

SHORT COMMUNICATION
BEHAVIOURAL EFFECTS OF INJECTION OF SMALL
CARDIOACTIVE PEPTIDE, SCP_B, ON THE SLUG
LIMAX MAXIMUS

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The molluscan neuropeptide, small cardioactive peptide B (SCP_B; Lloyd, 1978; Morris *et al.* 1982), has been shown to have excitatory effects on both central neurones and peripheral effectors associated with feeding behaviour in gastropod molluscs (Krajniak *et al.* 1985; Willows & Lloyd, 1983; Murphy *et al.* 1985; see Lloyd, 1988; Prior & Welsford, 1988, for reviews).

In the terrestrial slug, *Limax maximus*, application of exogenous SCP_B to an isolated CNS preparation can increase the responsiveness of the neuronal network underlying feeding, the feeding motor programme (Gelperin *et al.* 1978; Prior & Watson, 1988). Furthermore, SCP_B-like immunoreactive material has been localized to several neurones within the buccal ganglia of *Limax* (Prior & Watson, 1988). Activation of one of these neurones (B1) has been shown to mimic the excitatory effects of exogenous SCP_B on other central neurones (Prior & Delaney, 1986; Prior & Welsford, 1988) and on peripheral effectors (Welsford & Prior, 1987; Prior & Welsford, 1988). Although these observations suggest an involvement of SCP_B in the control of feeding in *Limax*, they are derived from *in vitro* experiments on isolated CNS preparations.

In intact *Limax*, feeding involves a stereotyped behavioural sequence which includes: (1) cessation of locomotion in the presence of food; (2) partial retraction of the superior tentacles; (3) eversion of the lips; (4) initiation of rhythmic lip movements; and (5) cyclical protraction and retraction of the radula against the food (see Gelperin *et al.* 1978).

The only effects of SCP_B reported have been from *in vitro* preparations. We have examined the effects of injection of SCP_B on the behaviour of intact slugs, and report that SCP_B can elicit specific behavioural responses that are associated with feeding.

Specimens of *Limax maximus* (0.5–1.5 g) were kept at 18°C in natural L:D conditions in large Petri plates and were fed rat chow *ad libitum*. Prior to

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experimentation, animals were fasted for 2–3 days. The fasted animals exhibited no feeding-related responses when presented with food. Control slugs received 25 μ l injections of saline into the posterior haemocoel, whereas the test slugs received injections of SCP_B (Peninsula) prepared in slug saline (Prior & Gelperin, 1977). Peptide injections were adjusted so that final haemolymph concentrations could be reported (see Prior *et al.* 1983). Observations were made between 10.00 h and 13.00 h or 14.00 h and 17.00 h and the order of treatments was randomized for each animal within each trial so that each slug was injected with each concentration of SCP_B and the control saline. The injections were counterbalanced over the two observation periods. Over the course of the experiments, each slug received a total of five injections, but an individual animal received only one injection per day. No significant difference in the behavioural responses of slugs was observed between the two observation periods or between different orders of injections. Observations were made by viewing the ventral side of the slugs through a clear Petri plate with the aid of an illuminated magnifying glass.

Preliminary studies indicated that feeding-related behaviour could be initiated by injection of SCP_B, but that the response was observed only within the 10 min following injection. Thus, in the present experiments, behavioural observations were limited to this 10 min period. The following responses were recorded: (1) the presence or absence of locomotor activity; (2) withdrawal of the superior tentacles (to at least half their fully extended length); (3) lip eversion (for a minimum of 45 s); (4) rhythmic lip movement (for a minimum of 45 s); (5) cyclical protraction and retraction of the radula. These data were analysed for significance using a non-parametric analogue to a two-way ANOVA, Friedman's test (Friedman, 1937), followed by a non-parametric multiple comparisons procedure (Zar, 1974). Since Friedman's test made comparisons between the overall behavioural scores of each treatment group, comparisons of the expression of individual responses (e.g. tentacular retraction) between groups was not possible in this analysis. In all comparisons, a probability of 0.05 was considered significant.

Animals injected with 10^{-5} mol l⁻¹ SCP_B (final haemolymph concentration) displayed: (1) reduced locomotor activity compared with controls; (2) retraction of the superior tentacles; (3) lip eversion; and (4) rhythmic lip movements (Table 1). Thus, when exposed to 10^{-5} mol l⁻¹ SCP_B, slugs displayed the initial sequence of responses known to be associated with feeding. However, cyclical radular movements were not observed. It is important to note that these responses were elicited even in the absence of food stimuli. Thus, SCP_B alone is capable of initiating the sequence of responses that are normally initiated by chemosensory cues.

