EFFECTS OF C-TERMINAL OCTAPEPTIDE OF
CHOLECYSTOKININ AND PROSTAGLANDINS ON
ADRENERGIC FUNCTIONS IN THE GUINEA-PIG
GALLBLADDER AND SPHINCTER OF ODDI

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Accepted August 11, 1980

Abstract—Effects of C-terminal octapeptide of cholecystokinin (C₈-CCK) and prostaglandins E₁, E₂ and F₂α on noradrenaline-induced responses and ³H-noradrenaline release in the gallbladder and sphincter of Oddi of guinea pigs were examined. In the sphincter of Oddi, noradrenaline in low concentrations induced a relaxation which was blocked by either phentolamine or propranolol, while noradrenaline in high concentrations induced a contraction which was blocked by phentolamine. These results suggest the existence of excitatory and inhibitory α-receptors and inhibitory β-receptors in the sphincter of Oddi. In the gallbladder, the adrenergic receptors are α-excitatory and β-inhibitory. C₈-CCK (10⁻⁹ g/ml) potentiated both contractile and relaxing responses to noradrenaline, in the gallbladder. The same concentration of prostaglandins potentiated only contractile response to noradrenaline. In the sphincter of Oddi, noradrenaline-induced responses were not affected by C₈-CCK and prostaglandins. Prostaglandins inhibited ³H-efflux evoked by electrical stimulation, while C₈-CCK had no effect on the ³H-efflux from both preparations. These results suggest that C₈-CCK enhances the contractile and relaxing responses to noradrenaline, and that prostaglandins act in a similar way on the postsynaptic response and, in addition, inhibit presynaptically the release of noradrenaline in the gallbladder. In the sphincter of Oddi, only prostaglandins inhibit the presynaptic event.

Muscle tone and motility of the biliary system are regulated by the autonomic nervous system, and the presence of cholinergic excitatory and adrenergic α-excitatory and β-inhibitory receptors has been proposed (1–4). The biliary system is also regulated by humoral agents such as cholecystokinin (CCK) and prostaglandins. The response to CCK has been considered to result from a direct action on the smooth muscle of the gallbladder and sphincter of Oddi, as the effects of CCK or C₈-CCK were not inhibited by pretreatment with autonomic blocking agents (5–7). E- and F-types of prostaglandins occur physiologically in the gastrointestinal tract of various species of animals (8, 9). It was also reported that prostaglandins, particularly prostaglandin E₂, contracted the gallbladder and relaxed the sphincter of Oddi (7), and that prostaglandins play a role in cholecystokininergic activity of the biliary system (10). Several investigators have demonstrated that prostaglandins act on the presynaptic site of adrenergic
nerve terminals in the spleen, oviduct, blood vessels, heart and vas deferens and modulate transmission mechanism (11–16). However, there is still a paucity in information concerning the effects of CCK and prostaglandins on the adrenergic mechanism in the gallbladder and sphincter of Oddi. We carried out studies in an attempt to elucidate the interactions between these substances and the adrenergic mechanism in the biliary system.

MATERIALS AND METHODS

The gallbladder and the terminal portion of the common bile duct (sphincter of Oddi) were isolated from either sex of guinea pigs weighing about 400 g. The gallbladder was cut longitudinally, divided into two portions and both sections used for experiment. The sphincter of Oddi was dissected free from the adjacent duodenum and a sample about 8 mm long from the duodenal end was used.

Mechanical response: Preparations were set vertically in 20-ml organ baths. The lower end was tied to a tissue holder and the upper end was connected to a strain gauge force-displacement transducer. A tension of 1.0 g was applied to the gallbladder and 1.5 g to the sphincter of Oddi. Experiments were started after a one-hour equilibration in normal medium. Responses were recorded isometrically on a biophysiograph. The preparations were mounted in the organ baths containing Krebs solution of the following composition (mM, in distilled and deionized water): NaCl, 120.7; KCl, 5.9; CaCl₂, 2.5; MgCl₂, 1.2; NaH₂PO₄, 1.2; NaHCO₃, 15.5; glucose, 11.5. The medium was adjusted to pH 7.4 at 37°C, and constantly bubbled with a gas mixture of 95% O₂ and 5% CO₂. For electrical transmural stimulation, the preparations were fixed between two platinum plate electrodes in the organ bath. Electrical stimulation was performed submaximally for 10 sec with rectangular pulses of 0.3 msec duration at 20 Hz.

