A Key Role of the Mammillary Body in Mediation of the Antianxiety Action of Zopiclone, a Cyclopyrrolone Derivative

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Accepted August 28, 1989

Abstract—The present study was designed to elucidate the brain site of the anti-conflict action of zopiclone (ZOP), a cyclopyrrolone derivative, using the rat conflict procedure. ZOP at 10 μg/μl, bilaterally injected into the mammillary body (MB), produced a significant increase in the punished responses, with no change in the unpunished responses. There were no significant changes in these responses when ZOP was injected into the central amygdala, frontal cortex or dorsal hippocampus. Attention should be given to the possibility that the MB is the site of the anti-conflict action of ZOP.

Our proposed neuroanatomical substrates regulating behavioral suppression include the following three major circuits: the mammillary body (MB)/Papez and the amygdaloid and septohippocampal systems (1). The MB and the central amygdala (ACE) are potential sites of the antianxiety action of benzodiazepines (BDZ) (2, 3). These regions comprise potentially large circuits which include the ventromedial hypothalamus, thalamus, frontal cortex (FC) and hippocampus (HIP). Lippa et al. suggested the possible involvement of the FC in mediation of the anxiolytic action of BDZ, as deduced from electrophysiological experiments (4). The septohippocampal system is regarded as a fundamental comparator or transducer component in the regulation of fear responses (5). We found that direct application of diazepam into the dorsal HIP (d-HIP) elicited a potent anticonflict action, determined using a Vogel type conflict test (6). Based on our observations (7, 8), BDZ seem to disinhibit behavioral suppression by modulating activities in these brain areas.

Zopiclone (ZOP), a cyclopyrrolone derivative, has a pharmacological property qualitatively similar to that of BDZ. However, ZOP is characterized by potent anticonflict and antiaggressive effects and has much weaker anticonvulsant, muscle relaxant, ataxiogenic and anesthesia-potentiating effects, as compared with BDZ (9). We addressed the question of whether localization of the site of the anticonflict action of a cyclopyrrolone derivative differed from that of BDZ. The approach was to inject ZOP directly into various brain regions associated with behavioral suppression such as ACE, MB, FC and d-HIP and observe their effects in a Geller type conflict test, which is known to correlate well with the clinical properties of anxiolytics.

Male Wistar rats (Kyushu University Institute of Laboratory Animals) aged 8 weeks and weighing about 200 g at the commencement of the experiment were maintained on a schedule of ad libitum feeding and drinking 15 ml of water a day; the rats were trained on a multiple continuous reinforcement (CRF)-CRF conflict schedule. In the unpunished period (10 min), lever pressings were rewarded with 0.02 ml of milk on CRF. In the punished period (5 min, signalled by a 1850 Hz), every response was...
rewarded with milk and also punished with a 0.1-sec electrical shock to the paws. The intensity of the shock was increased gradually, on an individual basis, until the response in the punished period was almost suppressed. Rats showing an established performance for at least 3 consecutive sessions were subjected to surgery for microinjection of drugs. Guide cannulae (0.7 mm diameter) were implanted bilaterally at a point 1 mm above ACE, MB, FC and d-HIP in rats anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The coordinates, anterior (A) from the bregma, lateral (L) to the middle and horizontal (H) below the dura selected with the aid of König and Klippel (10), were the following: ACE (A, -1.8; L, ±4.3; H, 6.6), MB (A, -4.4; L, ±2.3; H, 7.8 at an 80° angle on the frontal surface), FC (A, 3.4; L, ±1.5; H, 1.7) and d-HIP (A, -2.5~2.6; L, ±1.5; H, 2.9). Ten days after cannula implantation, the drug experiment was commenced. An injection cannula (0.35 mm in diameter) extending 1.0 mm below the tip of the guide cannula was used for microinjection of the drugs. After a pretest, 1 and 2 μl of drug was bilaterally injected into ACE or MB and into FC or d-HIP, respectively. The injection rate was 1 μl/2 min with the cannula left in place for one additional minute. Rats were subjected to the test immediately after the microinjection. The anticonflict action of the drug was rated as positive if rats pressed the lever more than 20 times during the punished period of the test session. This criterion was based on the results in which no rats showed more than 15 responses during the punished period when saline was injected into various brain regions. Localization of the cannula tips was verified histologically at the termination of the experiments. The rats in which both cannula tips were located out of ACE, MB, FC or d-HIP were excluded from the experimental data. Zopiclone was dissolved in 0.1 N HCl, and the pH was adjusted to 4.95 with 0.1 N NaOH. The 10 μg/μl of ZOP used here was the maximal concentration soluble under this condition. Saline instead of vehicle (pH 4.95) was injected into ACE, MB, FC or d-HIP of each rat as their own control, since this vehicle or saline injected into various brain areas did not influence conflict behavior (3).

