Reversal of Antinociceptive Effect of Cholecystokinin by Benzodiazepines and a Benzodiazepine Antagonist, Ro 15-1788

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Abstract—Intraperitoneally administered benzodiazepines, chlordiazepoxide (2–5 mg/kg), diazepam (1 mg/kg), flurazepam (1 mg/kg) and a benzodiazepine antagonist, Ro 15–1788 (0.5 mg/kg), reversed the antinociceptive effect in mice which was induced by intracisternal administration of 1 μg of sulfated cholecystokinin octapeptide. The antinociceptive effect of cholecystokinin was reversed by naloxone, suggesting that the antinociceptive action involves endogenous opioid peptides in its production. On the other hand, morphine-induced analgesia was not reversed by diazepam and Ro 15–1788. These facts rule out opioid receptors as the site of the antagonism between the benzodiazepines or Ro 15–1788 and cholecystokinin on the antinociceptive effect. Benzodiazepines and Ro 15–1788 seem to inhibit the release of opioid peptides induced by cholecystokinin.

In a previous report (1), it was stated that the contractile action of cholecystokinin in the isolated guinea-pig gall bladder was competitively antagonized by benzodiazepines such as chlordiazepoxide and diazepam. Therefore, it is of special interest to examine whether cholecystokinin actions in the central nervous system are also similarly antagonized by benzodiazepines since cholecystokinin has been well established to distribute widely in the brain (2) and various central actions of cholecystokinin have been reported (3–7). Zetler (3) and Jurna and Zetler (4) demonstrated that cholecystokinin induced naloxone reversible analgesia both in mice and rats. In the present paper, the authors found that the cholecystokinin induced antinociceptive effect in mice was more sensitively reversed by benzodiazepines than by naloxone. Ro 15–1788 was introduced by Hunkeler et al. (8) and Möhler and Richards (9) as a benzodiazepine antagonist, but interestingly, it was found to exert an effect that was similar to those of benzodiazepines with regard to the antagonism of the antinociceptive effect of cholecystokinin.

Materials and Methods
Materials: Chlordiazepoxide hydrochloride (CDP) and diazepam (DZP) were offered by Yamanouchi Pharmaceutical Co., Ltd., flurazepam (FZP) and Ro 15–1788 by Nippon Roche K.K., and Naloxone by Sankyo Co., Ltd. Synthetic sulfated cholecystokinin octapeptide (CCK8) was purchased from the Peptide Institute (Osaka, Japan).

Administration of drugs to mice: Male ddY mice weighing 19–21 g were used. Ten μl of a control solution or of solutions containing CCK8 or morphine hydrochloride was injected into the cerebellomedullary cistern by using a microsyringe attached with a J-shaped stainless needle, according to the method devised by Ueda et al. (10). The control solution contained Evans blue in distilled water at a concentration of 0.4%. CCK8 and morphine solution were prepared so that 10 μl of 0.4% Evans blue aqueous solution would contain 1 μg of CCK8 or 50 ng of morphine hydrochloride. CDP, DZP, FZP and Ro 15–1788 were injected intraperitoneally 10 min before the intracisternal
administration of the CCK8, morphine or control solution. CDP, FZP and Ro 15–1788 were dissolved in physiological saline. DZP was dissolved in physiological saline by adding 5% of Tween 80. Physiological saline containing 5% of Tween 80 was used as the control.

Measurement of pain threshold in mice:
Pain threshold of mice was measured by the tail flick method. Mice were restrained in a plastic holder specially made for this purpose, and their tail was stimulated by radiant heat focused on the surface of the tail. Heat stimulation was given at a stripe, 5 mm wide, on the tail drawn in black dye. The latency was about 3 sec in most mice. Heat stimulation was cut off if the latency after drug administration became more than three times the latency before the drug administration. Every mouse was killed after the experiments and subjected to confirmation of proper intracisternal injection through observing the spread of Evans blue. Abnormal behaviors like turning also served for checking proper injection.