Tritium noradrenaline release: To investigate the release of [³H]-noradrenaline from adrenergic nerve terminals of the gallbladder and sphincter of Oddi, we used a superfusion technique, as described by Kurahashi and Fujiwara (17). Samples were equilibrated for one hour in the organ bath containing Krebs solution, then were transferred to incubation medium containing 0.1 µM of 1-[³H]-noradrenaline (specific activity 6.6 Ci/mmol. Amersham/Searle, Illinois), ascorbic acid (0.1 mg/ml) and EDTA (1.5 µg/ml). After preincubation for one hour at 37°C, these samples were rinsed three times with fresh Krebs solution and mounted in the superfusion apparatus. For electrical stimulation, the preparations were suspended between two platinum wire electrodes and were superfused with Krebs solution. Transmural stimulation was applied supramaximally for 30 sec with rectangular pulses of 0.3 msec duration at 20 Hz. The flow rate was adjusted to 1.0 ml/min using a peristaltic pump. One ml of superfusate was collected into a vial containing 10 ml of Bray's scintillation solution and the radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer.

Since it was confirmed that the spontaneous efflux of ³H-compounds from samples became constant within 60 min, the first electrical stimulation was applied 60 min after the start of superfusion. Five minutes after the first electrical stimulation (control), superfusing Krebs solution was switched to that containing C₈-CCK or prostaglandins. Thirty minutes after this procedure, the second electrical stimulation was performed. Five minutes after the second stimulation the superfusion solution was again changed to normal Krebs medium. Thirty minutes were allowed to pass before the third electrical
The following drugs were used: noradrenaline bitartrate, C-terminal octapeptide of cholecystokinin (SQ 19,844, batch NN005NA, Sincalide, provided by Dr. Welch, the Squibb Institute for Medical Research, Princeton, U.S.A.), prostaglandin E₁, E₂ and F₂α (PGE₁, PGE₂ and PGF₂α) (provided by Ono Pharmaceutical Co. Ltd., Osaka). phen tolamine mesylate, propranolol hydrochloride, tetrodotoxin, atropine sulfate, bretylium tosylate, ascorbic acid, EDTA and ³H-noradrenaline. These drugs except for C₈-CCK, prostaglandins and ³H-noradrenaline were purchased from (Nakarai Chemical Co. Ltd., Kyoto).

RESULTS

Effects of noradrenaline on gallbladder and sphincter of Oddi: Noradrenaline (10⁻¹⁰ to 10⁻⁴ M) produced a relaxation in a dose-dependent manner in two of eleven gallbladder preparations, a contraction in one and no effect in the remaining eight preparations. After treatment with phen tolamine (10⁻⁶ M), however, the same agent induced relaxation in all preparations. By contrast, the agent produced contraction in all preparations after treatment with propranolol (10⁻⁶ M). After the combined treatment with phen tolamine (10⁻⁴ M) and propranolol (10⁻³ M), noradrenaline produced no effect in any of gallbladder preparations. These results confirm that adrenergic receptors in the gallbladder are α-excitatory and β-inhibitory.

In the sphincter of Oddi, noradrenaline at low concentrations (10⁻⁷ to 10⁻⁵ M) produced a relaxation, and with high concentrations (10⁻³ to 10⁻¹ M), a contraction. After treatment with propranolol (10⁻⁶ M), the relaxation was significantly reduced but not abolished, and the contractile response was enhanced (Fig. 1a). After treatment with phen tolamine (10⁻⁶ M), both relaxing and contractile responses were inhibited significantly (Fig. 1b). After combined treatment with both propranolol (10⁻⁶ M) and

![Graph](image-url)  
Fig. 1. Response of the sphincter of Oddi to noradrenaline applied cumulatively and the effects of propranolol (10⁻⁶ M), phen tolamine (10⁻⁶ M) and propranolol (10⁻⁶ M) plus phen tolamine (10⁻⁶ M). Closed circles indicate controls and open circles the treatment with propranolol (10⁻⁶ M) (a) or phen tolamine (10⁻⁶ M) (b). Open triangles indicate treatment with both propranolol (10⁻⁶ M) and phen tolamine (10⁻⁶ M) (a and b). The vertical bars represent the standard error of means. The sign of (*) and (**) mean p value when compared with corresponding controls. (*): p<0.05 and (***): p<0.01. (8 experiments)
Phentolamine (10^{-6} M), the relaxing response of the sphincter of Oddi to low concentrations of noradrenaline was abolished. Phenylephrine at low concentrations (10^{-7} M and 10^{-6} M) produced a relaxation (-0.02 ±0.01 g and -0.05±0.02 g, respectively), and at a high concentration (10^{-5} M) a contraction (0.07±0.02 g), and both responses were abolished by phentolamine (10^{-6} M).

