicuculline 5 min before muscimol (0.5 μg/rat) or CGP35348 (10 μg/rat).

Animals in Experiment 4: 12 groups of rats received ip injection of saline, bicuculline (0.25, 0.5, and 0.75 mg/kg) alone, bicuculline 30 min before muscimol (0.5 μg/rat icv), or CGP35348 (10 μg/rat icv).

Seven animals were used in each group. In all the experiments %OAE, %OAT, %CAT, and locomotor activity during the tests were recorded.

2.5. Statistical analysis

One-way ANOVA was used for comparison of effects of different doses of the drugs with saline (Experiments 1 and

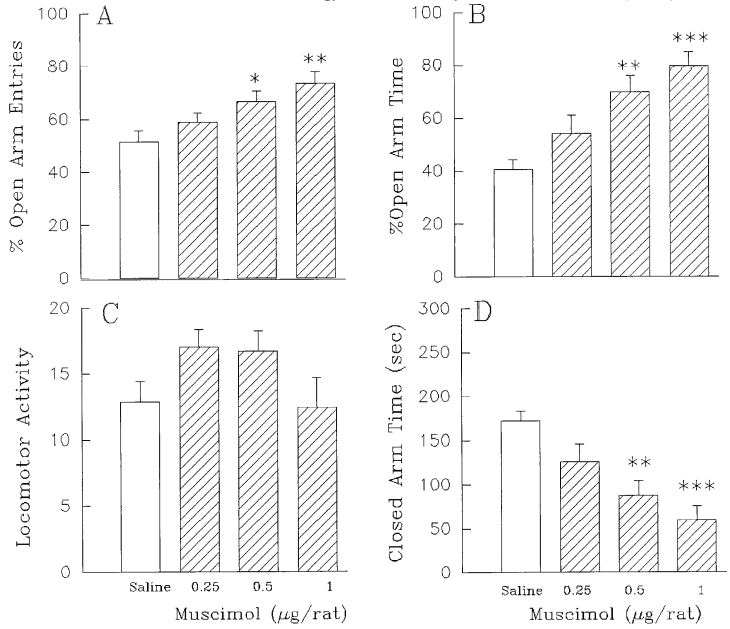

Fig. 1. Effect of icv injection of muscimol on elevated plus-maze (EPM). Rats were treated icv with either saline (2.5 μl/rat) or with muscimol (0.25, 0.5, and 1 μg/rat). Each bar is mean ± S.E.M. of %OAE (A), %OAT (B), %CAT (C), or locomotor activity (D). $N = 7$. * $P < .05$, ** $P < .01$, *** $P < .001$ different from saline-treated rats.

2). Two-way ANOVA was used for evaluation of interactions between the drugs (Experiments 3 and 4). Newman–Keuls test has been used for post hoc analysis of data, with significance level $P < .05$.

