evolve to disproportionately infect genetically uniform strains as they become common. The results are also consistent with our assumption of a genetic basis to the host–parasite interaction, and with the expectation that sexual reproduction provides a partial escape from biological enemies only in genotypically diverse populations. □

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A cholecystokinin-like hormone activates a feeding-related neural circuit in lobster

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THE peptide hormone cholecystokinin (CCK) contributes to the production of feeding-related behaviour in mammals, but the mechanism by which it exerts its effects remains unclear[1–6]. The gastric mill neural circuit of lobster is an experimentally accessible model system for studying the hormonal control of feeding-related behaviour[7,8]. Composed of 11 identified neurons, this circuit produces rhythmic movement of teeth within the stomach[9]. We have previously shown that the gastric mill motor pattern can be modulated by a cholecystokinin-like peptide *in vitro*[10]. We report here that (1) after feeding, levels of CCK-like peptide in haemolymph increase with the activation of the gastric mill, (2) injections of CCK activate the gastric mill, and (3) a specific CCK antagonist inhibits feeding-induced gastric mill activity. This neatly demonstrates a causal link between *in vivo* release of a peptide hormone and activation of a neural circuit.

In several vertebrates and invertebrates, blood levels of CCK-like peptide (CCKLP) rise after feeding[11–14]. We compared changes in blood CCKLP levels in the lobster *Panulirus interruptus* with the activation of the gastric mill (GM) following feeding. GM activity was monitored in freely moving lobsters by recording the activity of the gm1 muscle, which protracts the medial tooth of the GM. In individual experiments, the GM began cycling immediately after feeding, cycled continuously for 2–6 h, then the burst strength and frequency declined and activity became intermittent; this intermittent activity could continue for up to 48 h (Fig. 1*a*). On average, the GM reached its peak frequency during the first hour after feeding; the average frequency then declined over the next 3 h, but remained above base line for the next 4 h (Fig. 1*b*).

CCK-like peptide is found in lobster neurohaemal organs and haemolymph (G.G.T. and A.I.S., manuscript submitted). Here we use a radioimmunoassay to monitor the levels of CCKLP in haemolymph before and after feeding. There was a roughly fourfold increase in the levels of CCKLP in the first hour after feeding, peaking at an average of $(1.6 \pm 0.5) \times 10^{-9}$ M, and levels

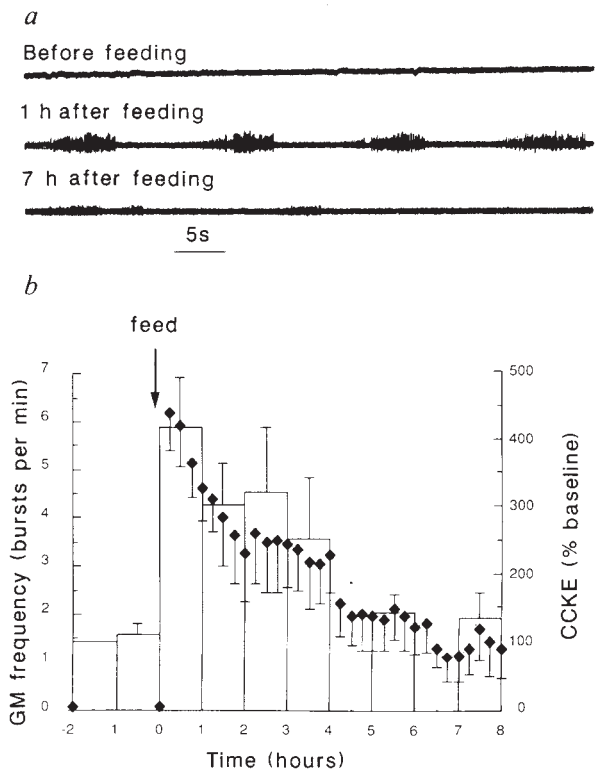

FIG. 1 Activity of the GM, and levels of CCKLP in haemolymph, before and after feeding. *a*, Gm1 muscle activity in one animal before feeding, at 1 h after feeding, and at 7 h after feeding. Before feeding there is no GM activity. At 1 h, the GM is cycling regularly, with a period of ~10 s. At 7 h the GM is cycling intermittently, and the individual muscle bursts are weaker than at 1 h. *b*, Average GM activity and average CCKLP levels in the haemolymph before and after feeding. Diamonds, average GM frequency, mean ±s.e.m. ($n = 6$). Histograms, average CCKLP levels in haemolymph, mean ±s.e.m. ($n = 7$); levels were significantly raised for the first 2 h after feeding ($P < 0.01$, Student's *t*-test).

