A glucagon-secretin-like peptide stimulates the intrinsic nervous plexus of guinea pig gallbladder

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Summary. The role of vasoactive intestinal peptide (VIP), as a possible neurotransmitter of the intrinsic nerve plexus in the guinea pig gallbladder, was investigated by monitoring spontaneous contractile activity. VIP receptor antagonist (4 Cl-D-Phe^6, Leu^{17})-VIP did not produce any effect on muscular tone and spontaneous activity, whereas (N-Ac-Tyr^1, D-Phe^2)-GRF-(1–29)-NH_2, (14-GRF analog), which is known to stimulate digestive enzyme secretion by interacting with the VIP-preferring receptors, greatly increased the amplitude and frequency of waves as well as the muscular tone. Since VIP receptor antagonist acts selectively as a competitive antagonist for the action of VIP, we conclude that the gallbladder inhibitory intrinsic plexus neurotransmitter is not VIP, but a member of the glucagon-secretin family of peptides.
Key words. VIP; gallbladder guinea pig; inhibitory intrinsic plexus; 14-GRF analog; VIP-antagonist.

The existence of non-cholinergic, non-adrenergic, inhibitory neurons has been demonstrated in the guinea pig gallbladder wall by Davison et al.[1]. These neurons can be activated by vagal preganglionic fibers across conventional nicotinic synapses[2].

Vasoactive intestinal peptide (VIP), a hormone found at high levels in the gastrointestinal tract, has recently been localized in a widely-distributed system of nerves in the gut wall[3,4], as well as in the guinea pig gallbladder[5]. Among known effects of VIP are the relaxation of the gallbladder[6,7] and the decrease of cholecystokinin-induced contractions[8].

In an effort to gain a better understanding of the nature of neurotransmitters of the intrinsic inhibitory plexus of the guinea pig gallbladder, we performed a systematic study involving the use of (4 Cl-D-Phe^6, Leu^{17}) VIP and 14-GRF analog as competitive antagonists of the action of VIP and other peptides of the glucagon-secretin family. (4 Cl-D-Phe^6, Leu^{17}) VIP[9] selectively antagonizes the action of the VIP receptors, whereas the 14-GRF analog[10] acts on both the VIP receptors and those of other members of the glucagon-secretin family of peptides.

Materials and methods

Experiments were performed using gallbladders isolated from 5 guinea pigs of both sexes (b.wt 300–500 g). The animals were anesthetized with Nembutal (30 mg/kg b.wt i.p.) and the gallbladder was quickly removed. Gallbladder strips, 3 mm wide and 10 mm long, were placed vertically in a 10-ml bath of Krebs solution (pH 7.4) containing (mMol/l): NaCl 118.0; KCl 4.70; $CaCl_2$ 2.52; $MgSO_4$ 1.64; $NaHCO_3$ 24.88 and glucose 5.55. The bath was maintained at 37 °C using a circulating immersion heater and bubbled with a 95% O_2 and 5% CO_2 gas mixture. Pre-warmed Krebs solution was continuously infused into the chamber at a rate of 1.5 ml/min throughout the experiment, except during the pharmacological trials.

One end of the gallbladder strip was tied to a hook on the bottom of the chamber and the opposite end was attached to an isometric force transducer (FTA-100 Sanborn, USA).

The transducer was connected to a chart recorder (JJ Instruments, Elis, Italy). A resting tension of 200 mg was applied to the strips and they were allowed to equilibrate for 30 min. Changes in muscle tension were measured in grams as deflection from baseline tension. The initial tension was assigned a value of zero.

Tetrodoxin (TTX) was purchased from Sigma (Dorset, England); vasoactive intestinal peptide (VIP, guinea pig), (N-Ac-Tyr^1, D-Phe^2)-GRF-(1–29)-NH_2 and VIP receptor antagonist (4 Cl-D-Phe^6, Leu^{17}) VIP were obtained from Peninsula Laboratories (St. Helens, England).

The effect of TTX (3.13×10^{-7} M) on spontaneous contractile activity of gallbladder strips was determined.

