J. exp. Biol. **142**, 473–478 (1989)
Printed in Great Britain © *The Company of Biologists Limited 1989*

SHORT COMMUNICATION

SMALL CARDIOACTIVE PEPTIDE B MODULATES *LIMAX* FEEDING MOTONEURONES

BY STEPHEN D. HESS* AND DAVID J. PRIOR†

Physiology Group, School of Biological Sciences, University of Kentucky, Lexington, KY 40506, USA

Accepted 4 October 1988

Although first described for its actions on the heart of the snail, *Helix* (Lloyd, 1978), small cardioactive peptide B (SCP_B) has since received attention primarily for its neuronal effects. The effects of SCP_B on the feeding neuronal networks of gastropods are particularly well characterized (e.g. Lloyd *et al.* 1984; Murphy *et al.* 1985; Sossin *et al.* 1987; Willows *et al.* 1988). However, less is known about cellular mechanisms by which the peptide enhances feeding responsiveness. The isolated central nervous system (CNS) preparation of the terrestrial slug *Limax maximus* is particularly useful for studies which examine the role of peptides in the expression of feeding, since the feeding motor programme can be reliably elicited by chemical stimulation of the lips (Gelperin *et al.* 1978) or electrical stimulation of the lip nerves (Phifer & Prior, 1985). SCP_B increases both the responsiveness of the feeding motor programme and the burst frequency of the fast salivary burster neurone of *Limax* (Prior *et al.* 1985; Prior & Watson, 1988). We therefore used intracellular recordings to examine the membrane responses which underlie the ability of SCP_B to increase the burst frequency of the fast salivary burster neurone (FSB). Portions of this work have appeared in an earlier abstract (Hess & Prior, 1986).

The isolated cerebral and buccal ganglia of *Limax* were pinned in a Sylgard-lined recording chamber and superfused with slug saline (Prior & Grega, 1982) at a rate of $1{\cdot}0\,ml\,min^{-1}$, an exchange rate equivalent to two volumes of the recording chamber every minute. Experiments with phenol red added to the superfusate showed that the dye attained an apparently uniform concentration in the chamber within 2 min. All experiments were made at room temperature (17–23 °C).

Fig. 1 shows the effects of a 2 min exposure to $2\times10^{-7}\,mol\,l^{-1}$ SCP_B on the endogenous bursting activity of the FSB neurone. SCP_B application caused an increase in the burst frequency (Fig. 1A). In addition, the peptide increased the

* Present address: Section of Neurobiology, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.

† Present address: Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA.

Key words: *Limax maximus*, slug, peptide, feeding motoneurone.

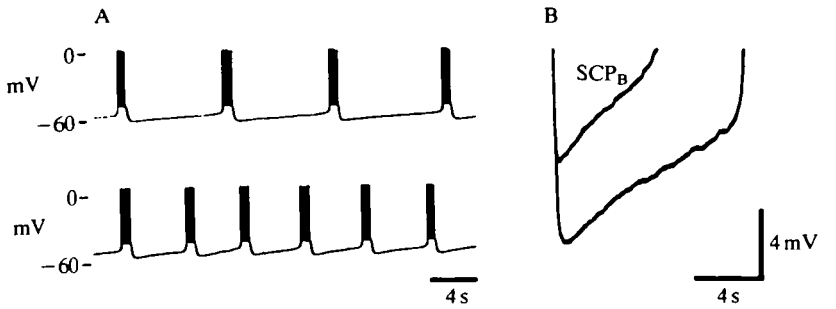

Fig. 1. Typical response of the fast salivary burster neurone (FSB) to application of 2×10^{-7} mol l^{-1} SCP_B. In A, intracellular recordings from the FSB are shown before (top) and 1·5 min after the start of SCP_B superfusion. SCP_B depolarized the FSB by 4 mV, increased the burst frequency and increased the rate of interburst depolarization. In B, traces recorded at a higher gain before and during SCP_B application are superimposed to show the effects on the interburst depolarization.

rate of interburst depolarization, changed the voltage trajectory immediately preceding the burst plateau (Fig. 1B), and caused a long-lasting depolarization. Each of these effects occurred within 50 s of the start of SCP_B administration. However, the recovery was always slower than the onset of the effects, often taking 6–8 min. Since the chamber volume was exchanged within 2 min, these recovery times presumably reflect the time necessary for dissociation of the peptide from receptors or the kinetics of possible peptide-triggered intracellular events, or both.

We chose the maximum interburst hyperpolarization as a reference point to examine the peptide's effect on the interburst membrane potential. Exposure of the isolated CNS to SCP_B for 2 min resulted in dose-dependent prolonged depolarization of the FSB in 27 of 31 trials (Table 1). In two trials the FSB hyperpolarized during SCP_B superfusion, and in two others there was no measurable change in membrane potential. During a 10 min superfusion with normal saline, the membrane potential of the FSB was stable; in no case did the FSB become depolarized during these control experiments.