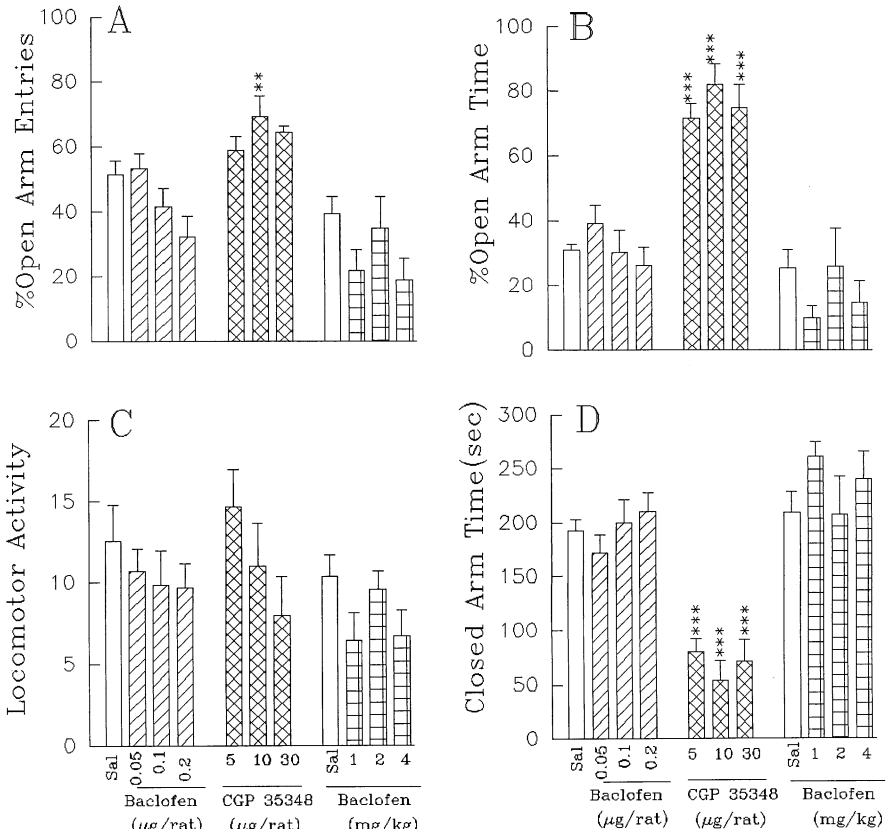

Fig. 2. Effects of icv or ip injection of $GABA_B$ receptor agonist and antagonist on EPM. Rats were administered saline (Sal; icv or ip as control), baclofen (0.05, 0.1, and 0.2 μg/rat icv), baclofen (1, 2, and 4 mg/kg ip) or CGP35348 (5, 10, and 30 μg/rat icv). Each bar is mean ± S.E.M. of %OAE (A), %OAT (B), %CAT (C), or locomotor activity (D). $N = 7$. ** $P < .01$, *** $P < .001$ different from respective saline control groups.

3. Results

3.1. Effects of $GABA_A$ receptor agonist on anxiety behavior

Fig. 1 shows the effect of icv injection of the $GABA_A$ receptor agonist muscimol (0.25, 0.5, and 1 μg/rat) on elevated plus-maze.

A one-way ANOVA shows that muscimol increased %OAE [$F(3,24)=5.4$, $P<.01$], %OAT [$F(3,24)=9.6$, $P<.001$], and decreased %CAT [$F(3,24)=9.5$, $P<.001$], indicating that muscimol induced an anxiolytic response. The drug did not alter locomotor activity [$F(3,24)=2.0$, $P>.05$].

3.2. Effect of $GABA_B$ receptor agonist and antagonist on anxiety behavior

Effect of the $GABA_B$ receptor agonist baclofen and $GABA_B$ receptor antagonist CGP35348 has been shown in Fig. 2.

A one-way ANOVA indicates a significant difference between the effects of icv injection of baclofen (0.05, 0.1, and 0.2 μg/rat) and CGP35348 (5, 10, and 30 μg/rat) on %OAE [$F(6,42)=101.5$, $P<.0001$], %OAT [$F(6,42)=18.2$, $P<.0001$], and %CAT [$F(6,42)=18.2$, $P<.0001$] but not on locomotion [$F(6,42)=1.1$, $P>.05$] with that of saline. Further analysis shows that baclofen had no influence on %OAE, %OAT, %CAT, or locomotion. However, CGP35348 increased the %OAT, %OAE and decreased %CAT, but did not alter locomotor activity. One-way ANOVA also indicates that ip administration of baclofen by itself (1, 2, and 4 mg/kg) had no effect on %OAE [$F(3,24)=1.9$, $P>.05$], %OAT [$F(3,24)=1.1$, $P>.05$], %CAT [$F(3,24)=1.1$, $P>.05$], or locomotion [$F(3,24)=1.9$, $P>.05$].

3.3. Effect of bicuculline on response induced by muscimol or CGP35348

Effects of icv administration of the $GABA_A$ receptor antagonist bicuculline on the response induced by icv injection of muscimol or CGP35348 are shown in Fig. 3.

