Activity-Dependent Peptidergic Modulation of the Plateau-Generating Neuron B64 in the Feeding Network of Aplysia

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Koh H-Y, Weiss KR. Activity-dependent peptidergic modulation of the plateau-generating neuron B64 in the feeding network of Aplysia. J Neurophysiol 97: 1862–1867, 2007. First published January 3, 2007; doi:10.1152/jn.01230.2006. Many behaviors display various forms of activity-dependent plasticity. An example of such plasticity is the progressive shortening of the duration of protraction phase of feeding responses of Aplysia that occurs when feeding responses are repeatedly elicited. A similar protraction-duration shortening is observed in isolated ganglia of Aplysia when feeding-like motor programs are elicited through a prolonged stimulation of the command-like neuron CBI-2. Here, we investigate a cellular mechanism that may underlie this activity-dependent shortening of protraction duration of feeding motor programs. CBI-2 contains two neuropeptides, CP2 and FCAP. Previous work showed that CP2 shortens protraction duration of CBI-2 elicited programs. We show here that the same is true for FCAP. We also show that both CP2 and FCAP modulated the biophysical properties of a plateau-generating neuron, B64, that plays an important role in terminating the protraction phase of feeding motor programs. We find that prestimulation of CBI-2, as well as superfusion of CP2 and FCAP, lowered the threshold for activation of the plateau potential in B64. The threshold-lowering actions of CBI-2 prestimulation were occluded by superfusion of FCAP and CP2. Furthermore, at elevated temperature, conditions under which peptide release is prevented in Aplysia, prestimulation of CBI-2 does not lower the plateau-potential threshold, whereas superfusion of CP2 and FCAP does. Our findings are consistent with the hypothesis that peptides released from CBI-2 lower the threshold for activation of plateau potential in B64, thereby contributing to the shortening of protraction duration when CBI-2 is repeatedly activated.

INTRODUCTION

Similar to the repeated stimulation of feeding responses with seaweed (Kupfermann 1974; Susswein et al. 1978), continuous stimulation of the feeding command-like neuron CBI-2 leads to a progressive and persistent shortening of the duration of the protraction phase of feeding cycles in Aplysia. Earlier work showed that superfusion of CP2, one of the peptides contained in CBI-2, shortens protraction duration, raising the possibility that peptidergic modulation of the network may be involved in this shortening (Morgan et al. 2000). Here, we investigate the possibility that peptides released from CBI-2 act to lower the plateau-potential threshold of the protraction-terminating neuron B64 (Hurwitz and Susswein 1996) and thereby may shorten the protraction-phase duration.

Stimulation of CBI-2 first elicits the protraction phase, which is then followed by the retraction phase. One of the neurons playing an important role in terminating protraction is neuron B64 (Hurwitz and Susswein 1996; Hurwitz et al. 2005; Jing and Weiss 2005). At the time when protraction termination occurs, neuron B64, which slowly depolarizes during protraction, reaches the threshold for activating a plateau potential and simultaneously inhibits protraction while exciting retraction phase neurons. Previous work (Morgan et al. 2000; Proekt et al. 2004) showed that, when prolonged, stimulation of CBI-2 alters the state of the feeding network. This altered state is associated with the shortening of protraction duration and also with a shortening of the latency to initiate protraction. Peptides are also involved in the shortening of protraction latency, but do so by a different mechanism, i.e., the enhancement of synaptic transmission (Koh et al. 2003). It was previously suggested (Marder et al. 1996) that establishment of activity-dependent network states may involve both the modulation of synaptic transmission and the alteration of intrinsic properties of neurons.

Because peptides can modulate the biophysical properties of plateau neurons and thereby alter network behavior (e.g., Golowasch and Marder 1992; Harris-Warrick and Marder 1991; Nusbaum et al. 2001; Swensen and Marder 2000), we investigated the possibility that peptides contained in CBI-2 may lower the plateau-potential threshold in B64. Such an action could advance in time plateau-potential generation, thereby contributing to the shortening of protraction duration in the aftermath of repeated stimulation of CBI-2. Here we provide—using a combination of experimental manipulations that affect peptide release and peptide actions—evidence that peptides released from CBI-2 lower the plateau-potential threshold of neuron B64.

