The Effect of Agonists at the GABA-Benzodiazepine-Receptor-Complex on the Proconflict Effect Induced by β-CCM and Pentetrazol in Rats

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Abstract—The effect of anxiolytics, benzodiazepine (BZP), diazepam (DZP), non-BZP zopiclone (ZOP) and phenobarbital (PBT), on the proconflict effect induced by methyl-β-carboline-3-carboxylate (β-CCM) or by pentetrazol (PTZ) was investigated. The proconflict effect of β-CCM and PTZ was reduced by these anxiolytics and aminooxyacetic acid (AOAA). In addition, isoniazid produced proconflict activity. Therefore, it is suggested that anxiolytics facilitate the GABA-ergic function, causing the inhibition of the proconflict effect. Although both propyl-β-carboline-3-carboxylate (β-CCP) and Rol5-1788 did not produce proconflict activity, they reduced the proconflict effect induced by β-CCM but not by PTZ. These data clearly show that β-CCM exerts the proconflict effect through interaction with BZP receptor and that there are behavioral similarities between β-CCP and Rol5-1788. In this study, we additionally observed the time latency until the rat began to drink the water. β-CCM and PTZ prolonged this latency in a dose-dependent manner. However, AOAA could not reduce the prolonged latency induced by β-CCM and by PTZ, and anxiolytics and β-CCP could not reduce the prolonged latency induced by β-CCM. The mechanism of the prolongation of latency induced by β-CCM and PTZ seems to be different from that of the proconflict effect.

Classical benzodiazepines (BZPs) are known to exert an anticonflict activity in the water lick method (1–3). It is considered that the pharmacological action exerted by BZPs is facilitated by GABAergic transmission on the binding site which form part of a macromolecular receptor complex with binding sites for GABA/benzodiazepine/barbiturate and a chloride ionophore (GBC-complex) (4). Non-BZP hypnotic zopiclone has been reported to possess a high affinity for BZP receptors (5) and to have a pharmacological profile similar to BZPs (6), which includes an anticonflict activity (7, 8). In addition, the barbiturate phenobarbital, which can not bind to BZP receptor directly, is well-known to increase GABAergic transmission by interacting with the GBC-complex (9, 10) and has an anticonflict activity. Recently, β-carboline compounds have been proposed as candidates for the endogenous ligands of the BZP receptor (11, 12), and propyl-β-carboline-3-carboxylate (β-CCP) has been reported to possess an anticonvulsant property similar to BZPs (13, 14).

On the other hand, methyl-β-carboline-3-carboxylate (β-CCM) has been reported to show a proconflict effect (3, 15, 16) which is in direct opposition to the anticonflict properties of BZPs. In addition, pentetrazol (PTZ), which is chemically unrelated to the β-CCM, also has been reported to have a proconflict effect (3, 16). It has been shown...
that the proconflict effect of a β-carboline (FG7142, amide type of β-CCM) is potentiated by isoniazid, an inhibitor of GABAergic function in the brain (17). β-CCM decreases the affinity for GABA binding (18). Moreover, the mechanism of action of PTZ probably consists of an inhibitory action of GABAergic function which is mediated through the GABA-ergic complex (19-21). Petersen and Jense (16) reported that the mode of the inhibitory activity on GABAergic function determined the degree of proconflict action.

All these findings indicate that the GABAergic function plays an important role in the anticonflict of anxiolytics and in the proconflict effects of β-CCM and PTZ. The present study was undertaken to examine the effect of β-CCM and anxiolytics, DZP, ZOP and PBT, on the proconflict effects of β-CCM and PTZ.

Materials and Methods

Animals: Male Wistar rats (Seiwa Experimental Animals, Ltd.), weighing about 200 g, were used. They were brought into the laboratory at least one week preceding each experiment and were housed in groups of 8 with free access to food and water in humidity and temperature controlled rooms (23±3°C and 60±5%, respectively).