A two-way ANOVA indicates the effect of bicuculline (Factor A) in the presence or absence of muscimol (Factor B) or CGP35348 (Factor C) and interaction (Factor A × B) or (Factor A × C). ANOVA shows that different doses of bicuculline (1, 2, and 4 μg/rat icv) altered the effect of muscimol (0.5 μg/rat icv) on %OAT [Factor A $F(3,48)=5.0$, $P<.01$; Factor B $F(1,48)=9.6$, $P<.01$; Factor A × B $F(3,48)=4.1$, $P<.05$] and %CAT [Factor A $F(3,48)=2.3$,

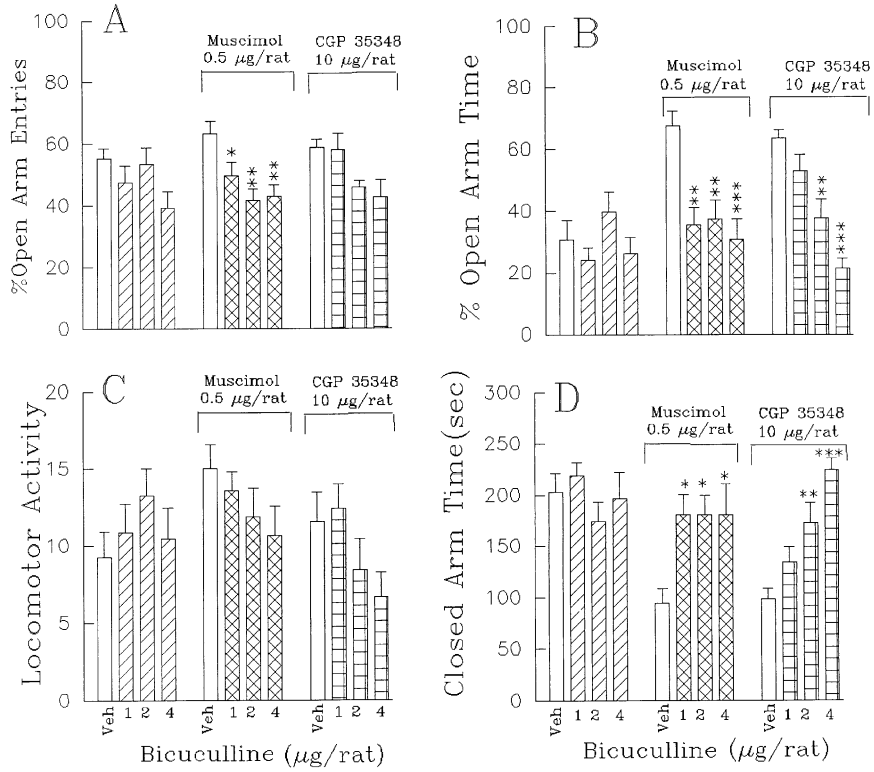

Fig. 3. Effects of icv administration of bicuculline in the presence or absence of muscimol (icv) or CGP35348 (icv) on EPM. Rats were injected (icv) vehicle (Veh; 2.5 μg/rat; a drop of glacial acetic acid in 10 ml distilled water) or bicuculline (1, 2, and 4 μg/rat), 5 min before muscimol (0.5 μg/rat) or CGP35348 (10 μg/rat). Each bar is mean ± S.E.M. of %OAE (A), %OAT (B), %CAT (C), or locomotor activity (D). $N=7$. $^{++}P<.01$ different from vehicle control groups. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ different from respective control groups.

$P>.05$; Factor B $F(1,48)=7.3$, $P>.01$; Factor A × B $F(3,48)=2.9$, $P<.05$]. Bicuculline did not show any interaction with muscimol on %OAE [Factor A $F(3,48)=5.6$, $P<.01$; Factor B $F(1,48)=0.2$, $P>.05$; Factor A × B $F(3,48)=1.8$, $P>.05$]. Further analysis indicates that muscimol (0.5 μg/rat icv) increased %OAT and decreased %CAT, but did not alter %OAE. Analysis also indicates that single administration of bicuculline did not alter %OAE, %OAT, and %CAT in the animals. The effects of muscimol on %OAT and %CAT was blocked by bicuculline. Analysis also shows that bicuculline did not show any response in combination with muscimol on locomotion [Factor A $F(3,48)=0.93$, $P>.05$; Factor B $F(1,48)=1.4$. $P>.5$; Factor A × B $F(3,48)=1.8$, $P>.05$]. Further analysis indicates that neither bicuculline nor muscimol alter the locomotor activity of the animals.