METHODS

Preparations of connected cerebral and buccal ganglia from Aplysia californica (125–175 g) were constantly superfused (0.3 ml/min) at 13.8–14.5 or 25°C. Normal artificial seawater (ASW) that contained (in mM) 460 NaCl, 11 CaCl₂, 10 KCl, 55 MgCl₂, and 10 HEPES buffer (pH 7.6) was used in experiments where protraction-duration measurements were made and in experiments involving comparisons of the effects of different temperatures. For B64 excitability experiments, 2.5 × Ca²⁺/1.5 × Mg²⁺ ASW (in mM: 25 CaCl₂ and 82.5 MgCl₂) was used. To suppress polysynaptic responses and spontaneous activity in occlusion experiments, 3 × Ca²⁺ ASW (33 mM CaCl₂) was used (Trudeau and...
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Castellucci 1992). Peptides were superfused at concentrations that produced maximum effects (Koh and Weiss 2005): 10^{-6} M FCAP and 10^{-5} M CP2 (both peptides were obtained from SynPep, Dublin, CA).

An AxoClamp 2A was used to amplify intracellular signals that were obtained using glass microelectrodes filled with 2 M potassium acetate and 100 mM KCl and beveled to 4–6 MOhms. Recordings were made in the bridge mode. Bridge balances were checked and, when needed, readjusted at the beginning of each series of measurements for each of the conditions, such as before, during peptide superfusion or high-frequency stimulation (HFS) of CBI-2, and after. CBI-2 was stimulated intracellularly with 17- to 20-ms current pulses, each of which elicited a single action potential. The HFS of CBI-2 used throughout this study was a 13-Hz train lasting 30 s; this stimulation frequency is within the normal physiological range of CBI-2 activity (Jing and Weiss 2005; Rosen et al. 1991).

Standard definitions of protraction and retraction phases of motor programs were used (Nargeot et al. 1997). The protraction phase (white bar) was defined by the activity of B61/62 and in I2 nerves (Fig. 1A). The retraction phase (black bar) was defined by a period of hyperpolarization of B61/62 after the protraction phase was terminated and also by a period of high-frequency activity in BN2.

When parametric data were analyzed, all statistical tests involving more than two conditions were performed using one-way ANOVA. Tests with the Bonferroni correction were used for post hoc comparisons. For the tests that involved dichotomous nominal scale variables, the Cochran Q test was used for overall differences, followed by McNemar post hoc tests for individual pairwise comparisons.

RESULTS

High-frequency prestimulation (HFS) of CBI-2 is known to cause shortening of protraction-phase duration of subsequent CBI-2 induced buccal motor programs (Morgan et al. 2000) and superfusion of CP-2 (Pahres and Loidl 1996) was also previously shown to shorten protraction duration (Morgan et al. 2000). We first sought to determine whether the second peptide contained in CBI-2, FCAP (Koh et al. 2003), also shortens protraction duration of CBI-2–elicited motor programs. Single buccal motor programs were elicited in ASW, every 3 min, by stimulating the command-like neuron CBI-2 at 6–7 Hz until the end of protraction phase, marked by the sharp inhibition of B61/62 firing and the abrupt ending of I2 nerve activity (Fig. 1A1). A concentration of 10^{-6} M FCAP reversibly reduced protraction duration (see Fig. 1A2 for grouped data). One-way ANOVA revealed a significant overall difference (P < 0.0001, F = 60.41, df = 2.8; n = 5) between the three conditions. In the presence of 10^{-6} M FCAP, protraction duration was significantly reduced (t = 9.38, P < 0.001) compared with control and to the postwash conditions (t = 9.66, P < 0.001), whereas there was no difference between the control and the postwash values (t = 0.28, P > 0.05). Thus similar to CP2, the second CBI-2 peptide, FCAP, also shortens protraction duration.

