RELAXING ACTION OF TREPIBUTONE (AA-149) ON THE ISOLATED SPHINCTER OF ODDI AND SMALL INTESTINE OF RABBITS

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Abstract—We investigated the smooth muscle relaxing action of trepibutone [3-(2,4,5-triethoxybenzoyl)propionic acid: AA-149] using assays of the isolated preparations of sphincter of Oddi, duodenum, jejunum and ileum of rabbits. Trepibutone (10^{-6} – 10^{-3} M) markedly relaxed the sphincter of Oddi and duodenum, while papaverine (10^{-6} – 10^{-4} M) exerted an equipotent relaxing activity in all these preparations. The actions of trepibutone were not altered by pretreatment with tetrodotoxin, phentolamine or propranolol. However, trepibutone at a concentration of 3×10^{-3} M, unlike papaverine, had no effect on the contractile response to ACh. These results indicate that trepibutone relaxes smooth muscle by a direct action which differs from the action of papaverine.

Trepibutone [3-(2,4,5-triethoxybenzoyl)-propionic acid: AA-149] is a novel compound with a prominent spasmolytic activity on the biliary tract and with a choleric activity in dogs (1, 2). We have already demonstrated that the spasmolytic action is not mediated *via* cholinergic and adrenergic receptors in anesthetized dogs (2). The present study was performed to elucidate the mechanism of the relaxing action of trepibutone on smooth muscle and findings in the isolated sphincter of Oddi and small intestine of rabbits were compared with data on autonomic drugs and papaverine.

MATERIALS AND METHODS

Isolated preparations of the sphincter of Oddi, duodenum, jejunum and ileum: Male albino rabbits weighing 3–4 kg were killed by injecting 10 ml of air into the ear vein, and the sphincter of Oddi and small intestine were isolated. Duodenal smooth muscles

attached to the sphincter of Oddi were removed. The duodenum just caudal to the sphincter of Oddi, the jejunum 100 cm caudal to the pylorus and the ileum 30 cm rostral to the ileo-caecal junction were used as intestinal preparations.

Assays: The assays were performed according to the method of Persson (3). Each of the 2 cm preparations was suspended in an organ bath containing 20 ml of Krebs solution, which was composed of 120.7 mM NaCl, 5.9 mM KCl, 15.5 mM NaHCO_3 , 1.2 mM NaH_2PO_4 , 2.5 mM CaCl_2 , 1.2 mM MgCl_2 and 11.5 mM glucose, continuously bubbled with 95% O_2 and 5% CO_2 , and maintained at 37°C. The preparations of the sphincter of Oddi, duodenum, jejunum and ileum were loaded with tensions of 0.25, 0.5, 0.5 and 0.5 g, respectively. Longitudinal movement of the preparations was measured with an isotonic transducer (Nihon Kohden, TD112S) and recorded on a polygraph

(Nihon Kohden, RM85).

Agents: The following agents were used: acetylcholine chloride (Daiichi), phenylephrine hydrochloride (Sigma), *l*-isoproterenol hydrochloride (Nikken), nicotine tartrate (Tokyo Kasei), cholecystokinin (pancreozymin grade II, Sigma), morphine hydrochloride (Takeda), papaverine hydrochloride (Hoei), atropine sulfate (Iwaki), phentolamine mesylate (Ciba-Geigy), propranolol hydrochloride (Sumitomo) and tetrodotoxin (Sankyo, crystal). Trepibutone was dissolved in 1N NaOH and the pH was adjusted to 7.0 with 1N HCl.

The values obtained are expressed as mean values and their standard errors (S.E.M.)

