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MODULATION OF HEART ACTIVITY IN THE TERRESTRIAL SLUG *LIMAX MAXIMUS* BY THE FEEDING MOTOR PROGRAM, SMALL CARDIOACTIVE PEPTIDES AND STIMULATION OF BUCCAL NEURON B1

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Summary

Activation of the feeding motor program (FMP) increases the force of ventricular contractions in heart/central nervous system (CNS) preparations of the terrestrial slug *Limax maximus* (Linnaeus). The FMP-induced increase in ventricular activity requires innervation of the heart by abdominal ganglion nerves N9 and N11. Application of the small cardioactive peptides SCP_A and SCP_B to isolated preparations of the heart causes dose-dependent increases in the force of ventricular contractions. In addition, the SCPs induce rhythmic contractions in quiescent heart preparations. The effects of the SCPs appear to be specific in that the neuropeptide FMRFamide has an inhibitory effect on ventricular activity. SCP-like and FMRFamide-like immunoreactive material is found in the heart, kidney and pericardium and in the nerves that innervate these organs. Unilateral intracellular stimulation of buccal neuron B1, which contains SCP-like and FMRFamide-like immunoreactive material, mimics the FMP- and SCP-induced increases in ventricular activity. The effect of B1 on ventricular activity is frequency dependent and requires innervation of the heart by N11. These results are consistent with the hypothesis that the SCPs are involved in feeding-related alterations in heart activity in *Limax* and that the control of this effect involves neuron B1.

Introduction

The molluscan neuropeptides, small cardioactive peptides A (SCP_A) and B (SCP_B; Lloyd, 1978, 1982; Morris *et al.* 1982; Lloyd *et al.* 1987), have been shown to modulate both the central neuronal and the peripheral effector organ activity associated with feeding in a number of molluscan species. For example, in *Tritonia*

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diomedia, superfusion of the isolated buccal ganglia with SCP_B causes 2–5 min of cyclical feeding motor output (Willows and Lloyd, 1983). SCP_B also has a positive modulatory effect on the patterned output from the buccal ganglia of *Dirona aurantia*, *Aeolidia papillosa*, *Archidoris montereyensis*, *Hermissenda crassicornis* and *Helisoma trivolvis* (Willows and Watson, 1986; Murphy *et al.* 1985). In addition, SCP_B can initiate patterned feeding motor output from quiescent buccal ganglia preparations of *Helisoma* (Murphy *et al.* 1985) and *Tritonia* (Willows and Lloyd, 1983). In *Aplysia californica*, SCP_B has a positive modulatory effect on buccal musculature associated with feeding (Lloyd *et al.* 1984; Richmond *et al.* 1986) and causes increases in gut motility (Lloyd, 1989). SCP_B also enhances the contractions and neurally mediated relaxation of the pharyngeal retractor muscle (PRM) in *Helix aspersa* (Lloyd, 1978, 1980*a*,*b*,*c*; Morris *et al.* 1982) and the size of EPSPs in salivary gland cells in *Helisoma* (Coates & Bulloch, 1985). In the terrestrial slug *Limax maximus* SCP_B increases the responsiveness of the neural network underlying feeding (the feeding motor program; FMP; Gelperin *et al.* 1978) to stimulation of chemosensory pathways, modulates the activity of individual buccal feeding motoneurons and increases crop motility (Krajniak *et al.* 1985; Prior and Watson, 1988; Hess and Prior, 1989).

Feeding has been shown to increase heart activity in a number of molluscs, including *Limax maximus*, *Agriolimax reticulatus* and *Aplysia californica* (Dieringer *et al.* 1978; Duval, 1983; Grega and Prior, 1985; Koester and Koch, 1987). SCP_B has similarly been shown to increase heart activity in several molluscan species. For example, SCP_B increases the force and rate of heart contraction when applied to isolated preparations of *Helix aspersa* (Lloyd, 1978) and *Aplysia californica* (Cawthorpe *et al.* 1985). Although the effects of exogenously applied SCPs on cardiac activity have been described in several molluscan species, SCP-ergic heart modulatory neural pathways have received considerably less attention. Since SCP_B has been implicated in the modulation of feeding responsiveness in *Limax*, we investigated its potential role in the control of feeding-related alterations in heart activity.

We report that, in semi-intact heart/CNS preparations of *Limax maximus*, activation of the FMP caused significant increases in the force, but not the rate, of ventricular contractions. The FMP modulation of ventricular force required that abdominal ganglion nerve N11 be intact. Application of the SCPs mimicked the FMP-induced increases in ventricular force. SCP-like immunoreactive fibers were identified throughout the heart, kidney and pericardium and in the nerves that innervate these organs. Furthermore, unilateral stimulation of buccal neuron B1, which contains both SCP-like and FMRFamide-like immunoreactive material (Prior and Watson, 1988; Cooke and Gelperin, 1988), mimicked the excitatory effects of both the FMP and the SCPs on the force of ventricular contraction. These results are consistent with the notion that the SCPs are involved in the FMP-induced increase in ventricular force and that the control of this effect may involve buccal neuron B1. A preliminary report of these results has appeared in abstract form (Welsford and Prior, 1987).

Materials and methods

Animals

The specimens of *Limax maximus* used in these experiments were collected in Lexington, Kentucky, placed in containers lined with moist paper towels and kept in an environmental chamber under a regulated L:D cycle (18 °C, 14 h day and 12 °C, 10 h night) and constant humidity (approximately 100 % relative humidity). Animals were fed laboratory food pellets (Purina) *ad libitum*.

Semi-intact preparations

Semi-intact heart/CNS preparations were used to study the effects of the FMP and identified buccal neurons on heart activity. These preparations consisted of the buccal, cerebral, fused parietal–visceral–pleural (i.e. abdominal ganglion; Mackay and Gelperin, 1972) and pedal ganglia, the nerves innervating the heart and kidney (N11 and N9, Grega, 1984; Fig. 1A) and the heart and kidney. Slugs were cold-anesthesized and a mid-dorsal incision was made in the body. The CNS, heart and kidney were removed from the slug and placed in a Sylgard-line (Dow Corning) recording chamber maintained at 20±1.5 °C. Preparations were continuously superfused with slug saline (55.6 mmol l^{-1} Na^+, 4.2 mmol l^{-1} K^+, 7.0 mmol l^{-1} Ca^{2+}, 4.6 mmol l^{-1} Mg^{2+}, 80.3 mmol l^{-1} Cl^-, 0.2 mmol l^{-1} $H_2PO_4^{-1}$, 0.5 mmol l^{-1} HCO_3^-, 5 mmol l^{-1} glucose; pH 7.4, osmolality of 140–147 mosmol kg^{-1} H_2O). The pattern of neuronal activity that comprises the FMP was recorded extracellularly from buccal nerve roots (Fig. 1B) using saline-filled polyprene suction electrodes. The FMP was initiated either by stimulating the lip nerves electrically or by applying potato extract to the innervated lips.