Results

Antinociceptive effect of CCK8 and its reversal by naloxone: Intracisternal administration of 0.4% Evans blue solution and intraperitoneal administration of physiological saline produced no significant prolongation of the latency of mice. However, intracisternal administration of 1 μg of CCK8 produced long lasting prolongation of latency, and the effect was significantly but incompletely reversed by 5 mg/kg of intraperitoneally injected naloxone. One mg/kg of naloxone could not reverse the antinociceptive effect of CCK8. (Fig. 1)

Effects of CDP, FZP, DZP and Ro 15–1788 on the antinociceptive effect of CCK8: Intraperitoneal injection of 2 to 5 mg/kg of CDP, 1 mg/kg of DZP or 0.5 mg/kg of Ro 15–1788 produced no significant prolongation of the latency of mice, as shown in Figs. 2–4, but all of them significantly reversed the effects of CCK8 on the latency. Although the data were not shown here, FZP (1 mg/kg) produced no significant antinociceptive effect and reversed the effects of CCK8 almost completely.

Effects of DZP and Ro 15–1788 on morphine-induced analgesia: Intracisternal administration of 50 ng of morphine prolonged the latency of mice by about 150% as compared with that of control mice, and the effect was almost the same as that induced by 1 μg of intracisternal CCK8. Intraperitoneal DZP (1 mg/kg) exerted no appreciable effect on the latency of mice which received intracisternal administration of control solution or 50 ng of morphine (Fig. 5). Although the data were omitted here, 0.5 mg/kg of Ro 15–1788 did not affect the morphine-induced analgesia.

Discussion

We previously demonstrated (1) that benzodiazepines such as CDP and DZP competitively antagonized the CCK8 action which induced the contraction of the guinea-
Fig. 2. Reversal of antinociceptive effect of CCK8 by CDP. Experimental procedure and conditions were as described in the legend for Fig. 1. — ▲ —: i.c. injection of 1 μg of CCK8 and i.p. injection of saline. — △ —: i.c. injection of 1 μg of CCK8 and i.p. injection of 1 mg/kg of CDP. — ▴ —: i.c. injection of 1 μg of CCK8 and i.p. injection of 2 mg/kg of CDP. — ■ —: i.c. injection of 1 μg of CCK8 and i.p. injection of 5 mg/kg of CDP. — ○ —: i.c. injection of control solution and i.p. injection of 1 mg/kg of CDP. — ■ —: i.c. injection of control solution and i.p. injection of 5 mg/kg of CDP. — ○ —: i.c. injection of control solution and i.p. injection of saline. **: P<0.01, in comparison of — ▲ — versus — △ —. P<0.01, versus — ▴ —. ▲: P<0.01, ●: P<0.05, — ■ — versus — ● —.

Fig. 3. Reversal of antinociceptive effect of CCK8 by DZP. Experimental procedure and conditions were as described in the legend for Fig. 1. — ▲ —: i.c. injection of 1 μg of CCK8 and i.p. injection of saline. — ■ —: i.c. injection of 1 μg of CCK8 and i.p. injection of 1 mg/kg of DZP. — ○ —: i.c. injection of control solution and i.p. injection of 1 mg/kg of DZP. — ■ —: i.c. injection of control solution and i.p. injection of saline. **: P<0.01, *: P<0.05, in comparison of — ▲ — versus — ■ —.

Fig. 4. Reversal of antinociceptive effect of CCK8 by Ro 15-1788. Experimental procedure and conditions were as described in the legend for Fig. 1. — ▲ —: i.c. injection of 1 μg of CCK8 and i.p. injection of saline. — ■ —: i.c. injection of 1 μg of CCK8 and i.p. injection of 0.5 mg/kg of Ro 15-1788. — ○ —: i.c. injection of control solution and i.p. injection of 0.5 mg/kg of Ro 15-1788. — △ —: i.c. injection of control solution and i.p. injection of saline. **: P<0.01, in comparison of — ▲ — versus — △ —. P<0.01, versus — ■ —.