RESULTS

Responses of the sphincter of Oddi and duodenum to autonomic drugs, cholecystokinin, and morphine: As the *in vitro* physiological properties of the sphincter of Oddi have been assessed in tissues from cats, the following responses of the isolated sphincter of Oddi of rabbits to autonomic drugs, cholecystokinin and morphine were clarified and comparisons made with findings in the case of the duodenum (Fig. 1). In patterns of the spontaneous contractions two populations of the sphincter of Oddi seemed apparent, i.e., a group with an irregular,

large contraction (0.5–3 per min in frequency) superimposed with rhythmic fluctuations and another which exhibited only a rhythmic contraction (16–18 per min in frequency). ACh (10^{-9} – 10^{-5} M) contracted the sphincter of Oddi and duodenum in a concentration-related manner. Atropine (3×10^{-6} M) elicited a slight relaxation of both tissues. This agent inhibited the spontaneous contraction of the sphincter of Oddi in 5 out of 6 preparations, but did not inhibit the spontaneous contraction of the duodenum. Phenylephrine (10^{-7} – 10^{-4} M) induced a contraction in 3 out of 5 preparations of the sphincter of Oddi and a relaxation in the other two. This agent, however, induced a relaxation in all the 5 duodenal preparations followed by a slight contraction in two. Treatment with isoproterenol (10^{-9} – 10^{-6} M) resulted in a concentration-related relaxation of both tissues. Nicotine (10^{-4} M) led to a marked contraction followed by a slight relaxation of the sphincter of Oddi in 7 out of 8 preparations, and in the remaining one only a slight relaxation was induced. Pre-treatment of the sphincter of Oddi with atropine (3×10^{-6} M) abolished the contractile response to nicotine, but enhanced the subsequent relaxation. This nicotine-induced relaxation was completely blocked by a combination of phentolamine (10^{-5} M) and

Fig. 1. Responses of the sphincter of Oddi and duodenum to pharmacologic stimuli.

propranolol (10^{-5} M). The duodenal preparations were obviously relaxed by nicotine in all of 8 preparations but in 3 preparations a slight contraction occurred prior to relaxation. Cholecystokinin (0.01–1 U/ml) induced a prominent contraction of the sphincter of Oddi in all of 12 preparations as well as in all the duodenum. Morphine (1.3×10^{-5} M) contracted the sphincter of Oddi in all of 4 preparations, but only slightly relaxed all of the duodenal preparations. The contractile effect of morphine on the sphincter of Oddi was not diminished in the presence of atropine (3×10^{-6} M).

Relaxing action of trepibutone and papaverine on the sphincter of Oddi, duodenum, jejunum and ileum: The sphincter of Oddi and intestinal preparations showed spontaneous rhythmic contractions in addition to the basal tones (Fig. 2). A cumulative application of trepibutone in the range of 10^{-6} – 10^{-3} M markedly reduced the tone of all the tissues in a concentration-related manner (Figs. 2 and 3). The potency in the relaxing activity differed among the tissues and decreased in the order of the sphincter of Oddi, duodenum, jejunum and ileum (Fig. 3). The relaxing effects of trepibutone at 10^{-4} M, expressed as % relaxation of the

maximum response induced by 10^{-6} M isoproterenol, were $77 \pm 8\%$ ($n=6$), $61 \pm 8\%$ ($n=7$), $45 \pm 6\%$ ($n=7$) and $7 \pm 9\%$ ($n=7$), respectively. Papaverine (10^{-6} – 10^{-4} M) exerted an equipotent relaxing activity in all the preparations (Fig. 3).

Effects of tetrodotoxin and adrenergic blocking agents on the relaxing action of trepibutone: The following experiments were performed to clarify whether trepibutone reduced the tone of the sphincter of Oddi *via* the intrinsic nervous system or through adrenergic receptors (Fig. 4). Tetrodotoxin diminished the response of the sphincter of Oddi to nicotine, but had no effect on the relaxing effect of trepibutone (10^{-4} M). Furthermore, the effect of trepibutone was not eliminated by pretreatment with a combination of propranolol (10^{-5} M) and phenolamine (10^{-5} M) which completely inhibited the relaxing effects of isoproterenol (10^{-7} M) and phenylephrine (10^{-5} M).

Effects of trepibutone and papaverine on the contractile response to ACh: ACh at 10^{-7} M showed 50–70% response of the

Fig. 2. Effects of trepibutone on the tone and spontaneous contraction of the sphincter of Oddi and intestinal preparations. Trepibutone was cumulatively added.