A two-way ANOVA shows that bicuculline (icv; Factor A) altered the influence of CGP35348 (icv; Factor C) on %OAT [Factor A $F(3,48)=7.1$, $P<.001$; Factor C $F(1,48)=14.1$, $P<.001$; Factor A × C $F(3,48)=7.4$, $P<.001$] but not the influence of CGP35348 on %CAT [Factor A $F(3,48)=4.2$, $P<.05$; Factor C $F(1,48)=11.3$, $P<.01$; Factor A × C $F(3,48)=6.98$, $P<.001$]. No interaction was found between bicuculline and CGP35348 on %OAE [Factor A $F(3,48)=4.4$, $P<.01$; Factor C $F(1,48)=0.6$, $P>.05$; Factor A × C $F(3,48)=1.3$, $P>.05$]. Further analysis shows that CGP35348 increased %OAT, which was blocked by bicuculline. CGP35348 also decreased %CAT. Bicuculline also tends to reverse the response of CGP35348 on %CAT. Bicuculline in combination with CGP35348 did not alter locomotor activity [Factor A $F(3,48)=0.99$, $P>.05$; Factor C $F(1,48)=0.8$, $P>.05$, Factor A × C $F(3,48)=1.98$, $P>.05$]. Further analysis showed that CGP35348 by itself did not alter the locomotor activity of the animals.

Effects of ip injection of bicuculline on the response of icv injection of muscimol or icv administration of CGP35348 is shown in Fig. 4.

A two-way ANOVA shows that bicuculline (Factor A 0.25, 0.5, and 0.75 mg/kg ip) altered the effect of muscimol (Factor B 0.5 μg/rat icv) on %OAE [Factor A $F(3,48)=8.3$, $P<.001$; Factor B $F(1,48)=6.6$, $P<.5$; Factor A × B $F(3,48)=7.6$, $P<.001$], %OAT [Factor A $F(3,48)=23.9$, $P<.0001$; Factor B $F(1,48)=109.0$, $P<.0001$; Factor A × B $F(3,48)=8.9$, $P<.0001$], and %CAT [Factor A $F(3,48)=15.6$, $P<.0001$; Factor B $F(1,48)=113.2$,