Intracellular recordings were obtained from neurones in semi-intact preparations using standard techniques with glass microelectrodes (5–35 MΩ). Neurons were induced to fire action potentials at various frequencies by passing current through a bridge circuit. Small pins were threaded through the wall of the ventricle and connected to a force transducer (Grass FT.03C) to monitor contractions. Ventricles were weighed after each experiment and contraction force was expressed as mg force mg^{-1} wet mass of heart.

Isolated heart experiments

The heart and kidney were removed from the slug and placed into a recording chamber containing slug saline (20±1.5 °C). The heart was dissected from the kidney and pericardium and pinned by the atrium to the bottom of the chamber. The heart was continuously superfused with saline at a rate of 1.0 ml min^{-1} with a gravity perfusion system. The exchange time of the chamber was approximately 50 s. A cannula was inserted into the atrium and secured with a suture and the heart was perfused at physiological pressures (i.e. 196–490 Pa; Welsford, 1989) with a peristaltic perfusion system. Heart contractions were recorded as described above for semi-intact preparations. The low-pressure perfusion of isolated hearts regularly resulted in stable contractions for several hours (Grega, 1984). The

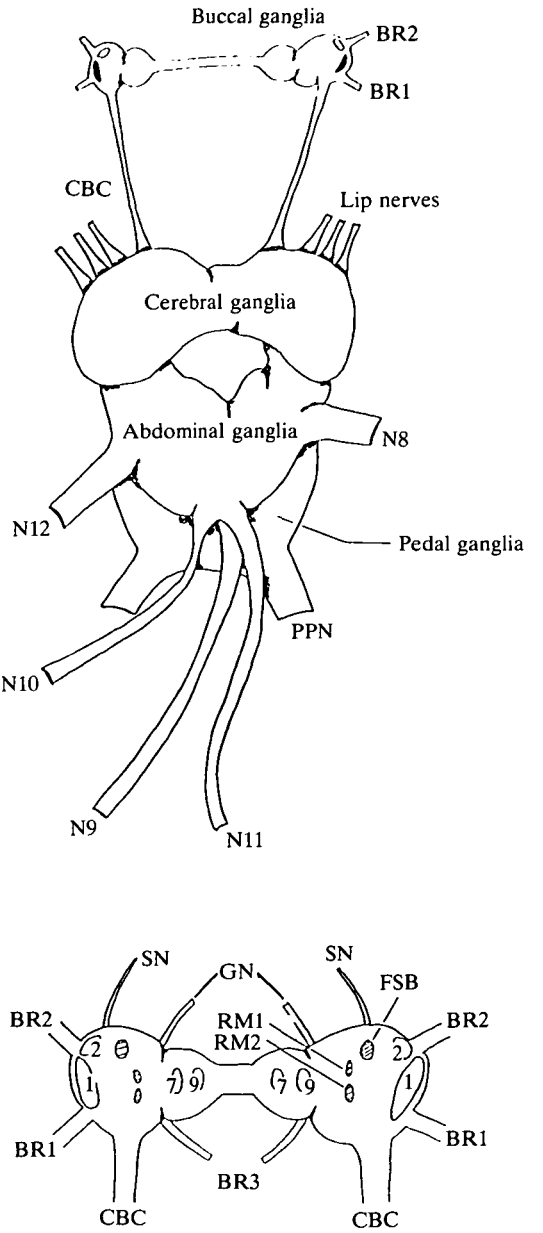

Fig. 1. (A) Diagram of the isolated central nervous system of *Limax maximus* including: the buccal, cerebral, abdominal and pedal ganglia; buccal nerve root 1 (a protractor nerve; BR1), buccal nerve root 2 (a retractor nerve; BR2), the cerebrobuccal connective (CBC), the lip nerves, abdominal nerves N8, N9, N10, N11, N12 and the posterior pedal nerves (PPN). N11 and N9 innervate both gut and heart. (B) A detailed drawing of the buccal ganglia of *Limax* illustrating the positions of buccal neurones B1 (1), B2 (2), the fast salivary burster (FSB), B7 (7), B9 (9), retractor motoneuron 1 (RM1) and retractor motoneuron 2 (RM2). Also shown are buccal roots 1 (BR1), 2 (BR2) and 3 (BR3), the salivary nerves (SN), the gastric nerves (GN) and the cerebrobuccal connective (CBC).

ventricular afterload was not controlled in these experiments and, therefore, the increase in contraction force cannot be reported as an increase in contractility since, by definition, this is an increase in force with both preload and afterload held constant (Milnor, 1975). However, the afterload in the present experiments was calculated to be no greater than 98 Pa.

Peptides

Peptide solutions (SCP_A, SCP_B and FMRFamide; Peninsula Labs) were prepared in slug saline from frozen 10^{-4} mol l^{-1} stock solutions (made in sterile water) immediately before use. Peptide solutions were applied to heart preparations in the superfusate. The time necessary for the peptide solutions to reach the preparation was determined by dye tracing. Preparations were superfused with saline for 20 min to establish baseline values for ventricular contractile activity (force and rate of contractions). Each concentration of each peptide was applied for 5 min, followed by a 25 min wash with saline. In all preparations contractile activity returned to baseline levels within 22 min. The order of peptide application was randomized in all experiments and had no significant effect on the response of the heart. The threshold concentration of a given peptide in the present experiments was defined as the lowest concentration capable of significantly affecting ventricular activity.

Immunohistochemistry

The method of Masinovsky *et al.* (1988) for the immunolabeling of tissue whole mounts was used to examine *Limax* CNS, heart, kidney and pericardium for the presence of SCP and/or FMRFamide-like immunoreactive material. The kidney was examined because it is known to share innervation with the heart (Grega, 1984). The primary antibody to SCP was monoclonal mouse anti-SCP serum (University of Washington Laboratories) and the primary antibody to FMRFamide was polyclonal rabbit anti-FMRFamide serum (INC.Star Inc.). The secondary antibody to SCP was goat anti-mouse serum conjugated to FITC and the secondary antibody to FMRFamide was goat anti-rabbit serum conjugated to TRITC (INC.Star Inc.). For the determination of co-localization of the SCP-like and FMRFamide-like immunoreactive material, tissues were treated with both primary and both secondary antisera simultaneously. Tissues were examined with fluorescence microscopy. The following controls were run to test for the specificity of immunolabeling: deletion of the primary antibody; deletion of the secondary antibody; and preincubation of the primary antibody with 10^{-4} mol l^{-1} arginine vasotocin (AVT), angiotensin II (AII), substance P (SP), SCP_A, SCP_B or FMRFamide for 24 h prior to application to the tissue. Positive controls for the detection of SCP-like immunoreactive material (examination of buccal ganglia and gut; Prior and Watson, 1988) and FMRFamide-like immunoreactive material (examination of the CNS; Cooke and Gelperin, 1988) were also performed.