Fig. 3. Concentration-relaxing activity relation curves of trepibutone (AA-149) and papaverine in the sphincter of Oddi, duodenum, jejunum and ileum. Agents were cumulatively added. The relaxing effects of drugs were expressed as % relaxation of the maximum response induced by 10^{-6} M isoproterenol. Each point represents mean $\pm$ S.E.M. of 6–7 experiments.

Fig. 4. Effects of tetrodotoxin (TTX) and adrenergic blocking agents on the relaxing action of trepibutone (AA-149) in the sphincter of Oddi.

Fig. 5. Effects of trepibutone (AA-149) and papaverine on the contractile response of the duodenum to ACh. Each point represents mean $\pm$ S.E.M. of 6–7 experiments. Ordinate in (b) shows the % inhibition by drugs of ACh-induced contraction, which is expressed as % change in contraction from the reduced tone elicited by 10^{-8} M isoproterenol.

maximum contraction induced by the concentration of 10^{-5} M in the duodenum. The contractile response to ACh was not affected by pretreatment with trepibutone (10^{-5} – 3×10^{-3} M), but was reduced by papaverine (10^{-6} – 3×10^{-4} M) in a concentration-related manner (Fig. 5). Similar results were

obtained with preparations of the sphincter of Oddi.

DISCUSSION

The motor function of the sphincter of Oddi is regulated *via* the autonomic nervous system and by gastrointestinal hormones, particularly cholecystikinin (4, 5). Histochemical studies have demonstrated adrenergic innervation (6) and cholinergic nerves (7). An inhibitory innervation by non-adrenergic nerves has been reported by Persson (8). In the present studies, the sphincter of Oddi of rabbits was contracted by ACh and α -adrenergic stimulating agents, and relaxed by atropine and β -adrenergic stimulating agents. Moreover, nicotine produced a strong contraction followed by a slight relaxation of the sphincter of Oddi and the effect was abolished by tetrodotoxin and a combination of atropine and α - and β -adrenergic blocking agents. These results indicate that the motility of the sphincter of Oddi of rabbits may be regulated through excitatory and inhibitory intrinsic nerves. On the other hand, morphine produces con-

traction of the sphincter of Oddi in dogs, cats and calves, and this contraction is not inhibited by atropine (2, 3, 9, 10). The same results were obtained in the present *in vitro* experiments using the sphincter of Oddi of rabbits. Cholecystokinin reduced the perfusion pressure of the sphincter of Oddi in dogs and cats, thereby revealing its relaxing action (2, 3, 11, 12). However, the isolated sphincter of Oddi of rabbits as well as the duodenum was contracted by cholecystokinin, though the responses to phenylephrine, nicotine, atropine and morphine were substantially distinct from those of the duodenal preparations. The difference in the response to cholecystokinin among preparations from dogs or cats and rabbits may be due to different experimental conditions and/or the animal species. On the basis of the findings, the relaxing effect of trepibutone on the sphincter of Oddi and small intestine of rabbits was determined.

Trepibutone at concentrations over 10^{-6} M exhibited a concentration-related reduction in the tone and spontaneous contraction of the sphincter of Oddi. The reducing effects of trepibutone were not altered by tetrodotoxin or α - and β -adrenergic receptor blocking agents. In addition, trepibutone did not abolish the contractile action of ACh. These findings indicate that the smooth muscle relaxing effect of trepibutone is mediated neither *via* intrinsic nerves nor *via* adrenergic and cholinergic receptors. This agent would appear to relax the smooth muscles by a direct action similar to that of papaverine. However, trepibutone even at a concentration of 3×10^{-3} M did not diminish the contractile response of the sphincter of Oddi and duodenum to ACh, whereas papaverine exerted a concentration-related inhibition. Moreover, trepibutone, unlike papaverine, inhibited only slightly the contractile response of the guinea-pig ileum to BaCl_2 , 5-hydroxytryptamine or histamine (unpublished data).