In an effort to determine a threshold concentration for the effect of SCP_B on intact *Limax*, animals were injected with either 10^{-6} or 10^{-7} mol l⁻¹ SCP_B. Injection of 10^{-6} mol l⁻¹ SCP_B caused increases in tentacular retraction, lip eversion and lip movements but no decrease in locomotion when compared with controls (Table 1). Although injection of 10^{-7} mol l⁻¹ SCP_B resulted in a slight increase in both tentacular retraction and lip eversion, there was no observable

Table 1. Behavioural effects of SCP_B injections in *Limax maximus*

Treatment	Behavioural observations			
	Locomotion	Tentacular retraction	Lip eversion	Lip movement
Saline	83 %	8.0 %	0.0 %	0.0 %
10 ⁻⁷ mol l ⁻¹ SCP _B	83 %	25 %	17 %	8.0 %
10 ⁻⁶ mol l ⁻¹ SCP _B *	92 %	33 %	42 %	25 %
10 ⁻⁵ mol l ⁻¹ SCP _B **	17 %	83 %	100 %	58 %

The percentage of animals that displayed each behaviour is presented in each case.

Each animal received injections of each concentration of SCP_B and the saline control.

The 0.05 probability level was accepted as significant (determined by a Friedman's test and a non-parametric multiple comparisons procedure).

All concentrations of SCP_B are the calculated final haemolymph concentrations.

The results with 10⁻⁵ and 10⁻⁶ mol l⁻¹ SCP_B injection were significantly different from those with injection of control saline and 10⁻⁷ mol l⁻¹ SCP_B. Furthermore, the results with 10⁻⁵ mol l⁻¹ SCP_B injection were significantly greater than those observed with injection of 10⁻⁶ mol l⁻¹ SCP_B.

* $P < 0.05$, ** $P < 0.01$. $N = 12$.

effect on locomotion or lip movement (Table 1). Highly significant differences existed between the experimental groups in the expression of feeding-related responses ($X^2_{0.5,3} = 49.35$; $P < 0.005$) (Table 1).

Since SCP_B is known to increase the responsiveness of the feeding motor programme in *Limax*, we tested the possibility that, together with exposure to an appropriate food stimulus, injections of SCP_B might initiate the entire feeding sequence, including cyclical radular movements. Slugs that had been injected with either 10⁻⁶ or 10⁻⁵ mol l⁻¹ SCP_B ($N = 12$) moved towards the food (5 cm), ceased locomotion, withdrew their tentacles, everted their lips and moved them rhythmically. None of these animals, however, initiated cyclical radular movements. Control animals, which had been injected with saline, exhibited no feeding-related responses.

As an initial test of the specificity of SCP_B in initiating feeding-related behaviour, we examined the effects of injections of SCP_A, a neuropeptide with close sequence homology to SCP_B. Injections of 10⁻⁶ or 10⁻⁵ mol l⁻¹ SCP_A had only slight behavioural effects, causing lip eversion and lip movements in only 25 % of the trials. Thus, in *Limax*, as in other molluscan systems, SCP_A has effects similar to those of SCP_B, albeit less marked (see Lloyd, 1988).

The present observations demonstrate that, regardless of the presence of food, SCP_B can elicit a sequence of feeding-related responses in *Limax* that include: (1) decrease in locomotor activity; (2) partial retraction of the superior tentacles; (3) lip eversion; and (4) rhythmic lip movements. That cyclical radular movements were not observed is not totally unexpected and is consistent with the observation that SCP_B does not directly elicit a feeding motor programme in *Limax*. Rather,

SCP_B has been shown to increase the responsiveness of the feeding motor programme to chemosensory stimulation (Prior & Watson, 1988).

The expression of a behaviour as complex as feeding must require the interaction of multiple modulatory systems (e.g. opiates, Kavaliers *et al.* 1984, 1986; FMRFamide, Cooke *et al.* 1985; dopamine, Weiland & Gelperin, 1983). The present results are particularly striking in that a single neuropeptide is capable of eliciting an entire series of feeding-related responses.

The apparent threshold concentration of SCP_B was 10^{-6} mol l⁻¹, but the actual haemolymph concentration of SCP_B resulting from injections has yet to be measured. If there is degradation or non-specific binding of the injected SCP_B, the haemolymph concentration may be lower than calculated. Consistent with this is the observation that the behavioural effects of SCP_B were observed only in the initial 10 min following injection, whereas the effects of SCP_B on isolated CNS preparations persist for up to 20–30 min (Prior & Watson, 1988).

This study has shown that, in intact *Limax*, a sequence of feeding-related responses can be initiated by SCP_B. These results, together with those from isolated CNS preparations, suggest that an SCP_B-like peptidergic system may be involved in the control of feeding behaviour in *Limax*.

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