Data analysis

Data from 15 active semi-intact preparations and 13 active isolated heart preparations were analysed (five isolated heart preparations were tested with SCP_A, five with SCP_B and three with FMRFamide). Separate one-way analyses of variance (ANOVAs) were performed on the initial values of contraction force and rate for each preparation. There were no statistically significant differences between the initial values of contractile activity for any of the preparations; thus, univariate statistical analyses were used (Sokal and Rohlf, 1982). The changes in the contraction force of the ventricle in response to peptide application, FMP activation or buccal neuronal stimulation were normalized as a percentage of the pretreatment level of activity. These data were square-root-arcsine transformed to approximate better a normal distribution and analysed with repeat-measure ANOVAs followed by *post-hoc* Fisher's LSD multiple comparisons procedures (Sokal and Rohlf, 1982). Separate analyses were performed on the data from the peptide application experiments, the FMP activation experiments and the buccal neuron stimulation experiments. The effect of peptide application was also examined in five quiescent heart preparations. The effect of buccal neuron stimulation on quiescent hearts was determined in three additional semi-intact preparations.

Results

Modulation of the heart by the feeding motor program

Phase-locked bursts of efferent neural activity in both salivary nerves and buccal retractor nerves is indicative of the FMP (Fig. 2). When this pattern was initiated

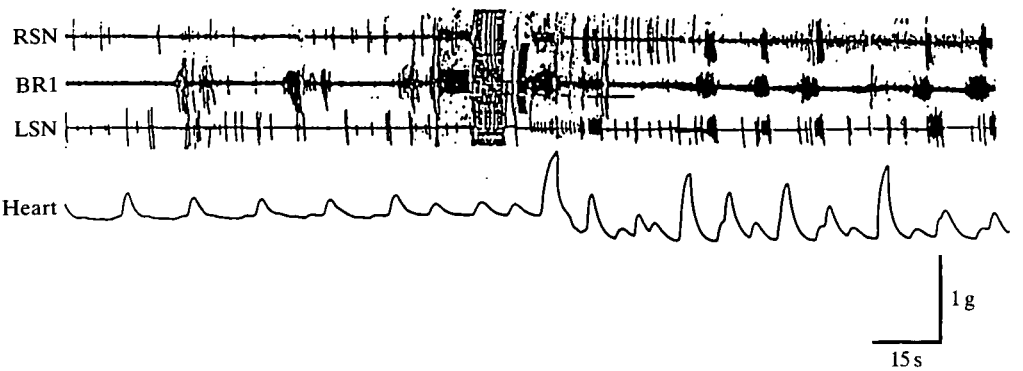

Fig. 2. Typical response of the heart in a semi-intact heart/CNS preparation to initiation of the feeding motor program (FMP). The figure shows simultaneous extracellular activity from both right and left salivary nerves (RSN, LSN) and buccal root 1 (BR1) as well as ventricular contractions (Heart). In this example, the FMP was initiated by stimulating an internal lip nerve for 7 s at a frequency of 7 Hz (high-frequency signal in middle of record). This stimulation elicited phase-locked bursts of neural activity in all three nerves, indicative of the FMP. Note the increase in ventricular force following initiation of the FMP.

by either chemosensory or electrical stimulation, the ventricle of the heart demonstrated a significant increase in contractile force (50±20% greater than controls; mean±S.D., N=6 preparations; $F_{2,10}$=17.92, P=0.0005) but not in the rate of contractions (5±15% increase over controls; $F_{2,10}$=1.9, P>0.1; Fig. 2). The latency between the onset of the FMP and the response of the heart was 13±5.6s and the increase in ventricular activity persisted for up to 10min following termination of the FMP. To determine if the increase in heart activity caused by stimulation of the lip nerves was due specifically to activation of the FMP, the lip nerves were stimulated after the cerebrobuccal connectives (CBCs) had been severed (see Fig. 1A,B). Under these conditions, the FMP was not activated since the buccal neurons responsible for the FMP were isolated from the rest of the CNS. After the CBCs had been severed, lip nerve stimulation caused only slight alterations in heart activity (10±13%; N=3).

The FMP-induced increase in ventricular force required innervation of the heart by both N9 and N11 (see Fig. 1A). When N11 alone was severed, the response of the ventricle to FMP activation was significantly less than that obtained when both N11 and N9 were intact. However, the force of ventricular contractions was still significantly greater than that of controls (i.e. in the absence of FMP activity; baseline in Fig. 3). This mild elevation in ventricular activity caused by activation

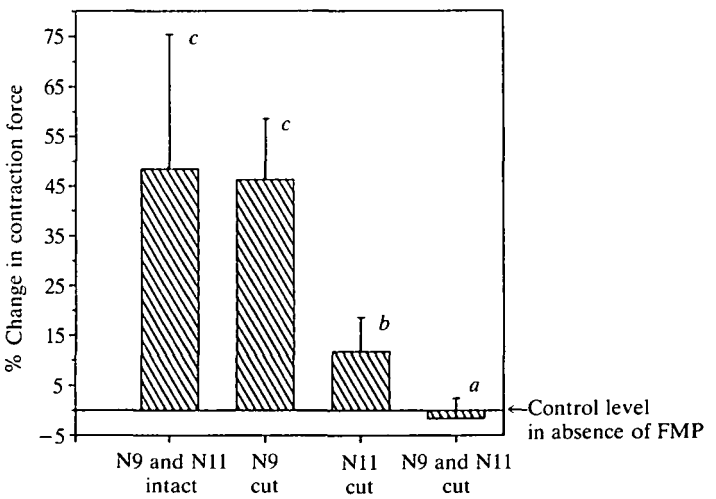

Fig. 3. The mean (+S.D.) response of the ventricle in six heart/CNS preparations after initiation of the FMP with both N11 and N9 intact, with only N9 cut, with only N11 cut, and with both N11 and N9 cut. The letters beside the bars indicate the statistical groupings within the ANOVA performed on these data ($F_{2,8}$=15.7, P<0.001). Different letters beside two bars indicate that they differ from one another at the 0.05 significance level. Note that when N11 alone is cut, the response of the ventricle to FMP activation is significantly reduced (but still greater than when both N11 and N9 are cut). In contrast, severing N9 alone did not significantly reduce the ventricular response to FMP activation.

of the FMP was abolished if N9 was also severed (Fig. 3). In contrast, when N9 alone was severed, the response of the ventricle to FMP activation was not significantly decreased compared with that observed with both N11 and N9 were intact (Fig. 3). Severing abdominal nerves other than N11 and N9 had no effect on the response of the ventricle to activation of the FMP.

