Inhibition of Cholecystokinin Response in the Gallbladder by Dibenamine and Its Protection by Benzodiazepines

Kazuhiko KUBOTA, Kiminobu SUGAYA, Fumio FUJII,
Masahiro ITONAGA and Nobuyoshi SUNAGANE

Department of Pharmacology, Faculty of Pharmaceutical Sciences,
Science University of Tokyo, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan

Accepted July 20, 1985

Abstract—The contractile response of the guinea-pig gallbladder to cholecystokinin (CCK) and acetylcholine (ACh) was irreversibly inhibited by 5×10^{-5} M dibenamine, and the dibenamine-induced inhibition in the CCK response was prevented by 10^{-4} M chlordiazepoxide (CDP) and diazepam (DZP), but not by 10^{-2} M proglumide or 10^{-6} M atropine. The dibenamine-induced inhibition in the ACh response was prevented by 10^{-6} M atropine, but not by 10^{-4} M CDP. These findings suggest that the binding of CCK to the CCK receptor can be inhibited by benzodiazepines.

Several CCK antagonists have been hitherto reported (1–3), and proglumide has been the most widely used as a selective antagonist against CCK. However, the potency of proglumide as a CCK antagonist is rather low. In our previous paper (4), it was shown that benzodiazepines like CDP and DZP antagonized cholecystokinin action to contract the guinea-pig gallbladder muscle in a competitive manner, and the potency of the benzodiazepines on the antagonism was much higher than that of proglumide.

In our other papers (5, 6), it was also demonstrated that the antinociceptive and satiety action of octapeptide cholecystokinin (CCK8) which was administered intracisternally to mice was significantly antagonized by very low intraperitoneal doses of benzodiazepines.

In this paper, authors intended to provide further evidence that benzodiazepines act on CCK receptors.

Male guinea pigs weighing 350 to 400 g were stunned by a blow on the head, and the gallbladder was excised. The gallbladder was cut helically, and strips about 10 mm long and 4 mm wide were prepared. The strips were suspended in Locke-Ringer solution which was maintained at 32°C and bubbled with air. The Locke-Ringer solution had the

following composition (mM): NaCl, 154.0; KCl, 5.6; CaCl_2 , 2.2; MgCl_2 , 2.1; NaHCO_3 , 5.9; and glucose, 2.8. The muscle strips were connected via surgical silk to a force displacement transducer for monitoring changes in isometric force. The muscle strips were equilibrated for 30 min in the Locke-Ringer solution and then applied repeatedly with agonists, 10^{-8} M CCK8 and 10^{-4} M ACh, until the contractile response to the agonists became constant. The strips were exposed to 5×10^{-5} M dibenamine sequentially twice for 30 min each, washed with Locke-Ringer solution for 90 min and applied with an agonist. The contractile responses of the muscle strips to an agonist before and after the exposure to dibenamine were compared. For the purpose of protecting receptors, antagonists such as atropine, benzodiazepine and proglumide were applied to the muscle strips for 15 min prior to the dibenamine treatment and during the dibenamine treatment.

CDP hydrochloride and DZP were provided from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo) and proglumide from Eisai Co., Ltd. (Tokyo). Dibenamine hydrochloride was purchased from Tokyo-Kasei Co. (Tokyo) and CCK8 from Protein Research Foundation (Osaka).

Significant differences between data were assessed by Student's *t*-test for paired samples.

The contractile response of the gallbladder muscle to CCK8 was significantly depressed by 60 min exposure of the muscle to 5×10^{-5} M of dibenamine, and the depression of the response by dibenamine was significantly prevented in the presence of 10^{-4} M CDP and DZP, but not prevented by 10^{-6} M atropine and 10^{-2} M proglumide. On the other hand, the inhibition of the contractile response of the gallbladder to ACh by dibenamine exposure was significantly prevented by 10^{-6} M atropine but not by 10^{-4} M CDP (Table 1).

In our present experiments, 10^{-8} M CCK8 was used, since this concentration of CCK8 was close to the ED₅₀ in the CCK8 response of the gallbladder. The contractile response of the gallbladder to 10^{-8} M CCK8 was

significantly ($P < 0.05$) inhibited to $56.5 \pm 5.3\%$ by application of 10^{-5} M dibenamine and more significantly ($P < 0.01$) to $39.6 \pm 1.7\%$ by 10^{-4} M dibenamine. Therefore, we used 5×10^{-5} M dibenamine in the present receptor protection experiments (Table 2).

Dibenamine has been widely used as an irreversible alkylating agent for ACh (7, 8), histamine (8, 9) and α -adrenergic receptors (8, 10, 11). In the present work, it was demonstrated that the CCK receptor of the guinea-pig gallbladder muscle was also irreversibly inhibited by dibenamine, and the inhibition was significantly protected in the presence of 10^{-4} M CDP and DZP. In the previous paper, authors demonstrated that CDP and DZP caused significant rightward parallel shift of the dose-response curve for CCK when they were present in concentrations of 10^{-5} M to 10^{-6} M, and Schild analysis of the parallel shift of the CCK

Table 1. Effect of chlordiazepoxide and atropine on the dibenamine (5×10^{-5} M)-induced inhibition of the contractile response of guinea-pig gallbladder to ACh (10^{-4} M)

Treatment	Contractile response (%) ^a
Dibenamine (control)	23.33 ± 2.82
Dibenamine+Chlordiazepoxide (10^{-4} M)	20.38 ± 2.05
Dibenamine (control)	28.84 ± 2.40
Dibenamine+Atropine (10^{-6} M)	$50.75 \pm 2.60^{**}$

^aThe contractile response of the gallbladder to ACh before dibenamine treatment was taken as 100%. The values are given as the mean $\pm$ S.E. ($n=6$). Significant difference from the control at $^{**}P < 0.01$.