Figure 1 shows changes in lever pressings during the punished and unpunished period after the microinjection of ZOP into ACE, MB, FC and d-HIP. ZOP at 10 μg bilaterally injected into the MB significantly increased the punished responses (P<0.01) without changing the unpunished responses and produced anticonflict action in 11 out of 17 rats. Bilateral microinjection of ZOP at 10 μg into ACE failed to significantly increase the punished responses, though positive anticonflict actions were observed in 3 of 15 rats. When ZOP at 20 μg was injected bilaterally into FC or d-HIP, the animals showed no change in the punished and unpunished responses. The anticonflict effect of ZOP injected into MB was completely antagonized by the oral administration of Ro 15-1788 at 20 mg/kg 15 min before the microinjection of ZOP (53.0±9.6 vs. 0.5±0.3 of 4 rats in the 5 min-punished period). This dose of Ro 15-1788 did not significantly decrease the unpunished responses. The positive and negative sites for the anticonflict effect of ZOP in various brain regions are shown in Fig. 2. There were no regional differences between the positive and negative sites for the anticonflict effects of ZOP injected into MB.

In the present study, ZOP elicited a potent anticonflict action in MB among the sites of this action of BDZ such as MB, ACE and d-HIP (2, 3, 6). These results suggest that MB in the pathway composed of MB→anterior thalamus→frontal cortex may have a key role in the neuroanatomical mechanisms of the antianxiety action of the cyclopyrrolone ZOP. The anticonflict effect of ZOP injected into MB was antagonized by Ro 15-1788, a BDZ antagonist, thereby suggesting that BDZ recognition sites in MB are required for the anticonflict action of ZOP. The BDZ receptor is known to have at least two distinct subtypes. One distinguishing feature of these two sites is that BDZ 1 (BDZ receptor subtype 1) has a high affinity for the triazolopyridazines and β-carbolines, whereas BDZ 2 (BDZ receptor subtype 2) has a low affinity for these drugs (11, 12). Taking brain areas injected with ZOP in the present study into consideration, FC, ACE and d-HIP appear to
Fig. 1. Effect of zopiclone injected into ACE, MB, FC and d-HIP on punished and unpunished responses in rats. Punished responses indicate the total lever pressings of the punishment period during a 5-min session (punishment). Unpunished responses are expressed as a percentage of the predrug response rate (control) (unpunishment). Dots in the bar graph of the punishment indicate the number of responses in each rat. The number of rats rated as positive anticonflict effect/the number of rats used are shown at the right-hand of each bar column in the punishment. ACE, MB, FC and d-HIP: central amygdala, mammillary body, frontal cortex and dorsal hippocampus, respectively. **P<0.01 : significantly different from saline treatment (one and two-tailed paired t-test for punished and unpunished responses, respectively).

| UNPUNISHMENT (% of control) | PUNISHMENT (mean responses/5min) |
|-----------------------------|----------------------------------|
| 150 100 50 0 0 20 30 40 50 | 0 10 20 30 40 50 60 70 80 90 100 |

| ACE | SALINE | 0/15 |
| ZOP10 | 3/15 |

| MB | SALINE | 0/17 |
| ZOP10 | 11/17 |

| FC | SALINE | 0/7 |
| ZOP20 | 0/7 |

| d-HIP | SALINE | 0/5 |
| ZOP20 | 0/5 |

be the mixed BDZ 1+2, the preferential BDZ 2 and the mixed BDZ 1+2 sites, respectively, as determined by the displacement of 1 nM [³H]flunitrazepam binding from each brain site by 200 nM CL 218,872 or 1 nM methyl β-carboline-3-carboxylate (β-CCM) using the quantitative receptor autoradiographic technique (6, 11, 13). Our recent study showed that [³H]flunitrazepam binding sites in the MB were less sensitive to the displacement by CL 218,872, but were most sensitive to that by β-CCM (6). This would suggest that the atypical BDZ 1 or a subtype other than BDZ 1 and 2 may be enriched in the MB. The functional activity of MB, in the mediation of behavioral suppression, does not seem to be regulated by the γ-amino-butyric acid (GABA) system, as deduced from the following results (14): The injection of GABA and muscimol into ACE produced a dose-dependent increase in the punished responses, but that into MB did not. On the other hand, ZOP has been reported to have about a 2.5-fold higher affinity for BDZ 1
than BDZ 2 (15). It is, therefore, likely that the atypical BDZ 1 with a high and low affinity for β-CCM and CL 218,872, respectively, may mediate the anticonflict action of ZOP in MB, in a GABA-independent manner.