Neuropeptide modulation of heart activity

Application of SCP_B increased the force of ventricular contractions in active isolated heart preparations (Fig. 4). The effect of SCP_B was observed within 3–5 min after application and persisted for 10–11 min after initiation of the saline wash. The time required for the activity of the preparation to return to baseline levels was not correlated with SCP_B concentration or with the previous treatment with SCP_B. The irregularity in contraction force seen in Fig. 4 immediately after SCP_B application and prior to the return of heart activity to baseline levels was observed with suprathreshold concentrations (i.e. above 10^{-9} mol l^{-1} SCP_B; see below) of SCP_B in three of five preparations. SCP_B did not have a significant effect on the tonic (diastolic) force of contraction in isolated hearts (2.3±6.0 % increase; mean±S.E.M., N=35 trials; $F_{5,20}$=0.9, P>0.1). The effect of SCP_B on the force of ventricular contraction was concentration-dependent. The maximum increase in force was in response to 10^{-6} mol l^{-1} SCP_B, but at concentrations greater than this the effect was diminished. The threshold concentration of SCP_B was approximately 10^{-8} mol l^{-1} (Fig. 5A). In contrast to its effect on ventricular force, SCP_B had only small and highly variable effects on the rate of ventricular contractions, which, when summed across all trials, were not significant (Fig. 5B).

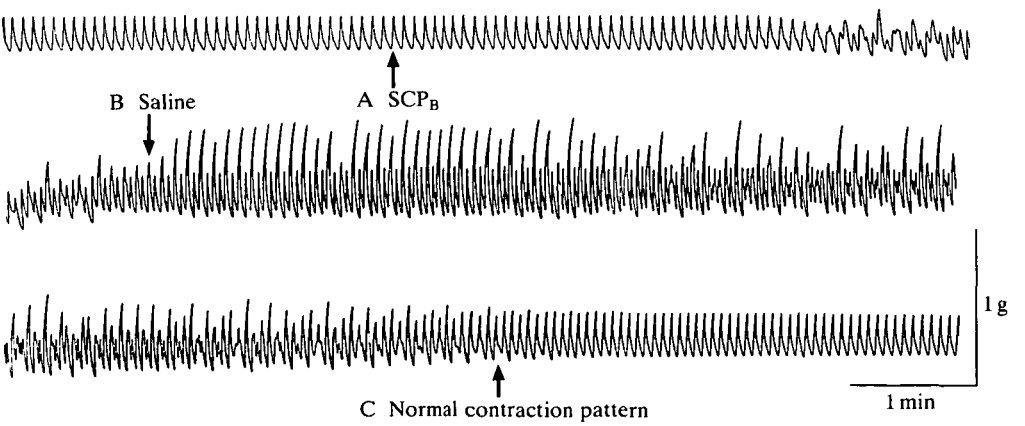

Fig. 4. The response of an actively contracting *Limax* heart to application of 10^{-8} mol l^{-1} SCP_B. At the first arrow (A), 10^{-8} mol l^{-1} SCP_B reached the preparation and at the second arrow (B) the saline wash began. Note the irregularity in ventricular activity following SCP_B application and prior to the return to baseline levels of activity (C).

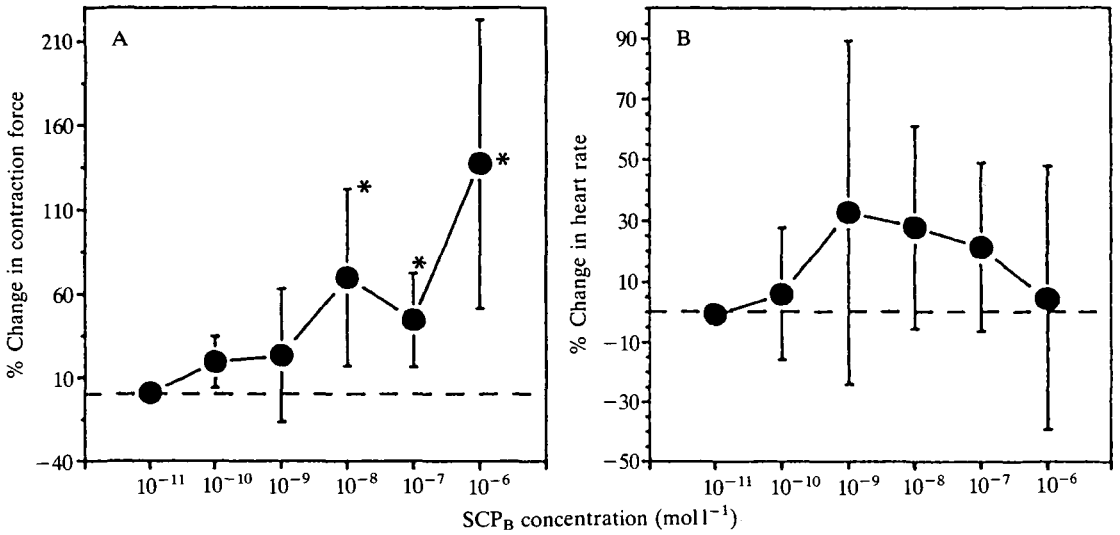

Fig. 5. The effect of various concentrations of SCP_B on ventricular contraction force. The change in contraction force of each heart was normalized as a percentage of its own pretreatment level of activity. Each point represents the mean (±S.E.M.) response of five isolated hearts. In A, the asterisks beside the points represent those means that are statistically different from control values ($F_{5,20}=7.61$, $P=0.0004$). (B) The effect of various concentrations of SCP_B on the rate of ventricular contraction. Data from the same five heart preparations shown in A are presented. In B, the results do not differ significantly from one another ($F_{5,20}=1.9$, $P=0.28$).

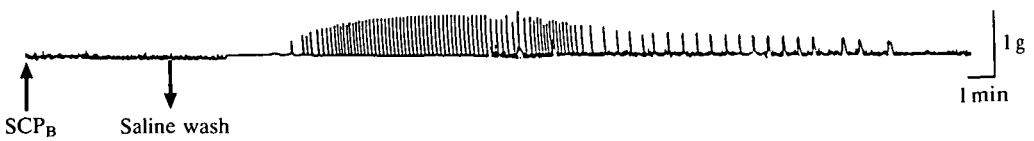

Fig. 6. Typical response of a quiescent heart to application of $10^{-8}\,mol\,l^{-1}$ SCP_B. At the first arrow, SCP_B reached the preparation and at the second, the saline wash began. Note that the time course of the effect was similar to that seen in active hearts (Fig. 2).