Fig. 2 illustrates the time course of the changes in the rate of interburst depolarization produced by various concentrations of SCP_B. The response to 2×10^{-6} mol l^{-1} SCP_B was evident 1 min after the introduction of peptide and reached its maximum 1 min after the start of the saline wash. The peak rates of depolarization caused by 2×10^{-6}, 2×10^{-7} and 2×10^{-8} mol l^{-1} SCP_B were significantly different from their pretrial mean ($P<0{\cdot}05$, 0·01, 0·005, respectively, $N=5$). The rate of interburst depolarization remained elevated for up to 6 min after the start of the saline wash.

In addition to modifying the membrane potential and the rate of interburst depolarization of the FSB, SCP_B also changed the amplitude of the interburst hyperpolarization. Although superfusion with 2×10^{-8} or 2×10^{-7} mol l^{-1} SCP_B did

Table 1. *SCP_B causes a dose-dependent prolonged depolarization of the fast salivary burster neurone*

SCP_B concentration ($mol\,l^{-1}$)	Mean depolarization (mV)	Range (mV)	P
2×10^{-8}	$1{\cdot}8 \pm 0{\cdot}3$ ($N = 7$ trials)	1·0–2·0	<0·005
2×10^{-7}	$2{\cdot}6 \pm 1{\cdot}5$ ($N = 6$ trials)	2·0–5·0	<0·025
2×10^{-6}	$6{\cdot}1 \pm 2{\cdot}9$ ($N = 14$ trials)	2·0–11·0	<0·01

Each value represents the mean ± S.D. of the maximum depolarization observed during 2 min trials from six CNS preparations.

For each dose, the depolarization caused by SCP_B was significantly different from zero at the given P value.

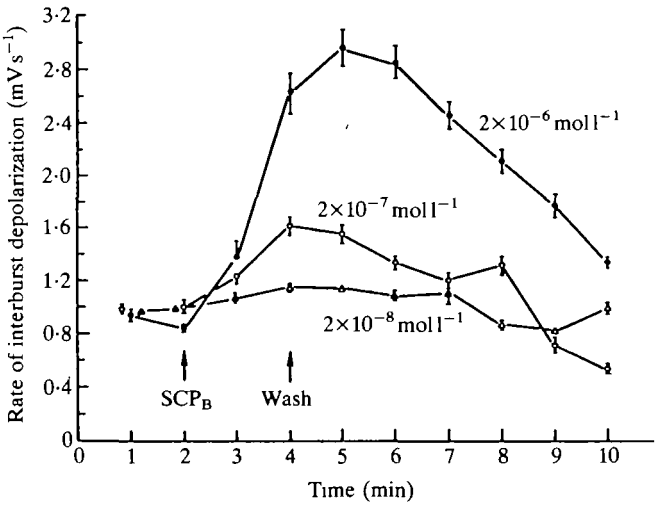

Fig. 2. The effect of SCP_B on the rate of interburst depolarization of the FSB. SCP_B was continuously superfused at the indicated concentrations beginning at the first arrow, and washed out at the second arrow. Each data point is the mean (±S.E.M.) of 1–3 trials from each of five CNS preparations.

not change the maximum hyperpolarization, $2\times10^{-6}\,mol\,l^{-1}$ SCP_B caused a significant decrease from the pretrial mean ($P < 0{\cdot}01$, $N = 5$).

In an analysis of the other FSB burst parameters, we found that SCP_B concentrations as high as $2\times10^{-6}\,mol\,l^{-1}$ had no statistically significant effect on the action potential amplitude, action potential duration, burst duration or the number of spikes per burst of the FSB. These results suggest that SCP_B has

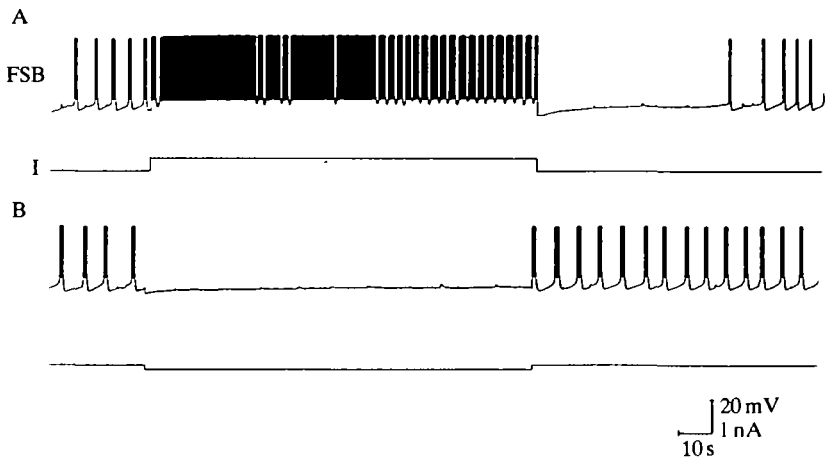

Fig. 3. Intracellular current injection does not mimic the effects of SCP_B on the bursting activity of the FSB. In A, a 2 min pulse of depolarizing current was injected (I). B shows that a 2 min pulse of hyperpolarizing current eliminated the bursting activity of the FSB. A and B were recorded from the same preparation.

