Benzodiazepine receptor antagonists flumazenil and CGS 8216 and inverse-agonist β -CCM enhance spatial learning in the rat: Dissociation from anxiogenic actions

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The effects of the benzodiazepine receptor antagonists flumazenil (10, 20, and 30 mg/kg) and CGS 8216 (10, 20, and 30 mg/kg), the benzodiazepine receptor inverse-agonist methyl β -carboline-3-carboxylate (β -CCM; 0.3, 0.6, and 1.0 mg/kg), and the benzodiazepine receptor agonist diazepam (3 mg/kg) were investigated on spatial learning in the Morris water maze and thigmotaxia in an open field. Flumazenil, CGS 8216, and β -CCM dose-dependently enhanced spatial learning, whereas diazepam impaired it. Flumazenil (10 mg/kg) antagonized both the enhancement produced by β -CCM (0.3 mg/kg) and the impairment produced by diazepam. The impairment produced by diazepam was not blocked by β -CCM (0.3 mg/kg). In the open field, flumazenil, CGS 8216, and β -CCM dose-dependently increased thigmotaxia (anxiogenesis), whereas diazepam had no effect. Flumazenil (10 mg/kg) blocked the anxiogenic effect of β -CCM (0.6 mg/kg) and increased anxiety when combined with diazepam; the anxiogenic effect of β -CCM (0.6 mg/kg) was not antagonized by diazepam. Since the dose-response profiles of flumazenil, CGS 8216, and β -CCM on spatial learning and thigmotaxia were highly dissimilar, it is unlikely that the mnemonic-enhancing actions of these drugs are due to their anxiogenic actions.

Benzodiazepine (BZ) drugs exert their actions by either increasing or decreasing the affinity of the GABA_A receptor-chloride ionophore complex in an allosteric manner (Ticku, 1983). For example, BZ receptor agonists, such as diazepam and chlordiazepoxide, increase GABA_A receptor affinity, whereas BZ receptor inverse-agonists, such as methyl β -carboline-3-carboxylate (β -CCM), decrease it. In contrast, BZ receptor antagonists, such as flumazenil (FLU; formerly Ro 15-1788) or CGS 8216 (CGS), block the receptor and neither increase nor decrease GABA_A receptor affinity (Haefely, 1990; Hunkeler et al., 1981). However, FLU, at high concentrations, has been shown to increase GABAergic responses to a moderate degree (e.g., Skerrett & Macdonald, 1983).

At the behavioral level, BZ inverse-agonists and antagonists have intrinsic actions that are opposite to those characteristic of BZ agonists. For example, β -carbolines have been found to be anxiogenic (Corda, Blaker, Mendelson, Guidotti, & Costa, 1983; Cutler & Aitken, 1991; Dorow, Horowski, Paschelke, & Amin, 1983; File, Lister, & Nutt, 1982; Novas, Wolfman, Medina, & De Robertis, 1988; Prado de Carvalho, Grecksck, Chapouthier, & Rossier, 1983), proconvulsant (Cowan, Green, Nutt, &

Martin, 1981; Jones & Oakley, 1981; Novas et al., 1988; Venault, Prado de Carvalho, et al., 1986), and mnemonic enhancing (Chapouthier, Raffalli-Sebille, Venault, Simiand, & Dodd, 1991; File & Pellow, 1988; Izquierdo, Pereira, & Medina, 1990; Raffalli-Sebille & Chapouthier, 1991; Venault, Chapouthier, et al., 1986). FLU has anxiogenic actions on some tests of experimental anxiety (File et al., 1982; Lee & Rodgers, 1991), but not others (Crawley, Skolnick, & Paul, 1984; Prado de Carvalho et al., 1983; Treit, 1987), and mnemonic-enhancing properties (Brioni, Arolfo, Jerusalinsky, Medina, & Izquierdo, 1991; Izquierdo et al., 1990; Lal, Kumar, & Forster, 1988; Raffalli-Sebille & Chapouthier, 1991) but lacks the proconvulsant properties found with β -carbolines (e.g., Marescaux et al., 1984). The behavioral properties of CGS 8216 more closely resemble the β -carbolines in that they possess proconvulsant (File, 1983), as well as anxiogenic (File & Lister, 1983) and mnemonic-enhancing (Kumar, Forster, & Lal, 1988) properties. Thus, unlike their actions at the GABA_A receptor, at the behavioral level BZ receptor antagonists act like inverse-agonists, suggesting that they are blocking an endogenously released BZ receptor agonist.