Application of SCP_B at concentrations of at least $10^{-8}\,mol\,l^{-1}$ elicited ventricular contractions in quiescent preparations (Fig. 6). The effect of SCP_B on quiescent hearts was evident within 10–12 min after application and persisted for up to 30 min after initiation of the saline wash. Two of the five quiescent hearts exhibited irregular contractions prior to returning to baseline levels of activity, even though none exhibited any irregularities immediately following SCP_B application. The lowest concentration of SCP_B that was capable of initiating rhythmic contractile activity from quiescent hearts was approximately $10^{-8}\,mol\,l^{-1}$. SCP_B did not significantly affect diastolic tone in quiescent hearts (1.6±4.0 % increase over controls; $F_{5,20}=2$, $P>0.07$).

The response of isolated hearts to application of SCP_A was similar to that seen

with SCP_B. At all concentrations, the magnitude of the increase in ventricular force caused by SCP_A was less than that observed with application of SCP_B (15–50 % increase over controls with SCP_A compared to 10–160 % increase with SCP_B). The threshold concentration of SCP_A was approximately 10^{-7} mol l^{-1} ($F_{5,20}=6.1$, $P<0.02$). As with SCP_B, SCP_A had no significant effect on the rate of ventricular contractions (2.0±6.7 % increase over controls; $F_{5,20}=2$, $P>0.07$). The effect of SCP_A could be observed within 5 min of application and persisted for up to 15 min after the initiation of the saline wash. In no case was the recovery time correlated with concentration. SCP_A did not significantly affect diastolic tone (1.0±6.5 % increase over controls; $F_{5,20}=1.3$, $P>0.07$), even though application of SCP_A, at concentrations of 10^{-6} mol l^{-1} or greater, elicited activity in quiescent hearts with a time course similar to that observed in active hearts.

In contrast to the excitatory effects of the SCPs, the effect of the peptide FMRFamide on heart rate and force varied greatly between different preparations and between different concentrations within a preparation. At low concentrations (10^{-12}–10^{-10} mol l^{-1}), FMRFamide had mild but significant ($F_{9,36}=32.38$, $P<0.0001$) inhibitory effects on ventricular activity. Although 10^{-9} mol l^{-1} FMRFamide had no effect on ventricular force, at concentrations near 10^{-7} mol l^{-1} there was a mild excitatory effect and at 10^{-6} mol l^{-1} ventricular activity was abolished. FMRFamide had similar effects on heart rate, being mildly inhibitory at low concentrations (10^{-12}–10^{-10} mol l^{-1}) and having no effect at concentrations from 10^{-9} to 10^{-7} mol l^{-1} ($F_{9,36}=22.32$, $P<0.0001$). The effects of FMRFamide on heart activity generally lasted 18–20 min after initiation of the saline wash.

Immunohistochemistry

Fibers containing SCP-like and FMRFamide-like immunoreactive material were observed throughout the myocardium (Fig. 7). Some of these fibers contained both SCP-like and FMRFamide-like immunoreactive material. SCP-like immunolabeling was diminished by preincubation with either 10^{-4} mol l^{-1} SCP_A or 10^{-4} mol l^{-1} SCP_B but not by preincubation with AII, AVT, SP or FMRFamide. Similarly, FMRFamide immunolabeling was only abolished by incubation with 10^{-4} mol l^{-1} FMRFamide. Deletion of the primary antibody abolished immunolabeling in all cases. The labeling was thus deemed specific for SCP-like and FMRFamide-like immunoreactive material. Immunolabeling for both SCP-like and FMRFamide-like immunoreactive material was also observed in the kidney and pericardium (Fig. 7). As with the heart, some of these fibers were labeled by both antisera. Consistent with the observations of others (Prior and Watson, 1988; Cooke and Gelperin, 1988), neurons containing SCP-like and FMRFamide-like immunoreactive material were observed throughout the CNS, including the two large lateral buccal neurones, B1 and B2. In addition, SCP-like and FMRFamide-like immunoreactive material was contained in fibers in the gastric nerves and in abdominal ganglion nerves, including N9 and N11 (Fig. 8).