METHODS. Myograms: Lobsters were starved for 1–3 weeks, kept on an artificial light–dark cycle, and fed ~10 g smelt or squid in the dark. Myograms were obtained from unrestrained animals by implanting teflon-coated silver wire electrodes into the gm1 muscle; electrode placement was verified at the end of the experiment[30]. Animals recovered 2–7 days before initiation of experiments. Signals were differentially amplified, recorded on videocassette, and displayed with a Gould ES1000 electrostatic recorder. Generally 16 h of activity were recorded; activity was then monitored intermittently for up to 36 h. Muscle spikes were converted into constant-voltage pulses using a Schmidt trigger; pulses were digitized and analysed using an IBM PC. Bursts were defined as more than 20 spikes less than 1 s apart. Data were averaged over 15-min time blocks; values are mean ±s.e.m. CCKLP levels: Lobsters were maintained and fed as above. Aliquots (1 ml) of haemolymph were withdrawn from the ventral artery, extracted with ethanol, microfuged, the supernatants dried in a Speed-Vac concentrator, then reconstituted in buffer and assayed in duplicate by radioimmunoassay as described[10]; values are expressed as CCK molar equivalents (CCKE). Several haemolymph samples were obtained before feeding, and at ~1 h intervals thereafter. Data were normalized to first baseline value, and averaged over 1 h time blocks.

remained elevated for 4 h. This correlates well with the time food remains in the stomach[15], and the time course of activation of the GM (Fig. 1*b*).

Injections of CCK in mammals produce changes in gut motility and satiety[4,16,17]. Injections of 500 μg CCK into lobster, which raised the haemolymph to between 10^{-7} and 10^{-6} M, initiated GM cycling in 9 out of 10 animals (Fig. 2); this concentration is higher than endogenous CCKLP after feeding. Preliminary data from reverse-phase analysis of CCKLPs in haemolymph and in the neurohaemal pericardial organs has revealed several species of CCKLP, none of which co-elutes with CCK, $CCK8SO_4$, the biologically active form of the peptide, or gastrin 17 (G.G.T and A.I.S., manuscript in preparation), suggesting that the endogenous peptide is not CCK. The endogenous peptide may be more potent than CCK at lower concentrations[10]. The two invertebrate CCKLPs sequenced to date are distinct from mammalian CCK[18,19]. The GM activity stimulated by CCK (Fig. 2*a*) was not identical to the activity produced by feeding (Fig. 1*a*), but was generally slower. This was also true for the occasionally observed spontaneous GM activity in starved animals, and for GM activity produced by injection of the peptides proctolin or red pigment concentrating hormone (RPCH). These observations suggest that GM activity peaks only when there is food in the stomach. Stomach distention may supply several sources of excitation through activation of stretch or chemoreceptors.

Proglumide, a specific competitive antagonist of CCK[20–22], blocks the behavioural effects of CCK in mammals, including the induction of satiety[5,23,24]. We tested whether proglumide could inhibit GM activity after feeding. Injections of proglumide (500 μg) following feeding produced a long-lasting inhibition of the GM, whereas there were only short-lived interruptions after injections of vehicle (Fig, 3*a*). Proglumide inhibited GM activity for an average of 122 ± 22 min, which is significantly longer than the effect of vehicle alone (11 ± 5 min) (Fig. 3*b*); results were independent of the order of injection. Minor disturbances of the animal, such as turning on a light transiently, caused brief interruptions in GM activity, comparable to those produced by vehicle injections (Fig. 3*a*). Injections of proglumide more than 2 h after the start of feeding had little effect on the GM (Fig. 3*a*, *b*), indicating that a CCKLP is probably necessary for early but not late GM activity. Two possible reasons for this are (1) that different mechanisms are responsible for GM activity after several hours, or (2) that longer exposures to CCKLP stimulate a second messenger system, the effects of which outlast the presence of the hormone.

To determine the specificity of proglumide in lobster, we investigated its ability to block the effects of CCK; we also tested two other peptides known to modulate the GM, proctolin[25] and RPCH[26]. Proglumide prevented CCK from initiating GM activity when injected into the haemolymph (Fig. 4*a*) but it had no effect on the ability of RPCH to activate the GM (Fig. 4*b*). Proglumide also blocked the *in vitro* effects of CCK on the GM, but did not affect its modulation by proctolin or RPCH.