Drugs: The following drugs were used: methyl-β-carboline-3-carboxylate (β-CCM, Asahi Chemical Ind. Co., Ltd.), propyl-β-carboline-3-carboxylate (β-CCP, Asahi Chemical Ind. Co., Ltd.), pentetrazol (Tokyo Kasei Kogyo Co., Ltd.), diazepam (Cercine®, Takeda Pharmaceutical Co.), zopiclone (Rhône-Poulenc Co.), phenobarbital (Tokyo Kasei Kogyo Co., Ltd.), Ro15-1788 (Roche Laboratories), aminoxyacetic acid (AOAA, Sigma) and isonicotinic acid hydrazide (isoniazid, Wako Pure Chemical Ind., Ltd.).

β-CCP and Ro15-1788 were suspended in 1% methylcellulose and 0.1% Tween 80, respectively. Zopiclone was suspended in 0.5% carboxymethylcellulose. β-CCM was dissolved in 0.9% NaCl with 1 drop of 1 N HCl/5 ml. Pentetrazol, phenobarbital, AOAA and isoniazid were dissolved in 0.9% NaCl. β-CCP and β-CCM were administered i.v., and the other drug was administered i.p. in the constant volume of 1 ml/kg.

Conflict test procedure: A modification of the thirsty rat conflict test (2) was used in this study. Rats were deprived of water for 24 hr prior to the test. The rats were placed in a test box enclosed in a sound-proof cage and allowed to drink water from the nipple for thirty sec after finding it. During this trial, the shock (1 mA, 2 sec) was delivered through the nipple every time the rat drank one drop (about 0.03 ml) of water from it. We selected the rats which drank water drops from 4 to 8 times during this trial; and after 5 hr of this selection, we used them in the conflict test. The selected rats were removed from the chamber, injected with a drug, and returned to the home cage during the drug treatment. After the drug administration, the rats were again placed in a test box. The lag time (latency) till the rat began to drink the water and the number of the shocks received during the test period were monitored. The 5 min test period started at the time when the rats began to drink the water. In this test period, the rats were delivered a shock (1.5 mA, 2 sec) for every drop they drank.

Results

About 80% of the rats passed the selection procedure. The number of water drops drunk by rats administered the vehicle (0.9% NaCl) was 43.8±1.2 (average±S.E.)/5 min. The number of shocks in the rats administered β-CCM (0.1-3 mg/kg) or PTZ (5-40 mg/kg) decreased-dose dependently; i.e., showing a proconflict effect (Fig. 1). We could not perform the study at a higher dose level than 3 mg/kg or 40 mg/kg for these two agents because at these levels, the rat did not move more than 15 min in the test box probably due to some toxic effect. Therefore, in the following study, we employed β-CCM (3 mg/kg) and PTZ (40 mg/kg) as optimum doses to evaluate the effect of anxiolytics.

Table 1 shows the effects of drugs on β-CCM (3 mg/kg) and PTZ (40 mg/kg)-induced proconflict effect. DZP, ZOP, PBT, β-CCP and Ro15-1788 dose-dependently reduced the proconflict effect of β-CCM.

The ED50 values were estimated to be 1.5 mg/kg, 13.0 mg/kg, 19.4 mg/kg, 21.5 mg/kg...
Fig. 1. Proconflict effects of β-CCM (A) and PTZ (B). β-CCM was administered intravenously, and the test was started 10 min after this. PTZ was administered intraperitoneally, and the test was started 15 min after this. Each value represents the mean±S.E. of 8 results. Abbreviations in the figure: β-CCM (methyl-β-carboline-3-carboxylate) and PTZ (pentetrazol). Asterisks denote a significant difference from the value of the saline-treated control. *P<0.05, **P<0.01 (by Student's t-test)
Table 1. Antagonistic effects of anxiolytics on the proconflict effect induced by β-CCM and by PTZ

| Drugs | ED50 (95% C.L. mg/kg) of recovery effect | β-CCM (3 mg/kg) | PTZ (40 mg/kg) |
|-------|----------------------------------------|-----------------|----------------|
| Diazepam | 1.5 (1.0–2.3) | 0.7 (0.5–0.9) |
| Zopiclone | 13.0 (11.1–15.3) | 7.6 (6.7–8.5) |
| Phenobarbital | 19.4 (17.2–21.8) | 16.3 (15.1–17.7) |
| β-CCP | 21.5 (15.9–23.6) | No effect |
| Ro15-1788 | 18.8 (16.7–21.1) | No effect |