Table 2. Effect of chlordiazepoxide, diazepam, proglumide and atropine on the dibenamine (5×10^{-5} M)-induced inhibition of the contractile response of guinea-pig gallbladder to CCK8 (10^{-8} M)

Treatment	Contractile response (%) ^a
Dibenamine (control) (6)	64.11 ± 2.35
Dibenamine+Chlordiazepoxide (10^{-4} M) (6)	$76.07 \pm 2.30^{**}$
Dibenamine (control) (12)	48.08 ± 3.56
Dibenamine+Diazepam (10^{-5} M) (6)	62.76 ± 8.59
Dibenamine+Diazepam (10^{-4} M) (6)	$70.28 \pm 3.45^{**}$
Dibenamine (control) (12)	55.47 ± 2.92
Dibenamine+Proglumide (10^{-3} M) (6)	56.85 ± 3.26
Dibenamine+Proglumide (10^{-2} M) (6)	58.27 ± 1.32
Dibenamine (control) (6)	60.04 ± 4.64
Dibenamine+Atropine (10^{-6} M) (6)	58.52 ± 2.57

^aThe contractile response of the gallbladder to CCK8 before dibenamine treatment was taken as 100%. The numbers of experiments are in parentheses. The values are given as the mean $\pm$ S.E. Significant difference from the control at $^{**}P < 0.01$.

dose-response curve supported the competitive antagonism between benzodiazepines and CCK (4). The present results provide further evidence that benzodiazepines can bind to CCK receptors and selectively protect it from inhibition by dibenamine, since cross-protection of ACh and CCK receptors by benzodiazepines and atropine was not observed. Proglumide has been claimed to be a selective receptor antagonist (3) of CCK. In the present protection experiments, proglumide considerably prevented the decrease in the CCK response produced by dibenamine, but the prevention was not statistically significant. According to our calculation (4), the pA_2 value for proglumide which was obtained on the basis of K_B reported by Kaplita and Roebuch (2) was 3.82. By comparison of this pA_2 value with 7.33 for DZP and 6.70 for CDP (4), the activity of proglumide on CCK antagonism is estimated to be about one thousand times lower than those of DZP and CDP. Concentrations of proglumide higher than 10^{-2} M could not be used in the present experiments because of its solubility. The present results, therefore, demonstrated that proglumide was a very weak antagonist of CCK, giving further support to the previously reported (4–6) antagonism between CCK8 and benzodiazepines.

Acknowledgements: The authors wish to thank Yamanouchi Pharmaceutical Co., Ltd. and Eisai Co., Ltd. for kindly providing us with the benzodiazepines and proglumide.

References

- 1 Hutchison, J.B. and Dockray, D.J.: Inhibition of the action of cholecystokinin octapeptide on the guinea pig ileum myenteric plexus by dibutyryl cyclic guanosine monophosphate. *Brain Res.* **202**, 501 (1980)
- 2 Kaplita, P.V. and Roebuck, B.D.: Proglumide antagonizes the stimulation of rabbit gallbladder by cholecystokinin. *Arch. Int. Pharmacodyn. Ther.* **269**, 271 (1984)
- 3 Gardner, J.D. and Jensen, R.T.: Cholecystokinin receptor antagonist. *Am. J. Physiol.* **9**, G471–G476 (1984)
- 4 Kubota, K., Sugaya, K., Sunagane, N., Matsuda, I. and Uruno, T.: Cholecystokinin antagonism by benzodiazepines in the contractile response of the isolated guinea-pig gallbladder. *Eur. J. Pharmacol.* **110**, 225–231 (1985)
- 5 Kubota, K., Sugaya, K., Matsuda, I., Matsuoka, Y. and Terawaki, Y.: Reversal of antinociceptive effect of cholecystokinin by benzodiazepines and a benzodiazepine antagonist. *Ro* 15–1788. *Japan. J. Pharmacol.* **37**, 101–105 (1985)
- 6 Kubota, K., Matsuda, I., Sugaya, K. and Uruno, T.: Cholecystokinin antagonism by benzodiazepines in the food intake in mice. *Physiol. Behav.* (1985) (in press)
- 7 Araki, H. and Matsumoto, H.: Mode of antagonism of dibenamine against the acetylcholine-induced contraction of excised ileum from the rabbit. *Japan. J. Pharmacol.* **24**, Supp. 147 (1974)
- 8 Furchgott, R.F.: Dibenamine blockade in strips of rabbit aorta and its use in differentiating receptors. *J. Pharmacol. Exp. Ther.* **111**, 265–284 (1954)
- 9 Uchida, M. and Takagi, K.: Purification of histamine receptor. (II) Subcellular distribution of specifically bound dibenamine. *Japan. J. Pharmacol.* **23**, 337–347 (1973)
- 10 Swamy, V.C. and Triggle, D.J.: 2-Halogenoethylamines and the role of Ca^{2+} in adrenergic α -receptor activation in the rat vas deferens. *Eur. J. Pharmacol.* **19**, 67–78 (1972)
- 11 Tuttle, R.R. and Moran, N.C.: The effect of calcium depletion on the combination of agonists and competitive antagonists with alpha adrenergic and histaminergic receptors of rabbit aorta. *J. Pharmacol. Exp. Ther.* **169**, 255–263 (1969)