selective effects on the relatively slow currents that generate the burst rhythm (see Kramer & Zucker, 1985).

Neuromodulators can affect the activity of bursting neurones without changing the membrane potential (see Kupfermann, 1979, for examples). To determine if the long-lasting depolarization produced by SCP_B increased the burst frequency and the rate of interburst depolarization of the FSB, the prolonged changes in membrane potential produced by the peptide were simulated by current injection. When the FSB was depolarized by 6 mV by current injection, the cell ceased firing in a bursting mode and began firing continuously (Fig. 3A). Although the cell showed bursting activity during the last minute of the depolarization pulse, the burst duration was double the pretrial level and the amplitude of the interburst hyperpolarization decreased by 5 mV. In contrast, SCP_B application decreased the amplitude of the interburst hyperpolarization, but had no effect on the burst duration, even when the cell depolarized 11 mV during exposure to 2×10^{-6} mol l^{-1} SCP_B. In addition, the FSB was silent for approximately 1 min following the depolarization, an effect never observed following SCP_B application. These differences in the effects of the peptide and current injection suggest that SCP_B may alter the gating kinetics of the pacemaker currents of the FSB neurone in addition to changing the membrane potential.

The observation that SCP_B consistently increased the burst frequency and rate of interburst depolarization of the FSB, even in those trials in which the cell was hyperpolarized by the peptide, further supports this hypothesis. For example, the maximum hyperpolarization observed during superfusion of SCP_B was 2 mV, during which the burst frequency increased from 6 to 10 bursts min^{-1} and the rate of interburst depolarization increased to 155 % of the control value. In contrast,

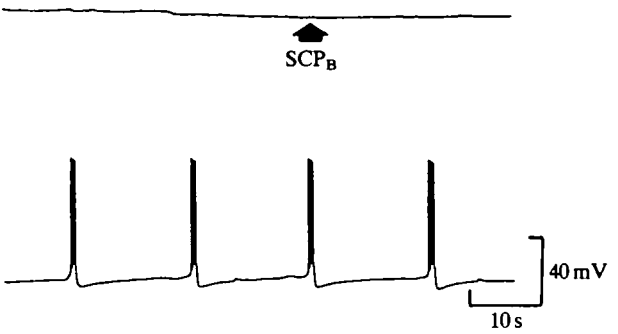

Fig. 4. SCP$_B$ can induce bursting in the FSB neurone. When 2×10^{-7} mol l^{-1} SCP$_B$ was superfused, the FSB hyperpolarized by 2·0 mV from a membrane potential of −60 mV and began bursting. The two traces are continuous. The cell again became silent after washout of the peptide (not shown).

bursting activity ceased when the FSB was hyperpolarized by 2 mV by current injection (Fig. 3B). Thus, prolonged changes in membrane potential cannot alone explain the effects of SCP$_B$ on the activity of the fast salivary burster. However, we do not know if SCP$_B$ acts at the soma, the site of our current injections, or on the axon, the site of spike initiation (Beltz & Gelperin, 1980). Thus, it is possible that some of the differences observed between the effects of the peptide and those due to current injection could be the result of the current affecting more membrane channels than are modulated by SCP$_B$.

Further evidence that SCP$_B$ alters the bursting activity of the FSB by modulating ion channel gating is shown in Fig. 4. In this preparation there was no spontaneous bursting activity until the neurone was exposed to 2×10^{-7} mol l^{-1} SCP$_B$. The peptide caused the cell to hyperpolarize by 2 mV and begin firing in a bursting mode. Approximately 2 min after a saline wash the FSB became silent again. Thus, SCP$_B$ is capable of initiating the endogenous burst pattern without depolarizing the FSB membrane potential, providing another indication that modulation of pacemaker currents may underlie the observed effects on the FSB.

Superfusion with high-Mg^{2+}, low-Ca^{2+} saline, a treatment which reduces chemical synaptic transmission in this preparation (Beltz & Gelperin, 1980), did not eliminate the response of the FSB to SCP$_B$. In two preparations, superfusion with 2×10^{-6} mol l^{-1} SCP$_B$ caused the normal increases in the rate of interburst depolarization and burst frequency. These data suggest that the effects of SCP$_B$ on the FSB are not dependent upon chemically mediated synaptic input, but cannot exclude the possibility that SCP$_B$ excites the FSB *via* an unknown electrical synapse.