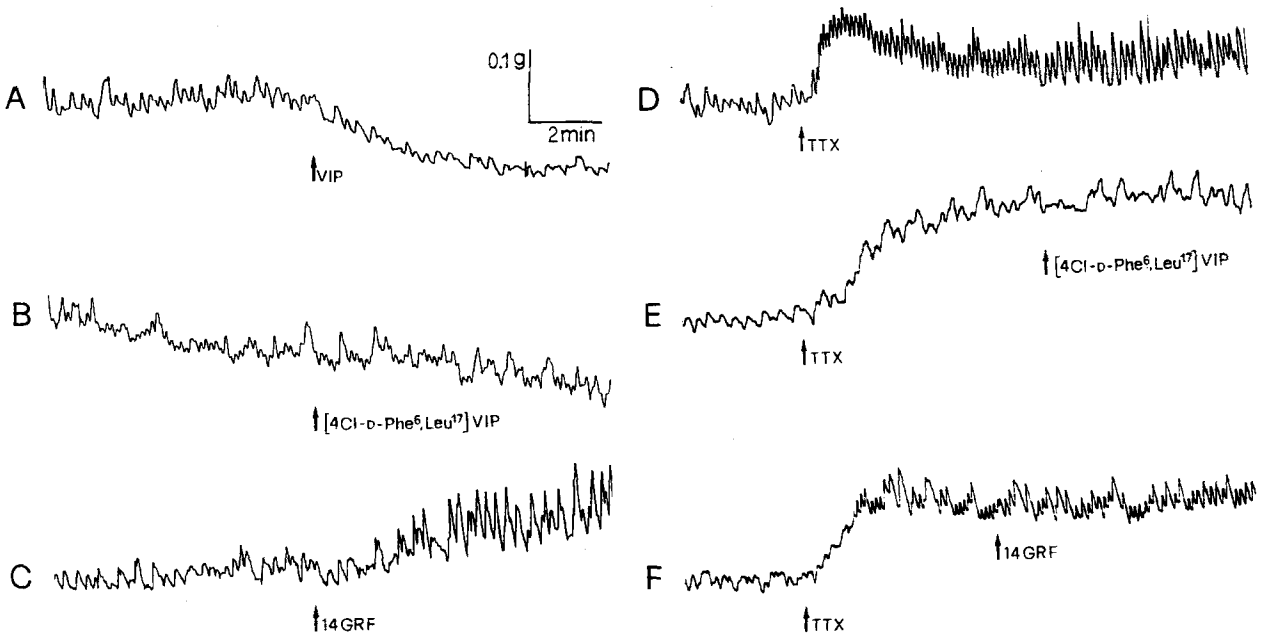
Increasing amounts of VIP, in a range from 10 to 100 ng, were tested on gallbladder strips by adding the peptide to the bath. However, the changes of both amplitude and frequency of spontaneous contractile waves and gallbladder tone were not evaluated.

Amounts of 14-GRF analog in a range of 10–100 ng or VIP-receptor antagonist in a range of 100–500 ng were added to the bath in a separate series of experiments.

Finally, the effect of the maximal doses of both 14-GRF analog (100 ng) and VIP-receptor antagonist (100 ng) on gallbladder strips was determined after TTX administration. Following each pharmacological manipulation the muscle chamber was flushed with at least 50 ml of fresh pre-warmed Krebs' solution and then equilibrated for 20 min before the next trial.

Results

After an equilibration period of about 30 min, the circular gallbladder strips showed rhythmic spontaneous contraction appearing as axial force waves. The wave-forms of the force developed in a single contraction were usually oscillatory, containing two or more peaks which were



Effects of VIP, receptor antagonist (4 Cl-D-Phe^6, Leu^{17})-VIP and VIP analog (14-GRF) on the spontaneous mechanical activity of guinea pig gallbladder strips. *A* VIP induced a decrease in resting tension and abolished spontaneous mechanical activity. *B* (4 Cl-D-Phe^6, Leu^{17})-VIP did not modify either the muscular tone or the spontaneous contractile activity. *C* Increased muscular tone and increased amplitude and frequency of spontaneous contraction waves after addition of VIP antagonist 14-GRF analog were found. *D* Tetrodoxin induced rise in tension of the muscular strip and an increase in the frequency and amplitude of spontaneous contraction waves. *E* (4 Cl-D-Phe^6, Leu^{17})-VIP did not modify mechanical activity recorded after TTX administration. *F* 14-GRF analog did not induce further increases in mechanical activity after TTX administration.

more or less separated. The number of oscillations and the overall pattern of these waveforms changed considerably during the course of the same experiments. No effect on muscle tone was observed when the medium flow was interrupted during the pharmacological trials.