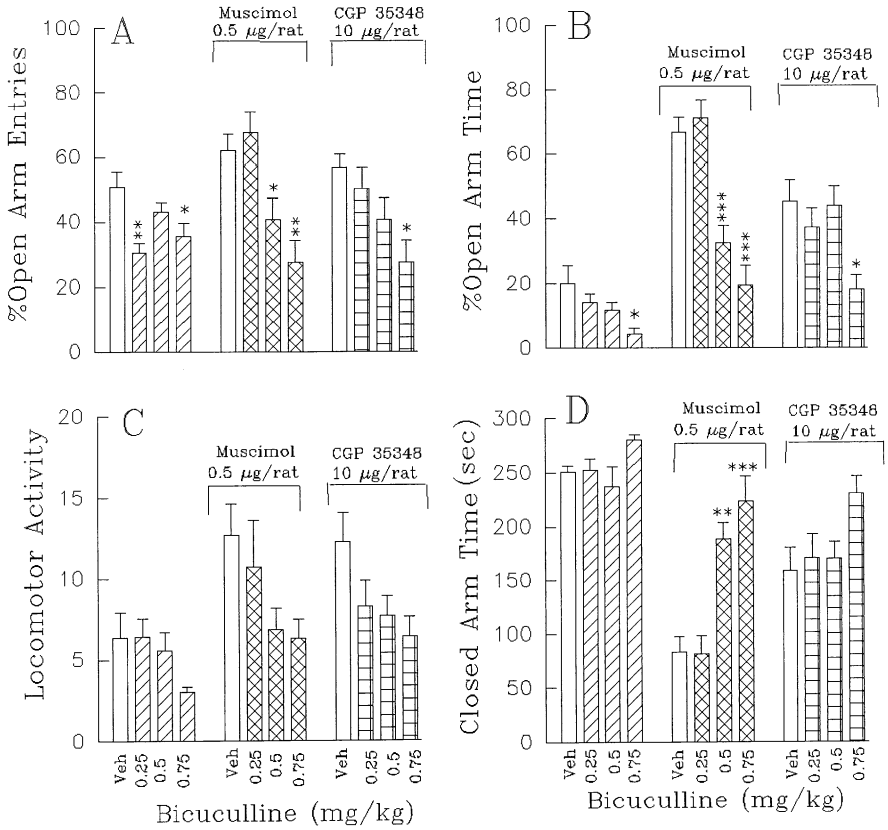

Fig. 4. Effects of ip injection of bicuculline in the presence or absence of muscimol (icv) or CGP35348 (icv) on EPM. Rats were injected (ip) vehicle (Veh; 10 ml/kg; a drop of glacial acetic acid in 10 ml distilled water) or bicuculline (0.25, 0.5, and 0.75 mg/kg), 30 min prior to vehicle, muscimol (0.5 μg/rat) or CGP35348 (10 μg/rat). Each bar is mean ± S.E.M. of %OAE (A), %OAT (B), %CAT (C), or locomotor activity (D). $N=7$. $^{+}P<.05$, $^{++}P<.01$, $^{+++}P<.001$, different from vehicle control groups. $^{*}P<.05$. $^{**}P<.01$, $^{***}P<.001$ different from respective control groups.

$P<.0001$; Factor A × B $F(3,48)=10.4$, $P<.0001$]. The bicuculline does not show interaction with muscimol on locomotion [Factor A $F(3,48)=3.7$, $P<.05$; Factor B $F(1,48)=12.4$, $P<.001$; Factor A × B [$F(3,48)=1.1$, $P>.05$]. Further analysis indicates that muscimol decreased %OAE or %OAT, increased %CAT, but did not alter locomotor activity. Bicuculline reduced %OAE and %OAT, but did not alter %CAT or locomotor activity, but the higher dose of bicuculline (0.75 mg/kg) tends to decrease locomotion, which is not statistically significant. Bicuculline blocked the effect of muscimol on %OAE or %OAT and reversed the effect of muscimol on %CAT.

Two-way ANOVA shows that bicuculline (ip) did not alter the response of icv injection of CGP35348 (Factor C 10 μg/rat) on %OAE [Factor A $F(3,48)=6.6$, $P<.05$; Factor C $F(1,48)=3.8$, $P>.05$; Factor A × C [$F(3,48)=2.3$, $P>.05$], %OAT [Factor A $F(3,48)=8.1$, $P<.001$; Factor C $F(1,48)=45.0$, $P<.0001$; Factor A × C [$F(3,48)=1.2$, $P>.05$], %CAT [Factor A $F(3,48)=5.0$, $P<.01$; Factor C $F(1,48)=43.0$, $P<.0001$; Factor A × C [$F(3,48)=0.7$, $P>.05$], or locomotor activity [Factor A $F(3,48)=3.9$, $P<.05$; Factor C $F(1,48)=17.3$, $P<.001$; Factor A × C [$F(3,48)=1.1$, $P>.05$]. Further analysis indicates that CGP35348 reduced %OAE or %OAT, but did not alter %CAT or locomotor activity. Ip administration of bicuculline by itself reduces %OAE and %OAT in the animals. The drug also did not show any effect on %CAT or locomotion. Post hoc analysis also shows that the higher dose of bicuculline (0.75 mg/kg ip) in combination with CGP35348 reduced %OAE or %OAT, which may be due to bicuculline effect.

4. Discussion

The elevated plus-maze test is a useful model for the selective identification of anxiolytic and anxiogenic drug effects in the rat (Menard and Treit, 1996; Pellow and File, 1986; Pellow et al., 1985; Treit et al., 1996).