Diazepam, zopiclone and phenobarbital were administered intraperitoneally 30 min before the test. β-CCM was administered intravenously 10 min before the test. The ED50 values and 95% confidence limits (C.L.) were calculated from the positive rate by the method of Litchfield-Wilcoxon. Abbreviations in the table: β-CCM (methyl-β-carboline-3-carboxylate) and PTZ (pentetrazol). No effect: No effect at doses up to 40 mg/kg of each drug.

and 18.8 mg/kg for DZP, ZOP, PBT, β-CCP and Ro15-1788, respectively.

In the same manner, DZP, ZOP and PBT suppressed the proconflict effect of PTZ; and the ED50 values were 0.7 mg/kg, 7.6 mg/kg and 16.3 mg/kg, respectively. However, β-CCP and Ro15-1788 at 10–40 mg/kg did not modify the proconflict effect of PTZ (Table 1).

AOAA at 15 mg/kg reduced the proconflict effect of both β-CCM and PTZ. Isoniazid, a GABA synthesis inhibitor, exerted a proconflict effect similar to β-CCM and PTZ (Fig. 2).

The drinking latency in saline administered rats was 94.2±7.3 sec (average±S.E.). β-CCM and PTZ prolonged dose-dependently the latency (Fig. 1). The prolonged latency induced by β-CCM was antagonized by Ro15-1788 (ED50=2.7 mg/kg), but DZP, ZOP, PBT and β-CCP failed to modify it. On the other hand, the prolonged latency induced by PTZ was antagonized by DZP (ED50=0.8 mg/kg), DOP (ED50=8.0 mg/kg) and PBT (ED50=9.5 mg/kg), but Ro15-1788 and β-CCP could not change the effect. AOAA could not antagonize the prolonged latency by both β-CCM and PTZ, and isoniazid did not induce such a prolongation of latency.

**Discussion**

In this study, β-CCM and PTZ decreased the water drinking which was punished by electric shock. As β-CCM and PTZ did not alter any pain thresholds or the water drinking behavior in a homecage in our study (data is not shown), it is suggested that the decrease in the number of shocks may result from a proconflict effect of these agents. In addition, DZP, ZOP, β-CCP and Ro15-1788 also did not alter any pain thresholds or the water drinking behavior at the dose used here. Ro15-1788 blocked the proconflict effect of β-CCM, but not that of PTZ. These results were in agreement with the results by Corda et al. (3). β-CCM seems to exert the proconflict effect on the Ro15-1788 sensitive site of the BZP receptor. It is well-known that the convulsion induced by β-CCM can be blocked by Ro15-1788 (14). Grecksch et al. (22) have reported that Ro15-1788 at doses from 1.0 to 10 mg/kg, s.c., suppressed the convulsion induced by submaximal doses of PTZ (55 and 65 mg/kg, s.c.). However, they described that with a dose of 100 mg/kg PTZ, Ro15-1788 was devoid of any anti-convulsive activity. The dose of PTZ employed here was 40 mg/kg, but Ro15-1788 could not reverse the proconflict effect of PTZ. In the action of PTZ, the mechanism of the convulsive effect may be different from the proconflict effect. The mechanism of action of PTZ on the convulsive effect and the proconflict effect remains unknown, but probably consists of the inhibitory action against the GABA function (18, 23). Considering this, it has been suggested that β-CCM and PTZ exert their proconflict effect in the end through the suppression of GABAergic function. Indeed, the proconflict effect induced...
Fig. 2. The effect of AOAA on the proconflict effect induced by \( \beta \)-CCM and by PTZ (A) and proconflict effect of isoniazid (B). AOAA was administered intraperitoneally 40 min before the test. Isoniazid was administered intraperitoneally 60 min before the test. Each value represents the mean±S.E. of 8 results. Abbreviations in the figure: AOAA (aminooxyacetic acid), \( \beta \)-CCM (methyl-\( \beta \)-caroline-3-carboxylate), and PTZ (pentetrazol). Asterisks denote a significant difference from the value when \( \beta \)-CCM or PTZ was administered singly (A) and from the value of the control (B). \(*P<0.05, **P<0.01\) (by Student's \( t \)-test).
by $\beta$-CCM and PTZ was reversed by AOAA in this study, and isoniazid, a GABA synthesis inhibitor, exerted a proconflict effect in a dose-dependent manner similar to $\beta$-CCM and PTZ.