**Effects of C8-CCK and prostaglandins on noradrenaline-induced responses:** Effects of C8-CCK and prostaglandins on contractile or relaxing responses to noradrenaline were examined. The concentrations of C8-CCK and prostaglandins were 10^{-9} g/ml, at which concentrations these agents themselves failed to induce any contractile or relaxing response.

In the gallbladder preparation, the relaxing response to noradrenaline in the presence of phentolamine (10^{-6} M) was enhanced by treatment with C8-CCK (10^{-8} g/ml), and the contractile response to noradrenaline in the presence of propranolol (10^{-6} M) was also enhanced by the same concentration of C8-CCK (Fig. 2a). PGE_{1}, E_{2} and F_{2α} also enhanced the contractile response to noradrenaline in the presence of propranolol (10^{-6} M), while these agents did not affect the relaxing response in the presence of phentolamine (10^{-6} M) (Fig. 3a). In the presence of both propranolol (10^{-6} M) and phentolamine (10^{-6} M), noradrenaline produced no response in the gallbladder and there were no apparent effects of C8-CCK.

**Fig. 2.** Effects of C8-CCK on the responses of the gallbladder (a) and sphincter of Oddi (b) to noradrenaline after treatment with propranolol (10^{-6} M) or phentolamine (10^{-6} M). Open circles, open triangles and open squares are responses to noradrenaline after treatment with propranolol (10^{-6} M), and closed circles, closed triangles and closed squares are those after treatment with phentolamine (10^{-6} M). Circles (both open and closed) are controls, triangles (both open and closed) are in the presence of C8-CCK (10^{-9} g/ml), and squares (both open and closed) are in the presence of C8-CCK (10^{-8} g/ml). The vertical bars represent the standard error of means. (*) means p<0.05 when compared with the control. Note that the scale of tension is larger than in Fig. 1.

**Fig. 3.** Effects of PGE_{1} on the responses of the gallbladder (a) and the sphincter of Oddi (b) to noradrenaline after treatment with propranolol (10^{-6} M) or phentolamine (10^{-6} M). Open circles, open triangles and open squares indicate responses to noradrenaline after treatment with propranolol (10^{-6} M), and closed circles, closed triangles and closed squares indicate those after treatment with phentolamine (10^{-6} M). Circles (both open and closed) are controls. Triangles (both open and closed) are in the presence of PGE_{1} (10^{-10} g/ml). Squares (both open and closed) are in the presence of PGE_{1} (10^{-9} g/ml). The sign of (*) and (**) mean p value when compared with control. (*) p<0.05, (**) p<0.01. Note that the scale of tension is larger than in Fig. 1.
and prostaglandins on the noradrenaline-induced response of the gallbladder.

On the other hand, the same treatment with C₈-CCK or prostaglandins little affected the response of the sphincter of Oddi to noradrenaline (Fig. 2b and Fig. 3b).

Effects of C₈-CCK and prostaglandins on tritium efflux: In the gallbladder and sphincter of Oddi which had been previously incubated with ³H-noradrenaline, spontaneous ³H-efflux from both tissues decreased sharply and reached a steady level within 60 min. Application of transmural stimulation (20 Hz, 0.3 msec duration, 20 V for 30 sec) produced phasic contractions in both preparations and such were blocked by atropine (10⁻⁶ M). There was an increase in tritium efflux from both the gallbladder and sphincter of Oddi, and such was blocked by treatment with 10⁻⁵ M bretylium or 10⁻⁷ M tetrodotoxin.

C₈-CCK (10⁻¹⁰ to 10⁻⁷ g/ml) affected neither the increase in tritium efflux induced by electrical stimulation nor spontaneous tritium efflux in both the gallbladder and sphincter of Oddi (Fig. 4). On the other hand, PGE₁, PGE₂ and PGF₂α (10⁻⁹ to 10⁻⁷ g/ml) significantly attenuated the increase in stimulation-induced tritium efflux in both preparations without affecting the spontaneous tritium efflux (Fig. 5). The inhibitory effects of prostaglandins on tritium release in the sphincter of Oddi were greater than those observed in the gallbladder (Table 1). C₈-CCK and prostaglandins potentiated the phasic contraction of the gallbladder, while that of the sphincter of Oddi was little affected.