Unlike the mnemonic-enhancing properties of BZ receptor antagonists and inverse-agonists, BZ receptor agonists produce anterograde amnesia in humans (Ghoneim & Mewaldt, 1990) and rodents (Cole, 1986). For example, diazepam, chlordiazepoxide, and the ω_1 receptor subtype agonist CL 218,872, impair spatial learning in the Morris water maze (McNamara & Skelton,

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1991, 1992b). It is possible that this task elicits anxiety, which in turn motivates the acquisition of an appropriate escape response. Thus, it is possible that BZ agonists impair acquisition by reducing the motivation to acquire an effective escape route—a notion supported by the findings discussed above that BZ receptor antagonists and inverse-agonists both increase anxiety and enhance mnemonic processes. However, to date definitive evidence for a relationship between the mnemonic-enhancing and anxiogenic actions of BZ agonists and inverse-agonists has yet to be obtained.

The present study tested the effects of several doses of the BZ receptor antagonists FLU and CGS and the BZ receptor inverse-agonist β -CCM on spatial learning in a task sensitive to BZ agonist-induced amnesia—namely, the Morris water maze (Morris, 1984). An amnesic dose of the BZ receptor agonist diazepam was also assessed for comparative purposes. Additionally, the same rats and doses of FLU, CGS, and β -CCM were assessed for anxiogenic actions using thigmotaxia (wall hugging), a robust and reliable defensive response that is selectively reduced by conventional anxiolytic agents (Treit & Fundytus, 1989).

METHOD

Animals

Eighty-eight hooded, male rats of the Long-Evans strain served as subjects. They were housed in pairs in shoebox cages and maintained on a 12:12-h light:dark cycle. Testing was conducted during the light phase of the cycle. The rats weighed approximately 400 g at the beginning of the experiment. Food and water were available ad lib.

Apparatus and Procedure

Water maze. The Morris water maze consisted of a circular pool (diameter, 150 cm; height, 45 cm), with a featureless white inner surface. The pool was filled to a height of 25 cm with 22°C ($\pm 1^\circ\text{C}$) water, in which 1,500 ml of powdered skim milk was dissolved. The hidden escape platform was a clear Plexiglas stand (13 $\times$ 13 cm) submerged 3 cm below the water surface so that it was invisible at water level. The visible platform was a black stand (13 $\times$ 13 cm) that protruded 5 cm above the surface of the water.

During initial acquisition, the submerged escape platform was located in the center of the northwest quadrant. All groups were given only two trials each day for 5 consecutive days. For each trial, the rat was placed in the water facing the pool wall at one of two quasi-randomly determined starting locations, with the only restriction being that daily sessions had to have one trial start from a location close to the platform and one start from farther away (e.g., S-W, N-S, E-N, etc.). During each trial, the rat's swim path length and escape latency were recorded with an overhead video-tracking system (Chromotrack; SD Instruments). Swim speed was calculated as the distance divided by the latency (centimeter/second) obtained for each trial. Once the rat located the platform, it was permitted to remain on it for 15 sec. If the rat did not locate the platform within 60 sec, it was placed on the platform for 15 sec. After each trial, the rat was returned to a holding cage positioned 90 cm under a 250-W brooding lamp (for warmth) and allowed to remain there for the intertrial interval (approximately 4 min).

On the day following the final day of acquisition, a drug-free probe trial was given to assess the strength and accuracy of initial learning and to determine the strategy adopted by the rat to locate the platform during training. The rats were required to swim in the

pool without an escape platform for 60 sec. All rats were released from the southern pole, and the time spent in each quadrant was recorded and analyzed with the video-tracking system.