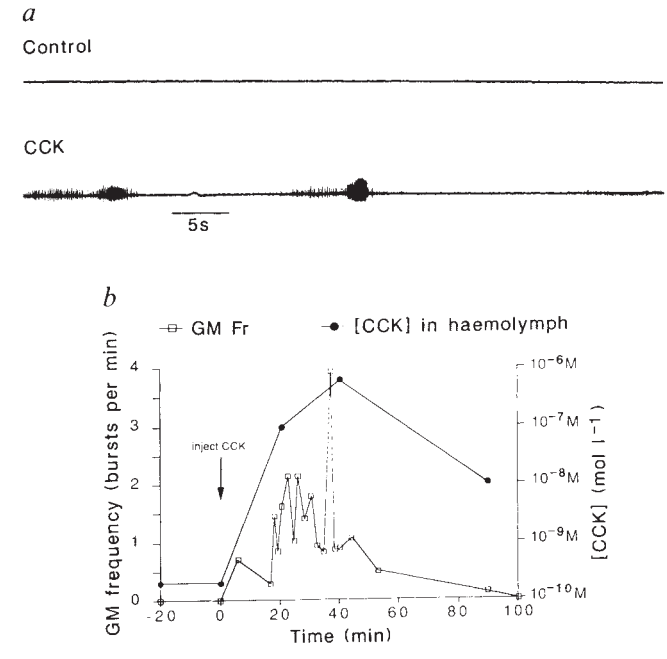

FIG. 2 GM activity before and after injections of CCK. *a*, Gm1 activity before injection of CCK (control), and following the injection of 500 μg $CCK8SO_4$ into the ventral artery. *b*, Injection of CCK raised the haemolymph CCK levels to between 10^{-7} and 10^{-6} M, and activated the GM for ~60 min. Similar results were obtained in 9 out of 10 experiments; average latency to activation was 4.4 ± 1.1 minutes, average peak frequency (averaged over five minute periods) was 1.8 ± 0.4 bursts per min, and average duration was 40 ± 3.2 min. The GM usually fired bursts in bouts of 2 to 6 bursts, separated by periods of no activity. CCK concentration and GM activity were measured and analysed as described in the legend to Fig. 1.

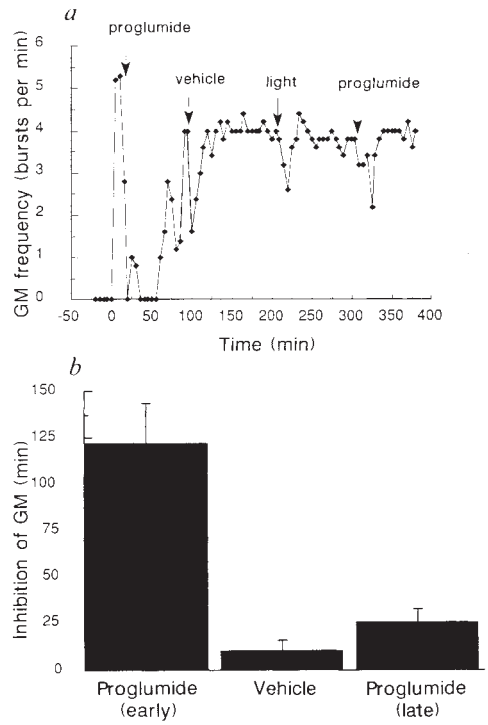

FIG. 3 The effect of the CCK antagonist proglumide on feeding-induced GM activity. *a*, Proglumide injected into the dorsal heart sinus soon after feeding produced a 70 min inhibition of GM activity; injection of vehicle, turning on the light, and injection of proglumide late in the experiment produced minor interruptions. *b*, Proglumide injections within the first 2 h after feeding produced a mean inhibition of 122 ± 22 min ($n = 7$, mean ± s.e.m.). Injections of vehicle (1 ml saline + 5 μl dimethyl sulphoxide) either before ($n = 4$) or after ($n = 3$) injections of proglumide, produced a mean inhibition of GM activity of 11 ± 5 min. Proglumide response was significantly different from that to the vehicle: $P < 0.001$, paired *t*-test. Injections of proglumide more than 2 h after the initiation of feeding produced a mean inhibition of 26 ± 7 min ($n = 3$), which is not significantly different from vehicle, paired *t*-test.

METHODS. GM activity was monitored and analysed as described in the legend to Fig. 1. Drugs were injected by a catheter implanted in the dorsal heart sinus. Inhibition of GM activity was defined as the amount of time after injection in which the GM frequency was below 50% of its pre-injection value; once the frequency rose above this value for over 10 min, the effect was considered to be over. Proglumide was dissolved in dimethyl sulphoxide (5 mg per 50 μl) immediately before use; 5 μl was then diluted into 1 ml saline and injected. Normal GM cycling is virtually uninterrupted during the first 2 h after feeding; on average there are 10 ± 4.5 min during which the GM frequency falls below half of the average frequency of the first half hour ($n = 6$).