DISCUSSION

Excitatory α- and inhibitory β-adrenoceptors have been demonstrated in tissues such as the sphincter of Oddi and gallbladder (1-4). However, the response of the sphincter of Oddi to noradrenaline seen in the present study cannot be simply explained by the α-excitatory and β-inhibitory mechanisms, since the relaxation seen with low concentrations of noradrenaline was reduced by propranolol or phentolamine. Furthermore, the combined treatment with both propranolol and phentolamine abolished the relaxation. Thus, it is tempting to assume that both α-
Table 1. Effects of C8-CCK and prostaglandins on the 3H-efflux from gallbladder and sphincter of Oddi

<table>
<thead>
<tr>
<th>Drug (g/ml)</th>
<th>Gallbladder</th>
<th>Sphincter of Oddi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>treated with</td>
<td>after washing with drug free medium</td>
</tr>
<tr>
<td></td>
<td>drug (N)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>91.4±3.8 (3)</td>
<td>70.5±0.9 (3)</td>
</tr>
<tr>
<td>C8-CCK (10^{-7})</td>
<td>92.6±2.2 (6)</td>
<td>70.3±1.6 (6)</td>
</tr>
<tr>
<td>PGE1 (10^{-7})</td>
<td>70.8±2.4* (4)</td>
<td>70.7±0.5 (4)</td>
</tr>
<tr>
<td>PGE2 (10^{-7})</td>
<td>65.2±3.2* (5)</td>
<td>79.7±7.7 (5)</td>
</tr>
<tr>
<td>PGF2α (10^{-7})</td>
<td>71.0±1.5* (3)</td>
<td>71.1±5.5 (3)</td>
</tr>
</tbody>
</table>

*: p<0.01 (when compared with respective control)
N: number of experiments

and β-adrenoceptors in the sphincter of Oddi are inhibitory, as is the case with these receptors in the gastrointestinal tract. On the other hand, the response of the sphincter of Oddi to high concentrations of noradrenaline (10^{-8} to 10^{-4} M) can be explained by α-excitative and β-inhibitory adrenergic mechanisms, as already reported, since the contractile response to high concentrations of noradrenaline was enhanced by pretreatment with propranolol and was inhibited by phentolamine. The discrepancy between our findings and those of other investigators may be due to different preparations and higher concentrations of sympathomimetic drugs (1-4).

Activation of the α-adrenoceptors in the sphincter of Oddi induced opposite responses, depending on the concentration of noradrenaline. A possible explanation is that there may be two types of α-receptors in the sphincter of Oddi. In this transitional region from the duodenum to the gallbladder, there may be an intermingling of a large number of inhibitory α- and a few excitatory α-receptors. Thus, the inhibitory α-receptors are activated first with low concentrations of noradrenaline, and the excitatory α-receptor activated only with high concentrations of this compound.

The application of C8-CCK or prostaglandins did not affect the response of the sphincter of Oddi to noradrenaline, while the responses of the gallbladder were variably affected by either C8-CCK or prostaglandins. C8-CCK affected not only α-receptor but also β-receptor mediated responses of the gallbladder, while prostaglandins affected only α-receptor mediated contractile responses of the gallbladder. Thus, adrenoceptors in the gallbladder are sensitized by humoral agents such as C8-CCK and prostaglandins. In preliminary experiments we found that these agents produced contractile responses in both the gallbladder and sphincter of Oddi preparations. However, the responses of the sphincter of Oddi were considerably smaller than those of the gallbladder. Together with a lower reactivity to noradrenaline, the sensitizing effect of C8-CCK and prostaglandins on adrenoceptors may also be weak in the sphincter of Oddi.

The question arises as to how C8-CCK and prostaglandins affect the adrenergic nerve terminals in the gallbladder and sphincter of Oddi. Application of transmural stimulation produced an increase in tritium efflux from both the gallbladder and sphincter of Oddi, and this increase was blocked by treatment with 10^{-5} M bretylium and/or 10^{-7} M tetrodotoxin. Thus, these increases in tritium
efflux are probably due to \(^{3}H\)-noradrenaline and metabolites released from the adrenergic nerve terminals. \(C_{6}\)-CCK had no effect on the stimulation-induced increase in tritium efflux from either preparation. On the other hand, PGE\(_1\), PGE\(_2\) and PGF\(_{2\alpha}\) significantly decreased the tritium efflux from both preparations. Such presynaptic inhibitory effects of prostaglandins in the adrenergic nerve terminals have also been observed in the vas deferens, oviduct, spleen and heart (11–16).

In conclusion, the adrenergic mechanisms in the biliary system of guinea pigs are modulated by humoral agents, that is, \(C_{6}\)-CCK and prostaglandins enhanced postsynaptically the responses of the gallbladder to noradrenaline, and prostaglandins exerted a presynaptic inhibitory effect on adrenergic nerve terminals in the gallbladder and sphincter of Oddi of guinea pigs.

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