Open field. Two days after the probe trial, potential anxiogenic drug effects were assessed by using the empty pool as a circular open field (diameter, 150 cm; height, 45 cm). The pool/open field was kept in the same room in which spatial learning was assessed to reduce novelty-induced anxiety. The field was illuminated to 100 fc by eight 100-W bulbs. Constant background noise was maintained with a stereo system. The inner surface was white and featureless except for a line drawn on the floor 6 cm from the wall around the periphery. This line divided the floor of the field into a central area (85% of field) and a peripheral area (15% of field). The rats were placed one at a time into the center of the open field and allowed to explore freely for 5 min. During this time, the rat's path in both the peripheral region and the central regions of the field were measured by the video-tracking system. Between trials, the wall and floor of the open field were wiped with ethanol (100%) to standardize the scent of the arena. Anxiety was assessed as thigmotaxia (the proportion of time spent in the peripheral region of the open field).

Drugs and Group Assignment

The subjects were randomly divided into the following eight treatment groups: (1) vehicle (1 ml/kg; 0.9% NaCl with a drop of Tween 80 [1 drop/10 ml]; $n = 10$), (2) FLU (10, 20, and 30 mg/kg; $n = 6$ /dose; Hoffmann-La Roche Inc.), (3) CGS (10, 20, and 30 mg/kg; $n = 6$ /dose; Ciba-Geigy Inc.), (4) β -CCM (0.3, 0.6, and 1.0 mg/kg; $n = 6$ /dose; Sigma Chemical Co.), (5) diazepam (3 mg/kg; $n = 6$; Hoffmann-La Roche Inc.), (6) diazepam (3 mg/kg) + β -CCM (0.6 mg/kg; $n = 6$), (7) diazepam (3 mg/kg) + FLU (10 mg/kg; $n = 6$), and (8) FLU (10 mg/kg) + β -CCM (0.6 mg/kg; $n = 6$). All drugs were suspended in physiological saline with Tween 80 (1 drop/10 ml) and injected i.p. in the rat's home cage 15 min prior to behavioral testing.

Data Analysis

Group differences in percentage distances in the peripheral region of the open field, probe test data, and swim speeds were assessed using Tukey's t test. Escape latencies and distances were assessed using an analysis of variance (ANOVA) procedure with repeated measures. Post hoc comparisons were assessed using Dunnett's t test. In every case, the acceptable level for statistical significance was $p < .05$.

RESULTS

Water Maze Acquisition

Rats treated with the drug vehicle showed a gradual improvement in performance over training but failed to reach asymptotic levels of proficiency by the end of testing. This pattern is consistent with previous observations that a two-trial-per-day protocol produces slower acquisition, allowing for potential enhancement effects to be revealed. Relative to the drug vehicle, the BZ receptor antagonists FLU and CGS and the inverse-agonist β -CCM all enhanced spatial learning in a dose-dependent, inverted U-shaped manner (Figures 1 and 2).

Rats treated with the two low doses of FLU (10 and 20 mg/kg) took shorter distances to locate the platform, though the high dose of FLU (30 mg/kg) had little effect relative to controls (Figures 1A and 2A). An overall ANOVA on the escape distances of groups treated with

Figure 1. Effects of (A) flumazenil (FLU), (B) CGS 8216 (CGS), and (C) β -CCM on the distance taken to locate the submerged escape platform over the course of training. Numbers represent the drug dose in milligrams/kilograms.

FLU revealed a significant group difference [$F(3,56) = 4.84, p < .005$] and a significant day difference [$F(4,224) = 25.2, p < .001$], but not a significant interaction between group and day [$F(12,224) = 0.40, p = .96$]. Post hoc tests revealed that rats treated with the low (10 mg/kg) and intermediate (20 mg/kg) doses of FLU, but not the high (30 mg/kg) dose, had significantly shorter distances relative to vehicle-treated controls ($p < .01$).

