POTENTIATING EFFECTS OF C-TERMINAL OCTAPEPTIDE OF CHOLECYSTOKININ ON CONTRACTILITY OF ISOLATED GUINEA-PIG GALLBLADDER*

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It was previously reported that the contractile response of isolated guinea-pig gallbladder and sphincter of Oddi to transmural stimulation is induced by release of acetylcholine (ACh) from the postganglionic cholinergic neuron and that angiotensin II (AG) potentiates the contractile response by facilitating the release of ACh (1, 2). Humoral as well as neural factors play an important role in the regulation of tone and motility of the biliary tract.

The present experiments were thus performed in an attempt to determine the effects of C-terminal octapeptide of cholecystokinin (C8-CCK) on the contractile response of isolated guinea-pig gallbladder and sphincter of Oddi to transmural stimulation and exogenously applied ACh.

Female guinea-pigs, weighing 250 to 300 g, were sacrificed by a blow on the back of the neck. The entire gallbladder was removed and suspended vertically in a 20-ml organ bath. The terminal portion of the common bile duct was resected from the remaining tissues, made tube-shaped and then suspended vertically in another bath. The composition of modified Ringer’s solution (mM) was as follows: Na+; 162.1, K+; 5.4, Ca++; 2.2, Cl−; 157.0, HCO3−; 14.9, and glucose; 5.6. Tension changes were recorded isometrically on a four-channel penrecorder by means of a force-displacement transducer. A 2 g tension was loaded to the tissues. Before measurements were started, the preparation was allowed to equilibrate for 1.5 to 2 hr in the bathing solution. The preparations were placed between a pair of platinum plated stimulating electrodes 5 x 7 mm in size and 3 to 5 mm apart. Gaps between the electrodes and the strips were wide enough to allow for undisturbed movement of the preparations and yet sufficiently narrow to effectively stimulate the intramural nerve terminals. For transmural stimulation, a train of square pulses delivered from an electronic stimulator were passed through the electrodes.

The following drugs were used: synthetic C-terminal octapeptide of cholecystokinin (C8-CCK) (SQ 19,844, batch 2NN005NA, Sincalide, provided by Dr. Welch, the Squibb Institute for Medical Research, Princeton, U.S.A.), a stock solution of 10 μg/ml of deionized and distilled water prepared and frozen in a creamstocker, acetylcholine chloride (ACh), carbamylcholine chloride (carbachol), atropine sulfate, physostigmine salicylate,

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KCl and tetrodotoxin (TTX) (provided by Dr. Sakai, the Sankyo Research Laboratories, Tokyo). All experimental solutions were freshly prepared by an appropriate dilution with deionized and distilled water. Concentrations given in the following refer to the final bath concentrations.

Transmural stimulation (0.3 msec duration, 20 Hz, 10 to 60 V, for 10 sec) applied every 3 min to the isolated preparation of the gallbladder and sphincter of Oddi caused a transient contraction. Threshold intensity of stimulation was approx. 10 V in both preparations. The degree of response increased with stimulus intensity until a plateau was attained at 40 V, and the response was also observed to increase with stimulus frequency and pulse duration. Pretreatment with atropine $10^{-6}$ M or TTX $3.1 \times 10^{-7}$ M completely blocked the contraction and conversely, physostigmine $3 \times 10^{-7}$ M markedly potentiated it in both preparations.

The application of C8-CCK in concentrations higher than $3 \times 10^{-10}$ g/ml gradually increased the tension of the gallbladder. Such was sustained until wash out, the tension was concentration-dependent, and hardly affected by treatment with atropine $10^{-6}$ M or TTX $3.1 \times 10^{-7}$ M. Higher concentrations were required to produce a transient, not sustained, increase in tension of the sphincter of Oddi as compared with the gallbladder. A transient contraction caused by C8-CCK ($3 \times 10^{-7}$ g/ml) in the sphincter of Oddi was abolished by pretreatment with atropine $10^{-6}$ M or TTX $3.1 \times 10^{-7}$ M. The contractile responses of the gallbladder to transmural stimulation as well as exogenously applied ACh were markedly potentiated (Fig. 1, upper), whereas those of the sphincter of Oddi were hardly affected in the presence of C8-CCK, $3 \times 10^{-10}$ to $3 \times 10^{-9}$ g/ml (Fig. 1, lower). The contractile re-

![Gallbladder from guinea pig](image)

![Sphincter of Oddi from guinea pig](image)

**Fig. 1.** Influence of C8-CCK on the contractile responses in the isolated gallbladder (upper) and sphincter of Oddi (lower) from guinea-pig caused by transmural stimulation and exogenously applied ACh.
The contractile responses of the gallbladder to carbachol 10^{-7} g/ml was also potentiated by C_{8}-CCK 3 \times 10^{-9} g/ml, the response to KCl 30 mM, however, was not affected (Fig. 2).

The present results demonstrate that C_{8}-CCK induces the sustained contraction of the gallbladder and potentiates the contractility. The sphincter of Oddi also responded to C_{8}-CCK, but the response was only transient and much weaker than that of the gallbladder. Andersson and associates (3, 4) showed the contractile effect of a low concentration of C_{8}-CCK in the isolated guinea-pig gallbladder, and concluded that local synthesis and release of prostaglandins do not play an essential role for the mediation of this effect and also that C_{8}-CCK had a direct effect on the muscle cells. Yau et al. (5) also showed that C_{8}-CCK exerted its action directly on the gallbladder muscle. In our present results, atropine or TTX had no effect on the contractile response caused by C_{8}-CCK in the gallbladder. On the other hand, as the contractile response of the sphincter of Oddi caused by C_{8}-CCK was abolished by atropine or TTX, it is speculated that C_{8}-CCK exerts its action through the release of ACh from intramural cholinergic nerve terminals in the sphincter of Oddi.

The contractile responses of gallbladder to exogenously applied ACh as well as to transmural stimulation were potentiated by treatment with low concentrations of C_{8}-CCK, whereas no potentiation occurred in the sphincter of Oddi. This finding suggests that unlike AG II, CCK potentiates the contractility of the gallbladder by sensitizing the post-junctional cholinergic mechanism. The view is further supported by the fact that the response to KCl, a non-specific stimulant, is not affected by C_{8}-CCK. The possibility that anticholinesterase activity plays a role in potentiation of ACh action is ruled out in light of the potentiation of contractile response to carbachol, a cholinesterase-resistant ester, following C_{8}-CCK. Why the responses of the gallbladder and sphincter of Oddi differed following application of C_{8}-CCK is now under investigation.

REFERENCES