Preliminary experiments demonstrated the effective action of VIP-receptor antagonist on VIP by abolishing the inhibitory effect of VIP on gallbladder muscular tone.

The figure (A) shows the effect of VIP on spontaneous contractile activity in guinea pig gallbladder strips. VIP (100 ng, the submaximal dose) induced both strip relaxation, as seen by a marked drop in the tone, and a decrease in the amplitude of the spontaneous contractile waves. (4 Cl-D-Phe^6, Leu^{17}) VIP (100–500 ng), which is a selective competitive inhibitor for guinea pig vasoactive intestinal peptide receptors [10] did not modify either the distensibility of the gallbladder strips or the amplitude of spontaneous contractile waves (fig., B). On the contrary, when the GRF analog (N-Ac-Tyr^1, D-Phe^2)-GRF-(1–29)-NH_2 (100 ng), which interacts with both pancreatic VIP receptors and receptors of other members of the glucagon-secretin family of peptides [9], was added to the bath, an increase in both the frequency and amplitude of force waves was observed simultaneously, in addition to an increase in the tone of the gallbladder strips (fig., C). The addition of TTX (3.13×10^{-7} M) to the bath caused a loss of gallbladder distensibility and increased the spontaneous contraction activity (fig., D). When VIP antagonist followed the addition of TTX to the bath, no increase in either amplitude or frequency of spontaneous contraction waves was found (fig., E).

Similarly, when 14-GRF analog was added to the bath after TTX, no further increase in the amplitude and frequency of spontaneous gallbladder waves was observed (fig., F).

Discussion

Guinea pig gallbladder strips showed a spontaneous mechanical activity characterized by rhythmic contraction waves, identical to those recently observed in whole gallbladders; the addition of TTX to the bath did not abolish spontaneous contractile waves, confirming the myogenic nature of this activity [11]. The loss of gallbladder distensibility after TTX treatment also suggests that the intrinsic neural activity was predominantly inhibitory. Davison et al. [1] proposed VIP as a possible neurotransmitter of the inhibitory plexus. VIP is a peptide chemically related to secretin [12, 13], which has been reported to relax isolated superfused guinea pig gallbladders [14] and to antagonize the contractile effects of CCK in vivo [15]. Moreover, VIP-like immunoreactivity has been localized in nerve fibers in the gallbladder wall [6].

In order to verify whether VIP is an endogenous inhibiting neurotransmitter of the gallbladder or not, we performed experiments using either a selective inhibitor of VIP receptors or an aspecific inhibitor of the secretin-glucagon family. Selective vasoactive intestinal receptor

antagonist (4 Cl-D-Phe^6, Leu^{17})-VIP did not produce any effect on spontaneous contractile activity. On the contrary, 14-GRF analog, which specifically interferes with VIP receptors, induced an evident increase in both tone and spontaneous contraction waves. The fact that the 14-GRF analog did not produce any increase in muscular tone after pharmacological blockade of the intrinsic nerve plexus, obtained by TTX administration, demonstrated that the action of this peptide is not myogenic, but is rather directed at the intrinsic nerve plexus. These findings suggest that a member of the glucagon-secretin family of peptides may be a neurotransmitter in the inhibitory plexus of guinea pig gallbladder, but VIP is not.