Rats treated with all three doses of CGS took shorter swim paths to locate the escape platform, with the intermediate dose of CGS (20 mg/kg) producing the most drastic reduction of distances (Figures 1B and 2B). An overall

ANOVA on the distances of groups treated with CGS revealed a significant group difference [$F(3,56) = 4.41, p < .001$] and a significant day difference [$F(4,224) = 30.6, p < .001$], but not a significant interaction between group and day [$F(12,224) = 0.47, p = 0.93$]. Post hoc tests revealed that rats treated with both the low (10 mg/kg; $p < .05$) and intermediate (20 mg/kg; $p < .01$) doses of CGS, but not the high dose (30 mg/kg), had significantly shorter distances relative to vehicle-treated rats.

Rats treated with the two low doses (0.3 and 0.6 mg/kg) of β -CCM showed distance reductions, whereas rats treated with the high dose (1.0 mg/kg) of β -CCM per-

Figure 2. Effects of (A) flumazenil (FLU), (B) CGS 8216 (CGS), and (C) β -CCM on the distance taken to locate the submerged escape platform averaged across days. Note the dose-dependent reductions of total distance in each treatment group. * $p < .05$, and ** $p < .01$, relative to vehicle. Error bars represent SEM.

formed at control levels (Figures 1C and 2C). An overall ANOVA on the distances of groups treated with β -CCM revealed a significant group difference [$F(3,56) = 3.61$, $p < .01$] and a significant day difference [$F(4,224) = 22.9$, $p < .001$], but not a significant interaction between group and day [$F(12,224) = 1.08$, $p = .37$]. Post hoc tests revealed that only those rats treated with the low (0.3 mg/kg; $p < .01$) and intermediate (0.6 mg/kg; $p < .05$) doses of β -CCM had significantly shorter distances relative to controls. Latency data (not shown) followed the same pattern of deficits and statistical significances.

The pattern of facilitated acquisition revealed during training was largely confirmed during the drug-free probe trial (Figure 3). Rats treated with the drug vehicle failed

Figure 3. Effects of (A) flumazenil (FLU), (B) CGS 8216 (CGS), and (C) β -CCM on the distance traveled in the correct quadrant during the 60-sec probe trial. * $p < .05$, and ** $p < .01$, relative to chance levels (25%; dotted line).

to demonstrate a bias for the goal quadrant, suggesting that the spatial location of the platform had not been acquired in so few trials (5×2). In contrast, rats treated with any dose of FLU (Figure 3A) demonstrated a clear bias for the goal quadrant, with those given the two lowest doses ($p < .01$) showing the greatest biases and those given the highest dose showing only a moderate quadrant bias ($p < .05$). Rats treated with any dose of CGS demonstrated a quadrant bias in a similar inverted-U dose-response curve, with the low (10 mg/kg) and high (30 mg/kg) doses resulting in a modest quadrant bias ($p < .05$) and the intermediate dose (20 mg/kg) resulting in a more robust quadrant bias ($p < .01$; Figure 3B). Rats treated with β -CCM, despite showing enhanced acquisition at the two lower doses, displayed surprisingly weak quadrant biases; only those rats treated with the lowest dose (0.3 mg/kg) showed a significant quadrant bias ($p < .05$), though this bias was not significantly different from the bias displayed by controls (Figure 3C).

Swim speeds (data not shown) varied little over the course of training; the data was therefore collapsed across days for clarity. Rats treated with the vehicle swam at an overall average swim speed of 31 ± 0.8 cm/sec, and the means of all other groups but one were not significantly different, ranging from 29.5 ± 0.8 cm/sec to 33.5 ± 0.85 cm/sec. Only the lowest dose of FLU (10 mg/kg) produced a significant swim speed reduction (28 ± 0.7 cm/sec; $p < .05$).

