Discussion. Changes in intracellular pH induced by changes in perfusate pCO_2 can be monitored in intact, toad ventricle strips by optical monitoring of neutral red absorption spectra. This technique allows simultaneous measurement of cytochrome chain redox state and pH_i. While quantitative values are not accurate, calibration of the changes in pH can be accomplished by comparison with changes in bath pCO_2.

In these muscles, almost half of the developed tension can be supported by the small amount of residual oxygen in the bath during nitrogen bubbling and anaerobic metabolism. The effect of the acidification of the tissue by bubbling with 5% CO_2 in oxygen on the developed tension is probably due to an effect on that part of the tension supported by anaerobic metabolism since there is little change in cytochrome redox state (figure 2B). Larger acidification has an effect on both aerobic and anaerobic processes as suggested by figure 2A.

The suggestion that the effects of hypoxia and acidosis on mechanical function are additive[14] is supported by the almost additive decrease in tension which accompanies both nitrogen bubbling and 5% CO_2 in nitrogen. This is probably due to the decreased uptake of substrates such as glucose and lactate[14]. The combination of both aerobic and anaerobic support of mechanical function may explain the finding that the oxidative metabolic change and decrease in mechanical function begin prior to the fall in intracellular pH[15].

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Enhancement of electrically stimulated guinea-pig gallbladder contraction by subthreshold concentrations of gastrointestinal hormones in vitro[1]

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Summary. Concentrations of the octapeptide of cholecystokinin and pentagastrin, which alone do not contract the gallbladder, enhance the gallbladder contraction in response to electrical stimulation in vitro.

At present the neural and hormonal interaction in regulating the motor activity of the gallbladder is poorly understood and has been studied mainly in vivo[2,3]. The present study was carried out to investigate how subthreshold concentrations of cholecystokinin-octapeptide (CCK-OP), pentagastrin (PG) or secretin (S) influence the effects of transmural electrical stimulation (to mimick vagal excitation) on the isolated guinea-pig gallbladder.

Methods. Isolated guinea-pig gallbladders were longitudinally fixed at 37 °C in an organ bath of 100 ml with modified Krebs-Henseleit solution (NaCl 111, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 0.92, $MgSO_4$ 0.88, glucose 16.7 mM) aerated with 95% O_2 and 5% CO_2. A small platinum wire electrode was inserted through a hole in the fundus, fixed by a purse string suture and connected to an isometric strain gauge transducer (Grass FT 03 B). A circular platinum wire electrode (25 mm ⌀) was placed around the gallbladder. The preload was adjusted between 2.5 and 6.5 g so that an optimal ratio between spontaneous and electrically stimulated activity (<0.75) was obtained. The gallbladder was stimulated with rectangular electrical impulses of 20 V and 1 msec duration at a frequency of 10 Hz for 5 min followed by 5 min rest. After 6 stimulation cycles, CCK-OP, S, or PG was added in decreasing concentrations to determine the threshold concentration. 5 min after each test dose the bath was rinsed 2 or 3 times. Subsequently the first subthreshold concentration (approximately half of the threshold) was added and another 6 stimulation cycles were completed with the hormones remaining in the bath. To rule out the possibility that the contractile response to electrical stimulation alone changed during the course of the experiment, another group of 8 experiments were carried out where after 6 cycles the bath was simply refilled with fresh solution.

The effect of atropine on stimulated gallbladder contraction was studied as follows: after all preliminaries (see above) electrical stimulation (2 5-min cycles with 5 min interval) was applied as described. After 5 min rest, acetylcholine (ACh) was added to the bath to give a final concentration of 7×10^{-5} M, and after washing out ACh CCK-OP was added at a final concentration of 2×10^{-9} M. After further washing, starting with the lowest concentration of atropine, the next group of stimulation was applied until no further increase in atropine concentration seemed to be useful.

The average contraction of each 5-min cycle was evaluated planimetrically. For each group of tests, the means of the 6 contractions before adding the hormone were compared with the mean of the 6 contractions after addition of the hormones and analysed by t-test for paired comparison.

Materials. Pentagastrin = Gastrodiagnost® (kindly supplied by Dr Wendt, E. Merck, Darmstadt), acetylcholine chloride, and atropine sulphate (E. Merck, Darmstadt), natural secretin, batch No. 17561 (Karolinska Institutet, Stockholm), synthetic cholecystokinin-octapeptide (kindly supplied by the Squibb Institute for medical Research).

Results. All preparations displayed rhythmical spontaneous activity with a frequency varying between 10 and 20 min^{-1}. The amplitude increased with increasing preload to maximally 1 g, but was usually around 0.1 g. The basal tone varied from preparation to preparation, and was on the average during the 2nd 6 cycles of electrical stimulation higher than during the 1st 6 cycles. This phenomenon was seen whether the hormones were given or not. Electrical stimulation caused the gallbladder to contract with a force equivalent to 0.875 ± 0.099 g (mean ± SEM). This contractile response was always less than the maximal contraction induced by ACh or CCK-OP. The contraction in response to electrical stimulation was blocked by concentrations of atropine which were smaller than those

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necessary to block the ACh response. The CCK-OP effect was reduced only by extremely high concentrations of atropine (figure 1).

PG caused a contraction of the gallbladder with a threshold concentration varying from 3.9 to 15.6×10^{-8} M. Accordingly the subthreshold concentration was $13–78 \times 10^{-9}$ M. Secretin in concentrations up to 2.9×10^{-8} M was ineffective. According to Chowdhury et al.[4], a background dose of 6×10^{-9} M was chosen. The threshold concentration of CCK-OP was $3–9 \times 10^{-11}$ M, the subthreshold concentrations used were $2–4 \times 10^{-11}$ M.