In summary, our data show that SCP$_B$ has profound effects on the bursting activity of the FSB neurone. Neurones that are immunoreactive for SCP$_B$-like peptides, such as the large laterally positioned B1, are present in the buccal ganglion of *Limax* (Prior & Watson, 1988). Because intracellular stimulation of B1 increases the burst frequency of the fast salivary burster neurone (Prior &

Delaney, 1986), it is possible that SCP_B or a related peptide increases the activity of the FSB *in vivo*, which is consistent with the peptide's ability to increase feeding responsiveness (Prior & Watson, 1988). Likewise, stimulation of SCP_B-containing neurones in *Tritonia* increases the neuronal activity involved in swallowing (Willows *et al.* 1988). Gastropods may use SCP_B or related peptides to modulate various aspects of the initiation and patterning of the neuronal output that underlies feeding.

This work was supported by a grant from the Whitehall Foundation to DJP and a University of Kentucky School of Biological Sciences Summer Research Fellowship to SDH. We thank G. Augustine for help with the figures, and J. Angstadt, G. Augustine, K. Delaney, A. Gelperin, W. Watson, I. Welsford and A. O. D. Willows for reading earlier versions of this manuscript.

References

BELTZ, B. & GELPERIN, A. (1980). Mechanism of peripheral modulation of the salivary burster in *Limax maximus*: a presumptive sensorimotor neuron. *J. Neurophysiol.* **44**, 675–686.

GELPERIN, A., CHANG, J. J. & REINGOLD, S. C. (1978). Feeding motor program in *Limax*. I. Neuromuscular correlates and control by chemosensory input. *J. Neurobiol.* **9**, 285–300.

HESS, S. D. & PRIOR, D. J. (1986). Small cardioactive peptide B modulates feeding neurons in the terrestrial slug, *Limax maximus*. *Soc. Neurosci. Abstr.* **12**, 1318.

KRAMER, R. H. & ZUCKER, R. S. (1985). Calcium-dependent inward current in *Aplysia* bursting pace-maker neurons. *J. Physiol., Lond.* **362**, 107–130.

KUPFERMANN, I. (1979). Modulatory actions of neurotransmitters. *A. Rev. Neurosci.* **2**, 447–465.

LLOYD, P. E. (1978). Distribution and molecular characteristics of cardioactive peptides in the snail, *Helix aspersa*. *J. comp. Physiol.* **128**, 269–276.

LLOYD, P. E., KUPFERMANN, I. & WEISS, K. R. (1984). Evidence for parallel actions of a molluscan neuropeptide and serotonin in mediating arousal in *Aplysia*. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2934–2937.

MURPHY, A. D., LUKOWIAK, K. & STELL, W. K. (1985). Peptidergic modulation of patterned motor activity in identified neurons of *Helisoma*. *Proc. natn. Acad. Sci. U.S.A.* **82**, 7140–7144.

PHIFER, C. B. & PRIOR, D. J. (1985). Body hydration and haemolymph osmolality affect feeding and its neural correlate in the terrestrial gastropod, *Limax maximus*. *J. exp. Biol.* **118**, 405–421.

PRIOR, D. J. & DELANEY, K. (1986). Activation of buccal neuron B1 in the edible slug, *Limax maximus*, mimics the action of exogenous SCP_B. *Am. Zool.* **26**, 126A.

PRIOR, D. J. & GREGA, D. S. (1982). Effects of temperature on the endogenous activity and synaptic interactions of the salivary burster neuron in the terrestrial slug, *Limax maximus*. *J. exp. Biol.* **98**, 415–528.

PRIOR, D. J. & WATSON, W. H. (1988). The molluscan neuropeptide, SCP_B, increases the responsiveness of the feeding motor program of *Limax maximus*. *J. Neurobiol.* **19**, 87–105.

PRIOR, D. J., WATSON, W. H. & HESS, S. D. (1985). The molluscan neuropeptide, SCP_B, increases the responsiveness of the feeding motor program of *Limax maximus*. *Am. Zool.* **25**, 16A.

SOSSIN, W. S., KIRK, M. D. & SCHELLER, R. H. (1987). Peptidergic modulation of neuronal circuitry controlling feeding in *Aplysia*. *J. Neurosci.* **7**, 671–681.

WILLOWS, A. O. D., LLOYD, P. E. & MASINOVSKY, B. P. (1988). Multiple transmitter types in *Tritonia*. III. Modulation of central pattern generator controlling feeding. *J. Neurobiol.* **19**, 69–86.