It has been reported that the GABA receptor mechanism can influence anxiety behavior in rats (Munro and Kokkinidis, 1997; Strzelczuk and Romaniuk, 1996). The aim of this study was to investigate the role of different GABA receptors in anxiety behavior by using elevated plus-maze in rats. Muscimol, which is a $GABA_A$ receptor agonist (Bowery et al., 1984), increased %OAE and %OAT but decreased %CAT, indicating the drug reduced anxiety in the present study. The response of the drug seems to be dose-dependent. This is in agreement with results obtained by others who showed that microinfusion of muscimol into the lateral septum induced an antianxiety response (Drugan et al., 1986). The administration of muscimol into lateral and basal amygdala nuclei prior to anxiety conditioning or testing also reduced freezing in rats (Muller et al., 1997). Both icv and ip administration of the bicuculline, which is a $GABA_A$ receptor antagonist (Ticku and Maksay, 1983), in combination with muscimol showed a decrease in %OAT and %OAE, and an increase in %CAT, but did not alter locomotion. The present data may indicate that $GABA_A$ receptor mechanisms mediate the anti-anxiety mechanism. This hypothesis is supported by other data that $GABA_A$ receptor agonists including muscimol induce an antianxiety response (Munro and Kokkinidis, 1997; Rodgers and Dalvi, 1997). Ip injection of bicuculline but not icv administration of the drug decreased %OAE and %OAT by itself. Neither ip nor icv administration of bicuculline altered %CAT or locomotion. Injection of the antagonist in the basolateral amygdala has been shown to produce anxiogenic-like effects in the social interaction paradigm and conflict paradigm. However, the same dose of the antagonist injected into the central nucleus of the amygdala did not elicit any response (Sanders and Shekhar, 1995). Therefore, it appears that bicuculline's effects depend on the sites of its administration. The reason why bicuculline induced a response by peripheral but not icv injection in the present study may reflect such pathways. It is shown that bicuculline applied to the ventral tegmental area neurons increases nucleus accumbens dopamine release (Westerink et al., 1996). Therefore, the response of the drug can be mediated through such a mechanism. However, it should be mentioned that ip administration of higher doses of bicuculline induced some muscle contraction. Whether this effect of bicuculline influenced the animals' behavior should be clarified by further experiments. Furthermore, the effects observed with bicuculline have been proposed to be mediated by an active metabolite of bicuculline such as bicucine (Dalvi and Rodgers, 1996).

Our study also showed that $GABA_B$ receptor agonist baclofen (Bowery et al., 1984) in the doses used did not alter anxiety response or locomotor activity. This is in agreement with results obtained by others that the $GABA_B$ receptor agonist baclofen has no effect on anxiety (Sanders and Shekhar, 1995). There are reports indicating that baclofen reduces anxiety due to ethanol withdrawal (File et al., 1991), withdrawal of diazepam (Andrews and File, 1993b), or handling (Andrew and File, 1993a), but even higher dose of the drug (3 mg/kg) induced an anxiogenic effect (Dalvi and Rodgers, 1996). There is a report indicating that the $GABA_B$ receptor antagonist CGP35348 (Olpe et al., 1990) is inactive in plus-maze behavior in mice (Dalvi and Rodgers, 1996). However, our results showed that CGP35348 increased %OAT or %OAE and decreased %CAT but did not alter locomotion. Thus the antianxiety effect of CGP35348 seems likely, since we have previously shown the currently used doses of CGP35348 to be active in other experiments (Zarrindast and Mousa-Ahmadi, 1999). The response of the $GABA_B$ receptor antagonist was reduced by bicuculline. It has been proposed that GABA autoreceptors ($GABA_B$) regulate the release of GABA (Bonnano et al., 1989; Bowery, 1989). Therefore, blockade of presynaptic $GABA_B$ receptor by CGP35348 may increase release of GABA, which in turn affects $GABA_A$ receptors,

reducing the anxiety. Overall, our data support the hypothesis that $GABA_A$ receptor mechanisms are involved in anxiety behavior.

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