In training trials, FLU antagonized both the impairment produced by diazepam and the enhancement produced by β -CCM, whereas β -CCM exacerbated the deficit produced by diazepam (Figure 4). Rats treated with diazepam alone (3 mg/kg) showed a small but significant increase in swim path lengths relative to vehicle controls ($p < .05$), an effect that was completely antagonized by the low dose of FLU (10 mg/kg; Figure 4A; $p < .05$, relative to rats treated with diazepam alone). FLU (10 mg/kg) also antagonized the enhancement produced by β -CCM (0.3 mg/kg; Figure 4B; $p < .01$, relative to either FLU or β -CCM alone). The low dose of β -CCM failed to antagonize, and actually appeared to exacerbate, the deficit produced by diazepam ($p < .01$, relative to controls; Figure 4A). Given the difficult training protocol, and the lack of a quadrant bias in vehicle-treated rats, it is not surprising that none of the groups given diazepam or the combination of FLU and β -CCM demonstrated a quadrant bias during the drug-free probe trial (Figure 4C). The combination of diazepam and β -CCM reduced the bias for the correct quadrant to a level significantly below that of vehicle-treated rats ($p < .05$).

Open-Field Behavior (Thigmotaxia)

The effects of each drug treatment on open-field behavior are summarized in Table 1. Several treatment groups showed a reduction in the total distance traveled in the open field. Most notably were the CGS groups in which all three doses of CGS significantly reduced the total distance traveled relative to controls ($p < .05$). Ad-

Figure 4. Effects of (A) diazepam (DZP; 3 mg/kg) on the distance taken to locate the submerged escape platform averaged across days either alone or in combination with FLU (10 mg/kg) and β -CCM (0.3 mg/kg). (B) Effects of FLU (10 mg/kg) and β -CCM (0.3 mg/kg) on the distance taken to locate the submerged escape platform averaged across days either alone or in combination. (C) Effects of drug combinations on the distance traveled in the correct quadrant during the 60-sec probe trial. Note that FLU, but not β -CCM, blocks the effects of diazepam in (A) and β -CCM in (B). * $p < .05$, and ** $p < .01$, relative to vehicle-treated controls.

ditionally, the moderate doses of FLU and β -CCM also reduced total distances relative to controls ($p < .05$).

Several treatment groups also showed a strong thigmotaxic response in the open field. Rats treated with the vehicle spent a large proportion (42%) of their total distance in the central region of the field. Rats treated with the

moderate dose of FLU (20 mg/kg), the low (10 mg/kg), and high (30 mg/kg) doses of CGS, and the intermediate (0.6 mg/kg) and high doses (1.0 mg/kg) of β -CCM all demonstrated a significant preference for the peripheral region (thigmotaxia; $p < .05$). The peripheral preference demonstrated by the moderate dose of β -CCM (0.6 mg/kg) was antagonized by FLU (10 mg/kg). Diazepam had little effect on regional preference relative to controls. Interestingly, the coadministration of a nonanxiogenic dose of FLU (10 mg/kg) and diazepam resulted in a significant preference for the peripheral region ($p < .05$). The anxiogenic effects of β -CCM (0.6 mg/kg) were unaltered when diazepam was given concomitantly.

The enhanced spatial learning produced by each drug treatment showed a poor relationship with their prothigmotaxic effects (Figure 5). In Figure 5, it can be seen that with each drug and dose, anxiogenesis (percentage distance spent in the periphery; thigmotaxia) and mnemonic enhancement (percentage time spent in the target quadrant during the probe trial) show little directional congruency. For example, and most notable, the dose-response curve of CGS for thigmotaxia is U-shaped, whereas the curve for spatial learning is an inverted-U shape. A similar lack of congruence between thigmotaxia and spatial learning can be seen with each drug and dose and is particularly evident in the drug combinations, which had exactly opposite effects on spatial learning and thigmotaxia.

DISCUSSION

The present investigation demonstrates that the BZ receptor antagonists FLU and CGS and the inverse-agonist β -CCM enhance spatial learning in the Morris water maze. Each of these compounds, in a dose-dependent manner, reduced the distance taken to locate the submerged escape platform and increased the bias for the correct quadrant during the probe trial. Conversely, diazepam impaired spatial learning, increasing the distance taken to reach the escape platform. The moderate dose of FLU antagonized the enhancement produced by the low dose of β -CCM and the impairment produced by diazepam. However, the low dose of β -CCM failed to block the impairment produced by diazepam. None of the drug treatments, either alone or in combination, altered swim speed except the low dose of FLU, suggesting that alterations in motivation or sensorimotor ability were not responsible for the observed changes in spatial learning. Together, these findings confirm earlier reports that diazepam impairs spatial learning in the Morris water maze in a FLU-reversible manner (McNamara & Skelton, 1991, 1992B) and that FLU can enhance spatial memory (Brioni et al., 1991). The present findings extend earlier reports by showing that FLU, CGS, and β -CCM also enhance spatial learning.