Fig. 5. Effects of DZP on morphine-induced analgesia. Experimental procedure and conditions were as described in the legend for Fig. 1. — ▲ —: i.c. injection of 50 ng of morphine and i.p. injection of saline. — ■ —: i.c. injection of 50 ng of morphine and i.p. injection of 1 mg/kg of DZP. — ○ —: i.c. injection of control solution and i.p. injection of 1 mg/kg of DZP. — △ —: i.c. injection of control solution and i.p. injection of saline.
pig gall bladder. This finding then suggested to us that benzodiazepines might also interact with cholecystokinin in the central nervous system.

Cholecystokinin-like immunoreactivity in the brain has been characterized as octa-peptides (11). Therefore, we used CCK8 in these experiments. Zetler (3) and Jurna and Zetler (4) reported that cholecystokinin induced naloxone reversible analgesia in mice and rats, while Itoh et al. (12) demonstrated that cholecystokinin suppressed β-endorphine-induced analgesia. Thus we first reconfirmed the characteristics of the antinociceptive action of cholecystokinin. As shown in the Figs., intracisternal administration of 1 μg of CCK8 produced significant antinociceptive effect in mice and the effect was reversed by naloxone (Fig. 1), although the dose of naloxone was as high as 5 mg/kg, and the reversal of the CCK8 action by naloxone was not complete. These facts suggest that CCK8 exerts its antinociceptive action via releasing endogenous opioid peptides. Benzodiazepines clearly reversed the antinociceptive effect of CCK8 at low doses of 1 to 5 mg/kg. For example, 2 to 5 mg/kg of CDP dose-dependently and 1 mg/kg of DZP or FZP almost completely reversed the antinociceptive effect of CCK8. CDP seems to have a little lower activity than DZP and FZP regarding the reversal of the CCK8 action. On the other hand, 1 mg/kg of DZP did not affect the morphine-induced analgesia (Fig. 5). Therefore, the antagonism between the benzodiazepines and CCK8 in the antinociceptive effect must take place at some other sites than opioid receptors. Inhibition of the CCK8-induced release of the opioid peptides by benzodiazepines seems to be most probable. Our previous report (1) evidenced that CCK8 action through which CCK8 contracts the smooth muscle of the isolated guinea-pig gall bladder was competitively antagonized by benzodiazepines such as CDP and DZP, suggesting that the antagonism of benzodiazepines to CCK8 action on the central nervous system may involve direct competition at the neuronal CCK8 receptor in the central nervous system.

However, according to the previous report (1) pA2 values of CDP and DZP, which represent the potency of a competitive antagonist, on the contractile action of CCK8 in the gall bladder muscle were of the orders of 6 or lower. Taking the low intraperitoneal dose of benzodiazepines used in the present experiments into account, it seems difficult to consider that the benzodiazepines exert their potent inhibitory effects on the CCK8 action in the central nervous system via directly competing with CCK8 at the cholecystokinin receptor as observed in the gall bladder.

It is noteworthy that a benzodiazepine antagonist, Ro 15–1788, also revealed antagonistic action to the antinociceptive effect of CCK8, and its potency was so high that at 0.5 mg/kg, it completely reversed the CCK8 action. Ro 15–1788 was discovered by Hunkeler et al. (8) and Möhler and Richards (9) to be able to specifically replace benzodiazepines bound to benzodiazepine receptors. The pharmacological properties of Ro 15–1788 as a benzodiazepine antagonist are well documented by Polc et al. (13). However, Robertson et al. (14) recently demonstrated that Ro 15–1788 acts as a partial agonist at an anticonvulsant benzodiazepine receptor. In the present experiment, Ro 15–1788 revealed a common feature to those of benzodiazepines with regard to reversing the antinociceptive action of CCK8 and failing to reverse morphine analgesia.

The mechanism of action of benzodiazepines has mainly been explained by the interaction of benzodiazepines with GABA receptor. Gallager (15) and Choi et al. (16), for example, showed augmentation of the response of the dorsal raphe and spinal cord to GABA by benzodiazepines. The present results regarding the interaction between benzodiazepines and CCK8, however, may provide a new aspect to the approach for elucidating the mechanism of benzodiazepine action.

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References