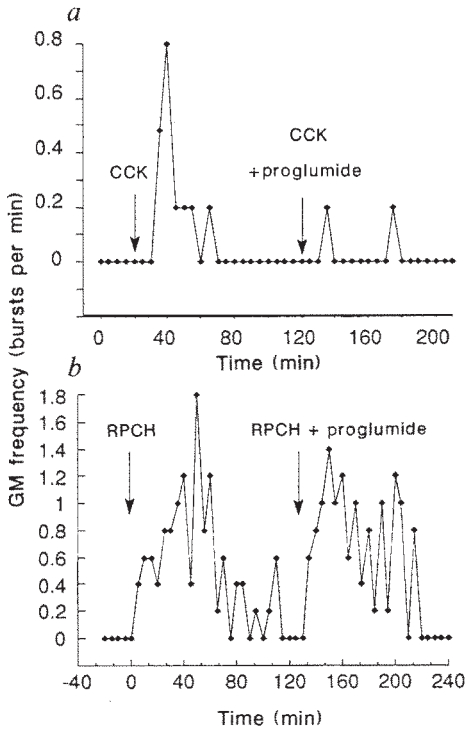

FIG. 4 The specificity of proglumide in lobster. *a*, Proglumide blocks the effects of CCK *in vivo*. Injection of CCK (500 μg) activates the GM; injection of CCK (500 μg) + proglumide (1 mg) has little or no effect. *b*, The ability of RPCH to activate the GM is unaffected by proglumide; injecting RPCH (200 μg) or RPCH (200 μg) + proglumide (1 mg) has the same effect. Controls were performed at least twice with similar results.

These data indicate that proglumide acts specifically to antagonize the effects of CCKLPs in lobster.

We infer that a CCKLP is released into the haemolymph after feeding, where it activates the GM. Because CCK antagonists inhibit the GM, a CCKLP must be necessary for feeding-induced GM activity, but as CCK injections cannot exactly reproduce the effect on GM following feeding, it is probably not sufficient. The GM receives a complex array of inputs, including several modulatory inputs and proprioceptive feedback[10,25–29]. Besides its direct excitatory effects on the GM[10], circulating CCKLP could act to prime the GM so that it can respond to these other inputs. Alternatively, circulating CCKLP may be responsible for directly activating other inputs which together induce vigorous GM activity.

We have previously identified fibres that can release CCKLP directly into the stomatogastric ganglion which contains the GM neural circuit[10]. It is unclear why the GM should receive both a hormonal and a modulatory CCK-like input. The two inputs may function together to raise CCKLP levels in the ganglion sufficiently to activate the GM. Alternatively, the two inputs may be independent, exerting different effects because of differences in concentration, in the number of targets, or as a result of co-release of several substances by the modulatory fibres.

The role of CCK in producing feeding-related behaviour in vertebrates is well documented. The similar role of CCKLP in lobster indicates a remarkable degree of conservation of function in this family of peptides. The identification of a specific antagonist for CCKLPs in lobster and the accessibility of the GM neural circuit, will allow us to resolve the cellular mechanisms by which this hormone exerts its behavioural effects to a level of detail difficult to attain in more complex systems. □

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Activin is a nerve cell survival molecule

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THE structures of five neurotrophic molecules have so far been published. Nerve growth factor[1], fibroblast growth factor[2,3] and purpurin[4], have been identified as nerve-cell survival molecules. More recently, brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor have been cloned and sequenced[5,6]. As all these proteins stimulate the survival of ciliary or sensory neurons, a new cell survival assay is required if novel neurotrophic molecules are to be discovered. P19 teratoma cells differentiate to nerve-like cells in the presence of 5×10^{-7} M retinoic acid (RA)[7,8]. But when P19 cells are plated in N_2 synthetic medium[9] without being exposed to RA, they die within 48 h. In an attempt to identify a molecule(s) that can substitute for RA in promoting P19 survival, we assayed serum-free growth-conditioned media for their ability to promote P19 survival. One cell line from the rat eye secreted a molecule that promoted the survival of P19 cells and some types of nerve cell. We identified this molecule as activin, better known for its role in hormone secretion.

When the serum-free growth-conditioned media of many clonal cell lines from the eye and central nervous system[10] were assayed for their ability to promote the survival of P19 cells, one cell line could do so in a dose-dependent manner. The clonal cell line, designated R33, was obtained from an eye tumour induced by nickel subsulphide[11]. The exact phenotype of R33 is unknown. Figure 1*a* shows that at concentrations >10%, medium conditioned by R33 completely maintained P19 viability for 3 days in N_2 medium. The resulting population of cells is 83% A_2B_5 positive and morphologically homogeneous. Time-lapse photography shows that they do not divide. The phenotype of the surviving cells is not known, nor is the response of the P19 clone used in these experiments likely to be typical of all embryonal carcinoma cell lines. As a negative control, medium conditioned by PC12 had no effect. The R33 survival