Acknowledgments: This work was supported by research grants from The Uehara Memorial Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We thank S. Hisazumi for technical assistance, T. Usui for secretarial assistance and Rhône-Poulenc (Tokyo, Japan) for kindley supplying us with zopiclone.

References
1 Yamashita, K., Kataoka, Y., Shibata, K., Ozaki, T., Miyazaki, A., Kagoshima, M. and Ueki, S.: Neuroanatomical substrates regulating rat conflict behavior evidenced by brain lesioning. Neurosci. Lett. (in press)
2 Kataoka, Y., Shibata, K., Gomita, Y. and Ueki, S.: The mammillary body is a potential site of anti-anxiety action of benzodiazepines. Brain Res. 241, 374–377 (1982)
3 Shibata, K., Kataoka, Y., Gomita, Y. and Ueki, S.: Localization of the site of the anticonflict action of benzodiazepines in the amygdaloid nucleus of rats. Brain Res. 234, 442–446 (1982)
4 Lippa, A.S., Critchett, D., Sano, M.C., Kiepner, C.A., Greenblatt, E.N., Coupet, J. and Beer, B.: Benzodiazepine receptors: Cellular and behavioral characteristics. Pharmacol. Biochem. Behav. 10, 831–843 (1979)
5 Gray, J.A.: Précis of the neuropsychology of anxiety: An enquiry into the functions of the
septo-hippocampal system. Behavioral and Brain Sciences 5, 469–534 (1982)

Kataoka, Y. and Ueki, S.: A neurochemical basis for conflict behavior in rats with special reference to benzodiazepine (BDZ) receptor subtypes. Japan. J. Pharmacol. 46, Supp. 17P (1988)

Shibata, K., Kataoka, Y., Yamashita, K. and Ueki, S.: An important role of the central amygdaloid nucleus and mammillary body in the mediation of conflict behavior in rats. Brain Res. 372, 159–162 (1986)

Sakurai-Yamashita, Y., Kataoka, Y., Yamashita, K., Miyazaki, A., Ushio, M., Mine, K., Niwa, M. and Ueki, S.: Conflict behavior and monoamine dynamics of various brain nuclei in rats. Neuropharmacology (in press)

Ueki, S., Watanabe, S., Yamamoto, T., Kataoka, Y., Shibata, S., Shibata, K., Ohta, H., Shimazoe, T. and Kawamoto, H.: Behavioral and electroencephalographic effects of zopiclone, a cyclopyrrolone derivative. Japan. J. Pharmacol. 43, 309–326 (1987)

Konig, J.F.R. and Klippel, R.A.: The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore (1963)

Young, W.S., III, Niehoff, D., Kuher, M.J., Beer, B. and Lippa, A.S.: Multiple benzodiazepine receptor localization by light microscopic radiohistochemistry. J. Pharmacol. Exp. Ther. 216, 425–430 (1987)

Nielsen, M. and Braestrup, C.: Ethyl-carboline-3-carboxylate shows differential benzodiazepine receptor interaction. Nature 286, 606–607 (1980)

Niehoff, D.L. and Kuher, K.J.: Benzodiazepine receptors: Localization in rat amygdala. J. Neurosci. 3, 2091–2097 (1983)

Kataoka, Y., Shibata, K., Yamashita, K. and Ueki, S.: Differential mechanisms involved in the anticonflict action of benzodiazepine injected into the central amygdala and mammillary body. Brain Res. 416, 243–247 (1987)

Niddam, R., Dubois, A., Scatton, B., Arbilla, S. and Langer, Z.: Autoradiographic localization of [3H]zolpidem binding sites in the rat CNS: Comparison with the distribution of [3H]-flunitrazepam binding sites. J. Neurochem. 49, 890–899 (1987)