The present study also confirms previous reports that FLU (Lee & Rodgers, 1991), CGS (File & Lister, 1983), and β -CCM (Corda et al., 1983) have anxiogenic properties. In our open-field test given in a familiar environ-

Table 1
Effects of Drugs on Total Distance and Thigmotaxia in the Open Field

Drug Treatment	Total Distance Traveled (cm)		Distance in Periphery (%)	
	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>
Vehicle	8336	172	58	2.7
FLU 10	8021	381	59	5.9
FLU 20	7561	167†	70	2.6*
FLU 30	8131	478	66	7.4
CGS 10	7531	133†	71	6.7†
CGS 20	7131	565*	57	8.2
CGS 30	7169	140†	71	4.0†
β -CCM 0.3	8295	327	62	4.9
β -CCM 0.6	7248	372*	67	5.1*
β -CCM 1.0	8069	404	66	4.0*
β -CCM 0.6+FLU 10	8098	277	63	5.7
DZP	8086	548	61	4.6
DZP+FLU 10	8211	416	68	2.3*
DZP+ β -CCM 0.6	8277	267	71	2.6†

* $p < .05$ compared with vehicle-treated controls. † $p < .01$ compared with vehicle-treated controls.

ment, vehicle-treated controls spent close to half of their total distance in the central region of the field. This contrasts sharply with the thigmotaxic tendencies of naive rats who spent the majority of their total distance in the peripheral region ($> 85\%$; thigmotaxia; see Figure 2 in McNamara & Skelton, 1992B) and suggests that control rats were less anxious in this familiar environment. FLU, CGS, and β -CCM all dose-dependently increased the time spent in the peripheral region and decreased the total distance traveled, indicating that all three of these compounds increased anxiety. This increase in anxiety cannot be attributed to a drug-induced impairment of habituation to

the spatial environment since rats treated with FLU, CGS, and β -CCM showed better spatial learning than did controls.

Several observations in the present study dissociate the mnemonic-enhancing effects of FLU, CGS, and β -CCM from their anxiogenic properties. First, the low dose of FLU enhanced learning but had no effect on anxiety. Second, the moderate dose of CGS produced the greatest enhancement of learning, but the least anxiogenesis. Third, the high dose of β -CCM did not enhance learning but did increase anxiety. Finally, β -CCM in combination with either diazepam or FLU impaired learning while increasing anxiety. Thus, it appears unlikely that the processes mediating the anxiogenic effects of FLU, CGS, and β -CCM in the open field are responsible for their enhancement of spatial learning, a notion supported by other findings. For example, Ro5-4864, a ligand at the peripheral BZ receptor, failed to enhance avoidance conditioning (Holmes & Drugan, 1991) at doses that are proconflict (anxiogenic) on a Vogel conflict test (Mizoule et al., 1985). Taken collectively, these results strongly argue that the memory-enhancing actions of FLU, CGS, and β -CCM are not due to their anxiogenic actions. However, it does remain possible that those drug doses that did not affect thigmotaxia did increase anxiety to enhance spatial learning. Further investigation employing a range of different behavioral tasks (learning and anxiety) would help to determine the generality of these findings.