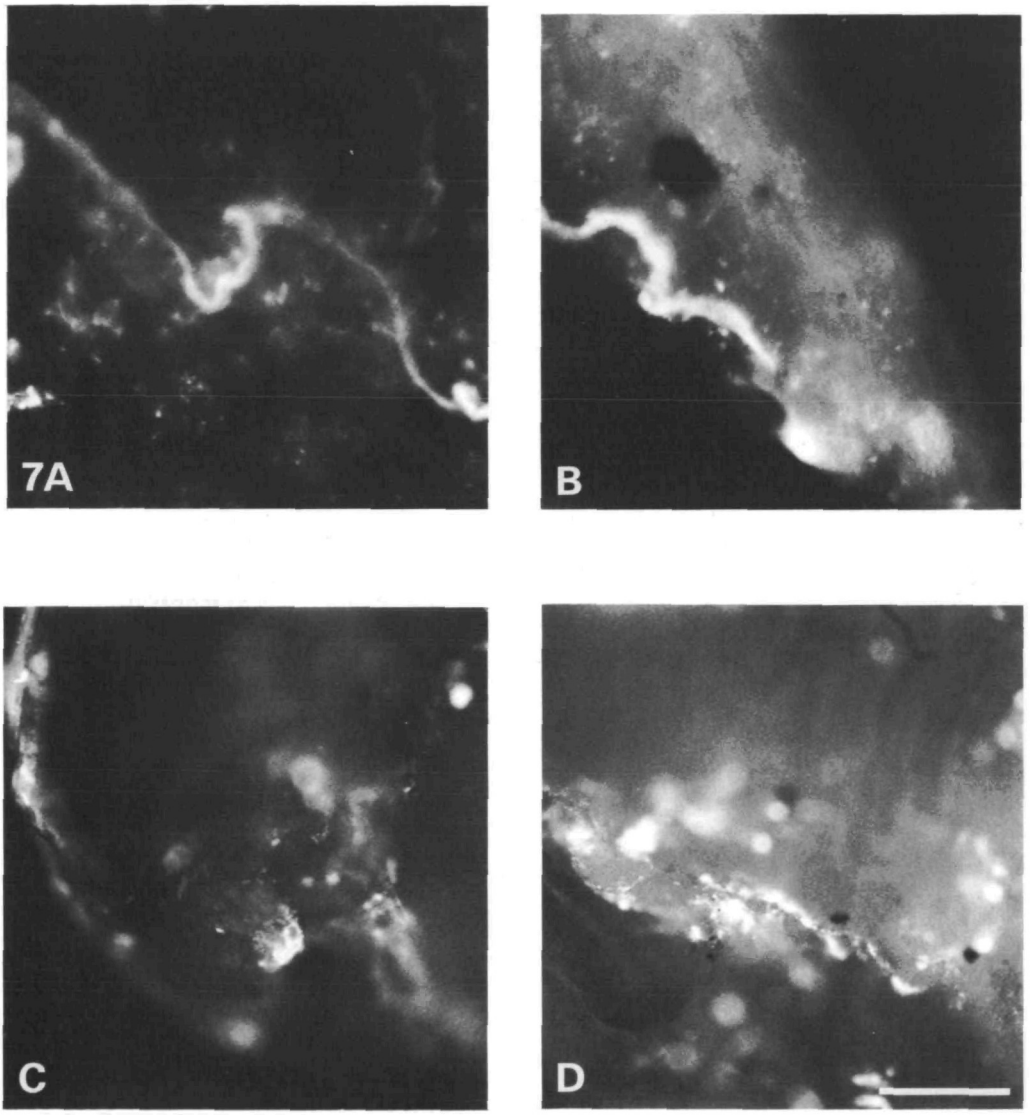

Fig. 7. (A) A photomicrograph of a fiber containing SCP-like immunoreactive material within the heart of *Limax* near the atrioventricular junction. (B) A photomicrograph of a fiber containing FMRFamide-like immunoreactive material within the heart of *Limax* in the same region in the heart as that shown in A. (C) SCP-like immunoreactive material in the kidney of *Limax*. (D) FMRFamide-like immunoreactive material in the kidney of *Limax* in the same region as that shown in C. Scale bar, 30 μm.

Effect of buccal neuron B1 on heart activity

Unilateral intracellular stimulation of buccal neuron B1 typically resulted in an increase in the force of ventricular contractions (Fig. 9). In the example shown in

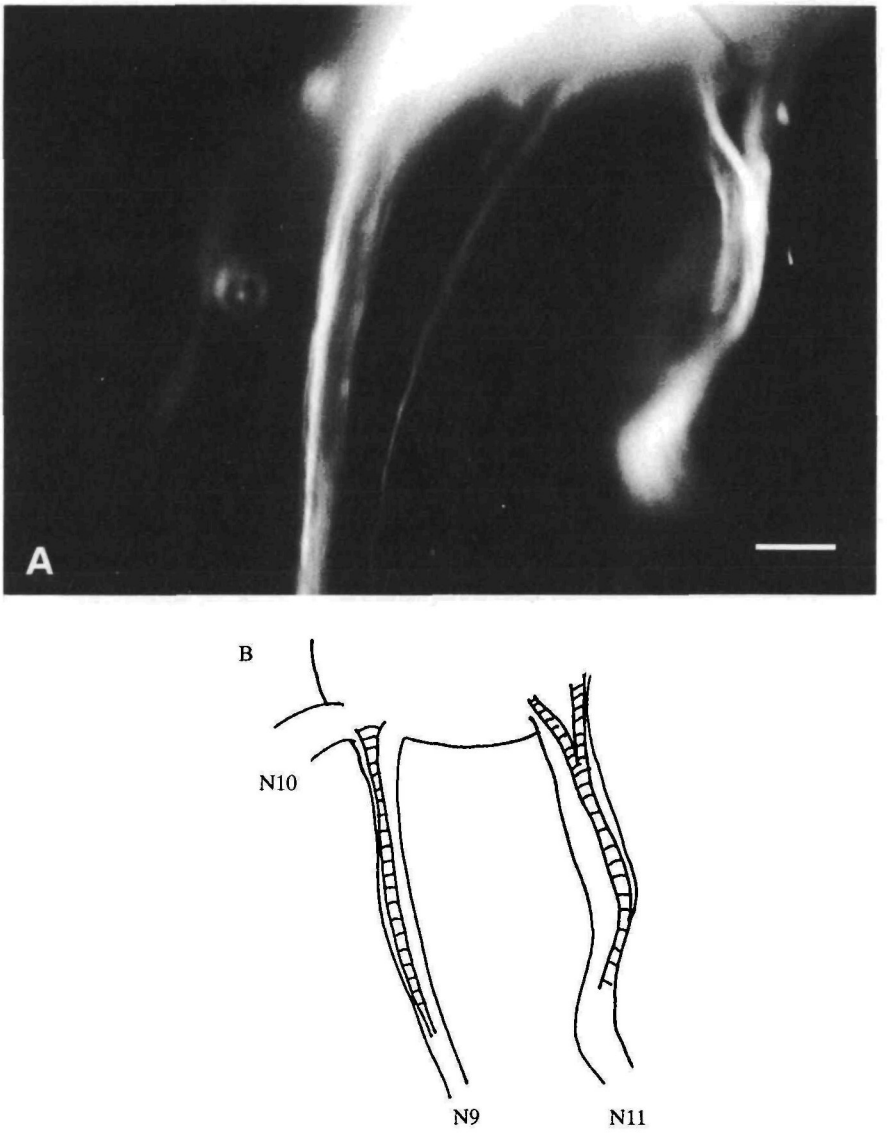

Fig. 8. (A) A photomicrograph of SCP-like immunoreactive fibers in abdominal ganglion nerves N11 and N9. Scale bar, 200 μm. (B) Diagram of the area photographed in A to illustrate the location of N11 and N9 and the fibers labeled within them.

Fig. 9, stimulation of B1 at 3 Hz caused a marked increase in ventricular force and in the level of tonic (diastolic) force between contractions, yet had no effect on the rate of contractions. However, when summed across all stimulation trials, B1 did not significantly affect diastolic tone (3±1.6% compared with controls; $F_{10,40}=1.7$, $P>0.07$). The mean increase in heart rate caused by stimulation of B1 was 16±15.4% (compared with controls; mean±S.E.M., $N=56$ trials), but was not

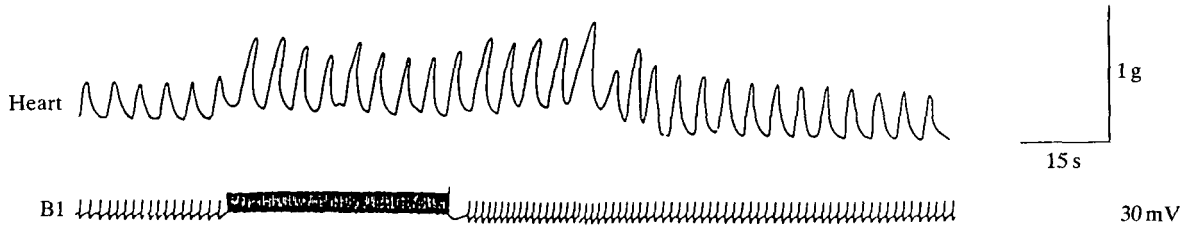

Fig. 9. A typical response of the ventricle to unilateral intracellular stimulation of B1 in a heart/CNS preparation. In the example shown, B1 was stimulated for 35 s at 3.5 Hz. Note the short-latency increase in both contractile force and diastolic tone, followed by the return of the heart to pretreatment levels of activity. Note also that contraction rate was unaffected.