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1 Davison, J. S., Al-Hassani, M., Crowe, R., and Burnstock, G., Pflügers Arch. *377* (1978) 43.
2 Davison, J. S., and Al-Hassani, M., in: Gastrointestinal Motility, p. 89. Ed. J. Christensen. Raven Press, New York 1980.
3 Larsson, L. I., Fahrenkrug, J., Schaffalitzky, De Muckadell, O., Sundler, F., Hakanson, R., and Rehfeld, J. F., Proc. natl Acad. Sci. USA *73* (1976) 3197.
4 Bryant, M. G., Bloom, S. R., Polak, J. M., Albuquerque, R. H., Modlin, J., and Pearse, A. G. E., Lancet *1* (1976) 991.
5 Sundler, F. J., Alumets, J., Hakanson, R., Ingemansson, S., Fahrenkrug, J., Schaffalitzky, D., and Muckadel, O., Gastroenterology *72* (1977) 1375.
6 Jansson, R., Steen, G., and Svanvik, J., Gastroenterology *75* (1978) 47.
7 Said, S. J., Gastroenterology *67* (1974) 735.
8 Said, S. J., and Makhlouf, G. M., in: Endocrinology of the Gut, p. 83. Eds W. J. Chey and F. P. Brooks. C. B. Slack, Thorofare, NJ 1974.
9 Pandol, S. J., Seifert, H., Thomas, M. W., Rivier, J., and Vale, W., Science *225* (1984) 326.
10 Pandol, S. J., Dharmsathaphorn, K., Schoeffield, M. S., Vale, W., and Rivier, J., Am. J. Physiol. *250* (1986) G553.
11 Mancinelli, R., Racanicchi, C., Mingrone, G., Arcieri-Mastromattei, E., and Greco, A. V., Q. J. exp. Physiol. *74* (1989) 429.
12 Said, S. J., and Mutt, V., Science *169* (1970) 1217.
13 Said, S. J., and Mutt, V., Eur. J. Biochem. *28* (1973) 199.
14 Piper, P. J., Said, S. J., and Vane, J. P., Nature *225* (1970) 1144.
15 Vagne, M., and Troitskoja, V., Digestion *14* (1976) 62.

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Changes in red blood cell choline and choline-bound lipids with oral lithium

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Summary. The influence of oral lithium on the concentration of red blood cell choline (Ch), lecithin, glycerophosphorylcholine (GPCh) and phosphorylcholine (PCh) was studied. The concentration of RBC Ch was significantly elevated and the concentration of lecithin, GPCh and PCh significantly depressed in 16 patients on oral lithium compared to 9 age-matched controls. We conclude that lithium markedly depletes the red blood cell of choline containing compounds including lecithin. These changes may be responsible for both the therapeutic efficacy and the toxicity of lithium.
Key words. Lithium; mechanism; choline; lecithin; glycerophosphorylcholine; phosphorylcholine.

Lithium (Li) is the treatment of choice for patients with bipolar disorders and is utilized by millions of people yearly worldwide[1]. Its mechanism of action is unknown although Janowsky et al. have proposed that it changes the relative balance between the cholinergic/adrenergic systems[2]. In addition, it alters phosphatidylinositol turnover[3] and increases red blood cell (RBC) glycine[4]. Li has dramatic effects on the transport and concentration of choline (Ch) in RBC. Martin was the first to demonstrate that Li blocked the transport of Ch into and out of the RBC in human subjects[5] and Jobe et al.[6] showed that Li led to an approximately tenfold rise of RBC Ch. Jenden[6,7] and Hanin[8] also demonstrated that the expected rise of RBC Ch could be predicted with a kinetic model that they developed. No sustained increases in brain Ch have been demonstrated in animals on chronic Li therapy. However, animal models may not be appropriate to study the effects of Li in man as Miller et al.[9] have demonstrated that in ten different non-human species, Li did not elevate RBC Ch even though the dosage administered resulted in what would have been 'therapeutic levels' of Li in human subjects.

The long-term effect of Li on Ch metabolism in man is unknown. Specifically there have been no studies to demonstrate what prolonged block of Ch transport will do to the concentration of lecithin in the membrane or other Ch containing compounds such as glycerophosphorylcholine (GPCh) or phosphorylcholine (PCh). We have measured RBC Ch, lecithin, GPCh, and PCh in 9 normals and 16 patients on Li therapy for more than one week. The changes in these compounds are described and possible implications for therapy and toxicity are discussed.