In the control group the contractile response remained unchanged: 1.061 ± 0.240 g (mean $\pm$ SEM) for the 1st 6 contractions and 1.055 ± 0.233 g for the 2nd. A significant increase in the contraction in response to electrical stimulation was found after subthreshold concentrations of PG ($p < 0.05$) and CCK-OP ($p < 0.01$). The slight increase after secretin was statistically not significant (figure 2).

Discussion. Since contractions of the gallbladder in response to both electrical stimulation and ACh are blocked by atropine almost to the same extent, electrical stimulation can be regarded as an equivalent to vagal stimulation in vivo. Concentrations of PG and CCK-OP which alone do not contract the isolated guinea-pig gallbladder after exposure of the organ to subthreshold concentrations of the hormones enhanced contraction in response to electrical stimulation. The conclusion seems justified that, even on a subthreshold level, at least on the hormonal side a synergistic action with the neural side is possible. The threshold concentration of CCK-OP is comparable to that found in the same species by Yau et al.[5]. It seems therefore likely that the neuro-hormonal potentiating effect found by Pallin and Skoglund[2] has to be attributed to the same principle of interaction as described above and – since obtained in vitro – takes place at the target organ. The failure of atropine to block in lower concentrations the CCK-OP effect confirms Hedner et al.[6] and Yau et al.[5] and excludes endogenously released ACh as a mediator of CCK-OP for gallbladder contraction.

Several authors have shown a spasmogenic effect of gastrin or PG on gallbladder muscle preparations in vivo and in vitro. However, this effect was not considered to be physiological because of the lower potency of gastrin and synthetic derivatives on a molar base as compared with CCK[4,7]. In contrast, Toouli and Watts[8], working with isolated human and canine gallbladder preparations, concluded that gastrin is a physiological rather than a pharmacological stimulant. The subthreshold concentration which was shown in our experiments to affect gallbladder contraction in vitro was smaller than the concentration necessary to stimulate gastric acid secretion in the isolated guinea-pig stomach (0.3–1.0 µg/ml) as reported by Holton and Spencer[9]. However, it must be emphasized that the threshold concentration of PG for contraction of the isolated gallbladder is approximately 1600 times higher than that of CCK-OP. Thus it seems unlikely that gastrin in combination with other factors, such as the cholinergic nervous system, plays a permissive role in regulating gallbladder contraction under physiological conditions, since these high plasma concentrations are unlikely to occur.

A cholecystokinetic activity of S in vivo was found by Lin and Spray[10], and Davidson and Foesel[3], but not by Vagne and Troitskaja[11]. From studies with human, feline and canine gallbladder strips, it is also controversial whether secretin is effective in vitro. The differences observed do not seem to be species-dependent. It is also controversial whether S in vitro contracts the gallbladder[8,12–14]. It is known that the effect of CCK is enhanced by S[4,11,14]. Although some of the S preparations contain CCK that alone cannot sufficiently explain the positive results.

From the present experiments it becomes likely that circulating concentrations of CCK, which alone are inactive can facilitate gallbladder contraction elicited by nervous excitation. This cooperative action could allow the neuroendocrine system to operate on a more economic level.

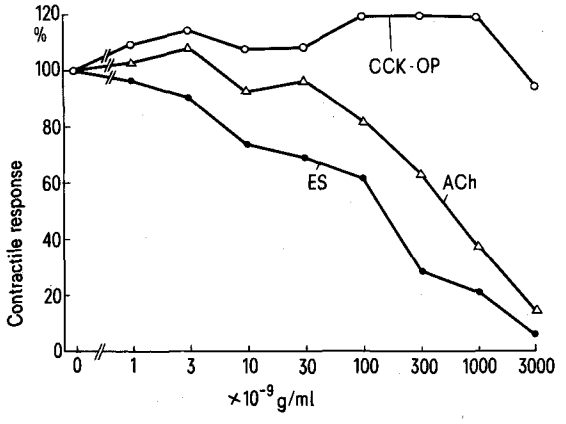

Fig. 1. Effect of increasing concentrations of atropine on maximally stimulated contraction of the isolated guinea-pig gallbladder in response to CCK-OP (○), ACh (△), and electrical stimulation (●) expressed as percentage of the response without atropine (N = 4).

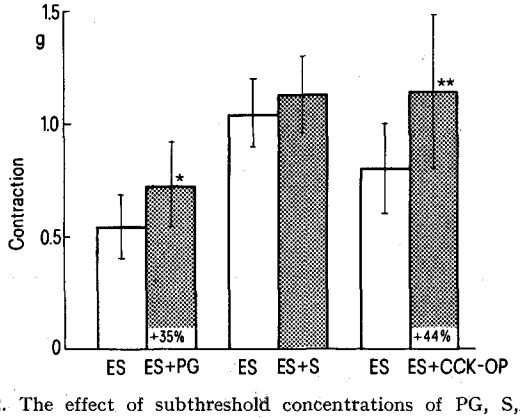

Fig. 2. The effect of subthreshold concentrations of PG, S, and CCK-OP on contraction of the isolated guinea-pig gallbladder in response to electrical stimulation (ES). N = 6; mean $\pm$ SEM; * $p < 0.05$; ** $p < 0.01$.

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