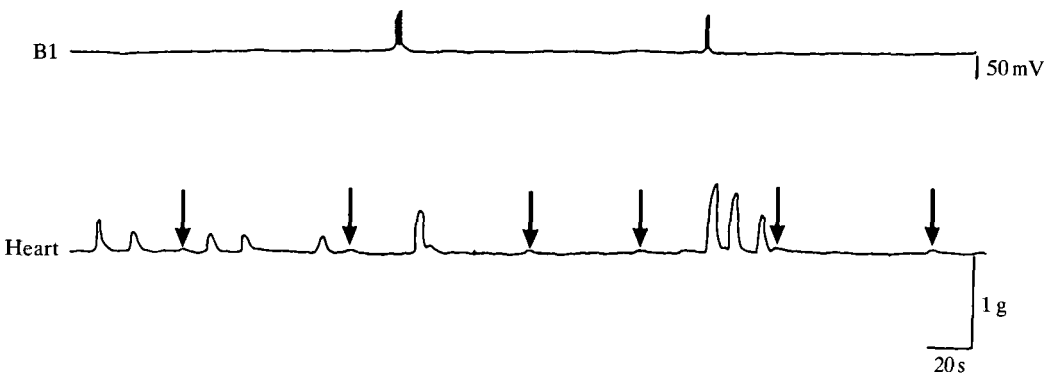

Fig. 10. A response of the ventricle to short-duration stimulation of B1 at 5 Hz. Ten action potentials were initiated in B1 in the first stimulation and five in the second. The augmentation of the ventricular response was not consistently observed in all preparations. The arrows in the figure denote regular low-amplitude ventricular contractions.

statistically different from control values ($F_{10,40}=1.2$, $P>0.07$). The latency between stimulation and the response of the heart was 0.64 ± 1.3 s and was not correlated with the impulse frequency of B1.

As few as five action potentials at an instantaneous frequency of at least 5 Hz were sufficient to elicit increases in heart force (Fig. 10). Even with this minimal stimulation, the response of the heart outlasted the period of B1 stimulation. To compare the effects of B1 on heart activity at different frequencies, we summed the results from those stimulation trials in which 10 action potentials were generated in B1 (Fig. 11). Although the response of the heart to this level of stimulation was variable, at frequencies at 5 Hz or above, 10 action potentials from a single B1 neuron were sufficient to cause sizeable increases in the force of heart contractions (up to 70 % over controls; Fig. 11). In contrast, stimulation of B1 for over 1 min at subthreshold frequencies (i.e. 0.5–4.0 Hz) did not have a reproduc-

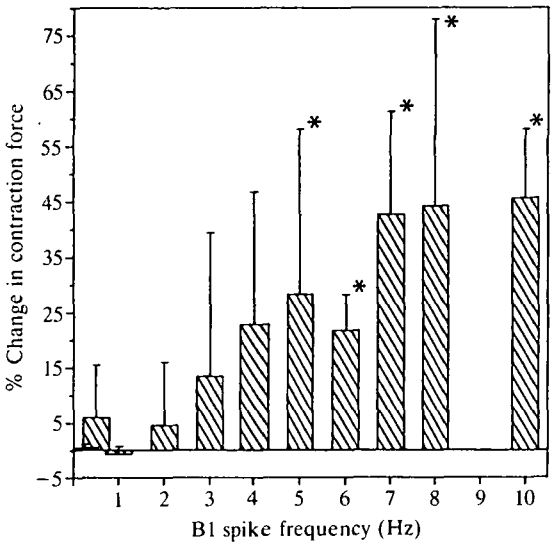

Fig. 11. The effect of unilateral stimulation of B1 at various frequencies on the force of ventricular contractions. Each bar represents the mean (+S.D.) response from 56 stimulation trials. Ten action potentials were elicited at each frequency. The asterisks beside the bars indicate those means that are significantly different from control values ($F_{10,40}$=6.5, P<0.001).

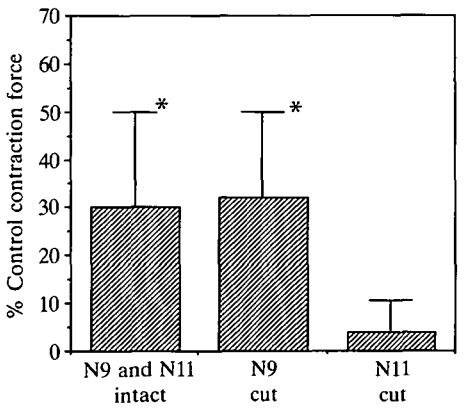

Fig. 12. The mean (+S.D.) increase in the force of heart contractions caused by unilateral stimulation of B1 at 5 Hz with both N9 and N11 intact, with only N9 cut and with only N11 cut. Note that the response of the heart was unaffected by cutting N9 but cutting N11 greatly reduced the response. The asterisks denote those means that are statistically different from control values ($F_{2,8}$=14.4, P=0.002).

ible effect on heart activity. The response of the ventricle to B1 stimulation was unaffected by cutting N9 but was abolished by cutting N11 (Fig. 12).

Buccal neuron B1 could elicit contractions from quiescent hearts in semi-intact

preparations. As few as 10 action potentials at a frequency of at least 5 Hz were sufficient to cause long-term alterations in heart activity. In quiescent preparations, the latency between B1 activation and heart response was 1.2±3.7 s. Since B1 is bilaterally paired in *Limax*, we investigated the possibility that the B1-induced increase in ventricular force was due to the combined effect of both B1 neurons. This was accomplished by severing the right CBC while recording from the left B1, thus severing the contralateral B1 axon. When both CBCs were intact, the response of the ventricle to B1 stimulation was similar to that described above: 43±23 % increase over controls (mean±S.E.M., N=5 preparations). The response of the ventricle to B1 stimulation was unaffected by severing the contralateral CBC (44±21 % increase over controls). In contrast, when the ipsilateral CBC was severed, the response of the ventricle to B1 stimulation was abolished. Thus, unilateral stimulation of B1 was sufficient to increase ventricular activity.