Therefore, the mechanisms of action of trepibutone and papaverine differ. Trepibutone may induce relaxation of smooth muscles by acting on mechanisms which maintain the tone, but probably does not act on the contractile system. Our recent findings in the guinea-pig taenia coli strongly suggest that the mechanism of action of trepibutone is not due to an inhibition of the Ca influx through cell membrane but rather to an increase in the Ca binding to intracellular storage sites (13).

The activity of trepibutone was potent in the sphincter of Oddi and duodenum, as compared with the action in the jejunum and ileum, while that of papaverine was equipotent in these preparations (Fig. 3). Although we have no direct evidence as to the organ specificity of trepibutone, there are reports that the capacity to store Ca and/or an affinity of the storage structure for Ca differs in various types of smooth muscles (14, 15).

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REFERENCES

- 1) Satoh, H., Kanno, M. and Maki, Y.: A possible mechanism of choleretic action of 3-(2,4,5-triethoxybenzoyl)propionic acid (AA-149) in dogs. *Europ. J. Pharmacol.* **48**, 125-132 (1978)
- 2) Satoh, H., Maki, Y. and Kanno, M.: Biliary spasmolytic action of 3-(2,4,5-triethoxybenzoyl) propionic acid (AA-149) in dogs. *Europ. J. Pharmacol.* **48**, 309-317 (1978)
- 3) Persson, C.G.A.: Adrenoceptor functions in the cat choledochoduodenal junction *in vitro*. *Brit. J. Pharmacol.* **42**, 447-461 (1971)
- 4) Hallenbeck, G.A.: Biliary and pancreatic intraductal pressures. *Handbook of Physiology, Alimentary Canal.*, Edited by Code, C.F., Vol. 2, p. 1007. American Physiological Society, D.C. (1967)
- 5) Persson, C.G.A.: Adrenergic, cholecystokinetic and morphine-induced effects on extra-hepatic

- biliary motility. *Acta Physiol. scand. Supp.* **383**, 5–32 (1972)
- 6) Mori, J., Azuma, H. and Fujiwara, M.: Adrenergic innervation and receptors in the sphincter of Oddi. *Europ. J. Pharmacol.* **14**, 365–373 (1971)
  - 7) Kyösola, K. and Rechart, L.: The anatomy and innervation of the sphincter of Oddi in the dog and cat. *Am. J. Anat.* **140**, 497–522 (1974)
  - 8) Persson, C.G.A.: Inhibitory innervation of cat sphincter of Oddi. *Brit. J. Pharmacol.* **58**, 479–482 (1976)
  - 9) Crema, A., Benzi, G., Frigo, G.M. and Berté, F.: The responses of the terminal bile duct to morphine and morphine-like drugs. *J. Pharmacol. exp. Ther.* **149**, 373–378 (1965)
  - 10) Persson, C.G.A. and Ekman, M.: Effect of morphine, cholecystokinin and sympathomimetics on the sphincter of Oddi and intramural pressure in cat duodenum. *Scand. J. Gastroent.* **7**, 345–351 (1972)
  - 11) Sandblom, P., Voegtlin, W.L. and Ivy, A.C.: The effect of cholecystokinin on the choledochoduodenal mechanism (sphincter of Oddi). *Am. J. Physiol.* **113**, 175–180 (1935)
  - 12) Hedner, P. and Rorsman, G.: On the mechanism of action for the effect of cholecystokinin on the choledochoduodenal junction in the cat. *Acta. Physiol. scand.* **76**, 248–254 (1969)
  - 13) Satoh, H., Inada, I. and Maki, Y.: Role of an intracellular Ca store in trepibutone (AA-149)-induced relaxation of the depolarized smooth muscle of the guinea-pig taenia coli. *Japan. J. Pharmacol.* **31**, 593–599 (1981)
  - 14) Davine, C.E., Somlyo, A.V. and Somlyo, A.P.: Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. *J. cell Biol.* **52**, 690–718 (1972)
  - 15) McGuffee, L.J. and Bagby, R.M.: Ultrastructure, calcium accumulation, and contractile response in smooth muscle. *Am. J. Physiol.* **230**, 1217–1224 (1976)