In addition to their anxiogenic actions, CGS (File, 1983) and β -CCM (e.g., Cowan et al., 1981) also have proconvulsant effects at higher doses. It is therefore possible that the enhancement of neuronal excitability that subserves seizure susceptibility also has a beneficial effect on mnemonic processes. However, this account seems unlikely since limbic kindling, a proconvulsant consequence of repetitive brain stimulation (Goddard, 1967), does not affect spatial learning in the Morris water maze, whereas

Figure 5. A composite illustration comparing the percentage time spent in the target quadrant during the probe trial (learning [probe]) and percentage distance spent in the peripheral region of the open field (anxiety [periphery %]) for each drug and dose (numbers). Values are expressed as percentage of control levels. Note the lack of congruence between the learning and anxiety measures.

limbic seizures impair, not enhance, it (McNamara, Kirkby, de Pape, & Corcoran, 1992). Moreover, the doses of FLU used in the present study have been reported to have a paradoxical anticonvulsant action (e.g., Robertson & Riives, 1983). Thus, although the enhanced neuronal excitability of the type subserving long-term seizure susceptibility does not appear to be responsible for the enhanced learning observed in the present study, these drugs may enhance spatial learning by enhancing a more subtle form of synaptic enhancement, such as long-term potentiation (e.g., Sorenson, Zwolshen, & Humphreys, 1990).

The findings that diazepam impairs spatial learning in a FLU-reversible manner and that FLU, CGS, and β -CCM enhance spatial learning suggest that endogenous BZ agonists (so-called *endopeptides*) activated during water maze training prevent optimal learning. Several endogenous substances with a high affinity for the BZ receptor have been identified in mammalian tissues, including inverse-agonists (Guidotti et al., 1983; Pena, Medina, Novas, Paladini, & De Robertis, 1986) and agonists, such as diazepam (Wildmann et al., 1987) and endozepine-2 (Rothstein, Garland, Puia, Guidotti, & Costa, 1991). It is unlikely that an endogenous inverse-agonist-like substance is activated during training since the dose of FLU that enhanced spatial learning when administered alone blocked the enhancement effect of β -CCM. Rather, the present results suggest that during the stress and anxiety of maze training, an endogenous BZ agonist, such as endozepine-2, is released, resulting in suboptimal learning. Surprising, then, is the finding that the amnesic effects of diazepam were not blocked by β -CCM. One possible account for this finding is that β -CCM binds selectively to the ω_1 receptor subtype, whereas diazepam and FLU bind with similar affinity to both the ω_1 and ω_2 receptor subtypes (Braestrup & Nielson 1981). Hence, diazepam, while in the presence of β -CCM, continued to exert its amnesic effects via the ω_2 receptor subtype.

Although it is assumed that FLU, CGS, and β -CCM enhanced mnemonic processes via an exclusive interaction with GABAergic systems, this facilitation might have been due to a secondary interaction with cholinergic systems. For example, cholinergic hypofunction is detrimental to spatial learning in the water maze (Sutherland, Whishaw, & Regehr, 1982), and the amnesic effects of scopolamine can be attenuated by both FLU (McNamara & Skelton, 1992a) and the inverse-agonist ZK 93 426 (Jensen, Stephens, Sarter, & Petersen, 1987). Indeed, biochemical studies have revealed that FLU and β -CCM enhance hippocampal high-affinity choline uptake when infused into the medial septum (Stackman, Emerich, Taylor, & Walsh, 1989). Hence, these findings suggest that the mnemonic-enhancing effects of FLU, CGS, and β -CCM may be due to an enhancement of cholinergic systems and might therefore be useful for treating cognitive disorders related to cholinergic hypofunction (see Sarter, Bruno, & Dudchenko, 1990, for a critique of this application).

In sum, the present experiment found that the pretraining administration of BZ receptor antagonists FLU or CGS and the BZ receptor inverse-agonist β -CCM dose-

dependently enhanced, while diazepam impaired, spatial learning in the Morris water maze. The low dose of FLU blocked both the enhancing effect of β -CCM and the impairing effect of diazepam. In the same rats, FLU, CGS, and β -CCM increased exploratory anxiety in a dose-dependent manner. However, the dose-response relationships between the observed mnemonic facilitation and the anxiogenesis were highly dissimilar. Taken together, these results support the view that BZ receptor activation, either endogenously or exogenously, is detrimental to optimal spatial learning and that blocking such activity can benefit mnemonic processes via a mechanism that is independent of anxiety.

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