Discussion

Feeding-related alterations in heart activity

In *Limax maximus*, stimulation of the lip nerves at levels sufficient to activate the FMP significantly increased the force of heart ventricle contractions. This increase was apparently mediated specifically *via* the buccal ganglion, since the response of the heart to lip nerve stimulation was abolished by severing the CBCs. The effect of the FMP on ventricular activity required innervation of the heart by abdominal ganglion nerves N11 and N9. However, N11 appeared to be more involved than N9 in regulating the feeding-related alterations. This was supported by the observation that, when only N9 was severed, the response of the heart to activation of the FMP was only mildly decreased compared to the significant decrease observed when both nerves were severed. In addition, when only N11 was severed, the response of the ventricle to FMP activation was markedly decreased compared with the response obtained when both N11 and N9 were intact.

In intact *Limax*, feeding has been shown to cause a significant increase in heart rate (Grega and Prior, 1985). However, in the present experiments, the FMP did not cause a significant elevation of ventricular contraction rate. The difference may be due to our having only recorded ventricular activity, while whole-heart activity was monitored in the earlier experiments. In addition, the present *in vitro* effect occurred within 4 min of initiation of the FMP, whereas in intact animals the increased heart rate occurred up to 20 min after feeding (Grega and Prior, 1985). Long-latency increases in heart activity were not observed in the present experiments. Thus, the long-term alterations in heart activity noted *in vivo* may be due to a separate modulatory system that requires proprioceptive feedback from the gut during food intake (Grega and Prior, 1985).

Neuropeptide modulation of the heart

The neuropeptides SCP_A and SCP_B caused dose-dependent increases in the

contractile force of the ventricle in a manner similar to the FMP. Although the effects of the two SCPs were essentially the same, in all instances SCP_A was less effective than SCP_B in increasing ventricular force. These results are consistent with the effect of the SCPs on the feeding system of *Limax* (Schagene *et al.* 1989). The qualitative similarity in the effects of the two known SCPs in *Limax* may be due to their close sequence homology, but the cause of the difference in potency between the SCPs is unclear.

In contrast to the excitatory effects of the SCPs, FMRFamide did not mimic the FMP-induced increase in ventricular force. Although the effects of FMRFamide were variable, it had a marked inhibitory effect on the heart and was effective at lower concentrations than the SCPs. Both SCP-like and FMRFamide-like immunoreactive material were present in the CNS, heart, kidney and percardium and in the nerves that innervate these structures. Although the immunolabeling was unaffected by incubation with peptides such as AII and SP, our immunohistochemical data do not rule out the possibility that the antisera were cross-reacting with other neuropeptides. This concern is important in that a variety of RFamide substances are known to be present in many invertebrates and at least one additional RFamide is known to be present in *Limax* (Krajniak *et al.* 1985).

If authentic SCPs are contained within the *Limax* CNS and peripheral effectors, the present data are consistent with a modulatory role for these neuropeptides in the physiology of peripheral effector activity associated with feeding. Recent results from whole-animal studies have provided correlative support for this hypothesis. Injection of SCP_B and SCP_A into intact *Limax* causes expression of feeding-related behavior even in the absence of food (Schagene *et al.* 1989). Since many other substances, including acetylcholine (King *et al.* 1987), opiate peptides (Kavaliers *et al.* 1984, 1986) and dopamine (Weiland and Gelperin, 1983), have also been implicated in the control of feeding, the SCPs and FMRFamide may be components of an integrated suite of neurotransmitters and neuromodulators.

In both central neurons and peripheral effectors, SCP-like and FMRFamide-like immunoreactive material were occasionally co-localized to the same neurones and/or fibers. This result is consistent with those from *Aplysia* buccal neurones (Lloyd, 1989) and crustacean neurones (Callaway *et al.* 1987). This is of particular interest in that, in *Limax*, SCPs and FMRFamide have opposing effects on central neuronal networks (e.g. the responsiveness of the FMP) and the contractile activity of peripheral effectors such as gut and heart (Krajniak *et al.* 1985).

B1 modulation of the heart

Stimulation of buccal neurone B1, which contains both SCP-like and FMRFamide-like immunoreactive material, caused a significant increase in ventricular force which was similar to that caused by both the FMP and application of the SCPs. The effect of B1 was frequency-dependent with a threshold of about 5 Hz. This threshold frequency is similar to the threshold for B1 excitation of the fast salivary burster neuron (Prior and Delaney, 1986). The effect of B1 on ventricular

activity occurred with a long latency and frequently outlasted the period of B1 stimulation. These results would be expected if B1 were exerting its effects by releasing a modulatory substance such as SCP (Lloyd, 1989).

If the physiological action of B1 on the heart is indeed due to release of authentic SCPs, this result is especially interesting because B1 also contains FMRFamide-like substance. Stimulation of B1 presumably also releases this FMRFamide-like material, yet when FMRFamide and SCP_A or SCP_B were simultaneously applied to isolated heart preparations, the effects were generally inhibitory and did not mimic those of either B1 stimulation or FMP activation (Welsford, 1989). Since the heart can respond to FMRFamide, the mechanism underlying the specificity of B1 activation may involve the physiology of B1 rather than an asymmetrical distribution of postsynaptic receptors.

It should be noted that the present results provide a conservative estimate of the role of B1 in heart modulation. Only results from unilateral short-duration stimulation have been reported. Although the activation of the contralateral B1 was apparently not necessary for the modulation of the heart, in intact slugs modulation of the heart probably involves the activity of both B1 neurones as well as other modulatory neurones. This notion is supported by the observation that the effect of B1 on the heart was apparently mediated *via* N11, yet the FMP was still able to increase ventricular activity slightly when only N9 was intact. Thus, although N11 appears to be the most important heart modulatory pathway, additional pathways exist.

B1 may have axonal projections in both N9 and N11. This is supported by the observation that 1:1 action potentials can be recorded in these nerves and in the soma of B1 (Welsford, 1989). Thus, some of the immunoreactive fibers in N11 and N9 could represent peripheral projections of B1. This raises the possibility that B1 could exert its effect on the heart *via* monosynaptic connections. Since the nerves that innervate the heart also appear to innervate the blood vessels surrounding it, the effect of B1 on ventricular activity could occur through actions on venous return (preload) or arterial resistance (afterload; Milnor, 1975).

Physiological significance of heart force modulation

The increase in contraction force caused by the FMP, SCPs and B1 stimulation would serve both to increase the circulation of hemolymph and to increase the driving force for the filtration of pro-urine across the atrium and ventricle of the heart in an intact slug (see Prior, 1989). Both of these effects would be advantageous after a feeding bout in that they would serve to increase nutrient delivery and waste removal, respectively. However, the filtration of pro-urine is primarily an osmoregulatory response. Thus, the present results, together with the fact that CNS-mediated heart activity in *Limax* is known to be affected by alterations in hemolymph osmolality (Grega and Prior, 1986), suggest that the SCP–B1–heart modulatory pathway may serve to mediate alterations in heart activity during dehydration as well as during feeding.

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