DZP reduced the proconflict effects, of $\beta$-CCM and PTZ dose-dependently. DZP is well-known to exert its pharmacological effect by a facilitation on the GABAergic function through BZP receptors. In this study, ZOP and PBT, which possesses anxiolytic activity, also reduced the proconflict effect of $\beta$-CCM and PTZ. It is known that non-BZP ZOP binds to BZP receptors and interacts with the GBC-complex (24, 25), and it exerts a similar pharmacological effect to BZPs (6, 26). Although PBT can not bind to BZP receptor directly, it has been known to have an ability to increase GABAergic transmission (9) and to interact with the GBC-complex (10). It is considered that ZOP and PBT can facilitate the GABAergic function on the GBC-complex in a manner similar to DZP.

In recent years, ligands to central BZP receptors have been classified into three overlapping groups: agonist, antagonist and inverse agonist (27, 28). $\beta$-CCM has been classified as an inverse agonist, but $\beta$-CCP has not been classified clearly. $\beta$-CCP, which is known to have a high affinity to the BZP receptor (29), did not show a proconflict effect different from that of $\beta$-CCM. On the contrary, $\beta$-CCP reduces the proconflict effect of $\beta$-CCM similar to Ro15-1788. File et al. (30) have reported that $\beta$-CCP showed an anxiogenic action in the social interaction test at small doses of 2 to 4 mg/kg. However, in this study $\beta$-CCP did not show any anxiogenic action with doses up to 20 mg/kg. However, at higher doses, e.g., 40 mg/kg, it showed a tendency of proconflict action, decrease in shocked drinking, but it was not significant. On the contrary, $\beta$-CCP showed a significant antagonistic effect on proconflict action of $\beta$-CCM and PTZ. These dissimilarities appear to be attributable mainly to the difference in the experimental systems. The presence of GABA increases the affinity of $\beta$-CCP to the BZP receptor (31, 32), and $\beta$-CCP enhances the GABA affinity (18). These findings lead us to predict that $\beta$-CCP would be an anxiolytic agent. However, in this study, $\beta$-CCP could not antagonize the proconflict effect of PTZ, unlike the reverse effect of anxiolytics used here. Braestrup et al. (28) reported that $\beta$-CCP can be classified into the same group as Ro15-1788 (i.e., antagonist) according to the GABA ratio. Our present data also suggest behaviorally that there are similarities between $\beta$-CCP and Ro15-1788.

In this study, we observed effects of drugs on the latency until the rat began to drink the water in the test box. $\beta$-CCM and PTZ prolonged the time latency in a dose-dependent manner. Therefore, it is interesting to know whether this prolonged latency is related to anxiogenic action. Both the prolonged latency and proconflict effect by PTZ were antagonized by anxiolytics dose-dependently. However, the present experiment demonstrated that the effects of test drugs on $\beta$-CCM- and PTZ-induced drinking latency were not parallel with those on the proconflict. Therefore, the prolonged latency induced by $\beta$-CCM and that by PTZ may be exerted by a mechanism different from that for the proconflict effect. In addition, although Ro15-1788 antagonized the drinking latency induced by $\beta$-CCM, $\beta$-CCP did not antagonize it. This is the only behavioral dissimilarity between $\beta$-CCP and Ro15-1788 observed in this study, but more detailed study will be needed to interpret this point.

In conclusion, $\beta$-CCM and PTZ appears to exert their proconflict effects through the depression of the GABAergic function by interacting with the GBC-complex. Anxiolytics used here reduce the proconflict effect induced by these two agents through a facilitation of GABAergic function. In this study, $\beta$-CCP seems to be an a'antagonist' like Ro15-1788, but unlike $\beta$-CCM. The mechanism for the prolonged latency induced by $\beta$-CCM and PTZ is different from that of the proconflict effect.

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