In a CBI-2–elicited motor program, it was previously shown that protraction is terminated when B64 generates a plateau potential and inhibits the protraction-phase interneurons, thereby terminating the protraction phase (Hurwitz and Susswein 1996). Pledopolarization of B64 advances and hyperpolarization delays the generation of plateau potentials, respectively shortening and lengthening protraction duration (Jing et al. 2003). We reasoned that peptides may lower the plateau-potential threshold and thereby contribute to protraction-duration shortening. We therefore sought to determine whether FCAP and CP2 affect the threshold of plateau generation in B64. B64 was injected with a repeated series of five current pulses (1-s duration, every 30 s). Current amplitudes were at the threshold and varying subthreshold levels that were applied in an ascending order. B1: superfusion of 10^{-6} M CP2 lowered the threshold for triggering the plateau potential. B2: 10^{-6} M FCAP also decreased the threshold. In B1 and B2, the same currents (shown on the left side of each row) were injected across all 3 conditions. C: ganglia were superfused with 2.5 × Ca^{2+}/1.5 × Mg^{2+} artificial seawater (AWT) to remove plateau potentials and to measure the number of spikes elicited by a constant depolarizing current pulse (1.5-s duration). Both CP2 (top) and FCAP (bottom) increased the number of spikes.

FIG. 1. A: protraction duration is shortened by the FCAP (feeding circuit activating peptide) neuropeptide. A1: every 3 min, single buccal motor programs were elicited by stimulating CBI-2 at 6–7 Hz until the end of protraction phase, marked by the sharp synaptic inhibition of B61/62 firing and the abrupt ending of I2 nerve activity (Hurwitz and Susswein 1996). Protraction phase (white bar) is defined by the activity of B61/62 and I2 nerve. Retraction phase (black bar) is defined by a period of hyperpolarization of B61/62 after the protraction phase is terminated and also by a period of high-frequency activity in BN2. A2: grouped data from 5 preparations. 10^{-6} M FCAP reduced the protraction-phase duration to 50.7 ± 3.93% its duration measured before the superfusion of FCAP (before). On wash, protraction duration returned to its control value (to 101.5 ± 4.75% control). B: exogenous FCAP and cerebral peptide 2 (CP2) lower plateau-potential threshold and increase excitability of B64. B64 was injected with a repeated series of five current pulses (1-s duration, every 30 s). Current amplitudes were at the threshold and varying subthreshold levels that were applied in an ascending order. B1: superfusion of 10^{-6} M CP2 lowered the thresholds for triggering the plateau potential. B2: 10^{-6} M FCAP also decreased the threshold. In B1 and B2, the same currents (shown on the left side of each row) were injected across all 3 conditions. C: ganglia were superfused with 2.5 × Ca^{2+}/1.5 × Mg^{2+} artificial seawater (ASW) to remove plateau potentials and to measure the number of spikes elicited by a constant depolarizing current pulse (1.5-s duration). Both CP2 (top) and FCAP (bottom) increased the number of spikes.
plied in ascending order (2.2–2.4–2.6–2.8–3.0 nA in the case of threshold = 3.0 nA) (Fig. 1B). In the presence of 10−6 M CP2, the threshold for triggering plateau potentials was lowered to 82.5 ± 0.01% of control, i.e., before CP2, threshold (overall, P < 0.0001, F = 112.7, df = 2,8, n = 5; before vs. CP2, P < 0.001; CP2 vs. washout, P < 0.001; before vs. washout, P > 0.05) (Fig. 1B). Similarly, 10−6 M FCAP (n = 5) decreased the threshold to 83.1 ± 0.01% of control values (overall, P < 0.0001, F = 112.7, df = 2,8: before vs. FCAP, P < 0.001; FCAP vs. washout, P < 0.001; before vs. washout, P > 0.05) (Fig. 1B).

To study the effects of peptides on B64 excitability, we used high-divalent ASW (2.5 × Ca2+/1.5 × Mg2+), a solution that suppresses the ability of B64 to generate plateau potentials. We measured the change in the number of action potentials elicited by a constant depolarizing current pulse (1.5-s duration). Current size in individual experiments was selected to generate nearly five action potentials under control conditions. CP2 increased the number of spikes from 5.0 ± 0.48 to 1.73 ± 3.2 (Fig. 1C, top) (overall, P < 0.01, F = 23.75, df = 2,4, n = 3: control vs. CP2, t = 5.88, P < 0.05; CP2 vs. washout, t = 6.06, P < 0.05; control vs. washout, t = 0.18, P > 0.05). FCAP also increased the number of spikes from 4.7 ± 0.5 to 16.3 ± 2.4 (Fig. 1C, bottom) (overall, P < 0.05, F = 12, df = 2,4, n = 3: before vs. CP2, t = 4.24, P < 0.05; CP2 vs. washout, t = 4.24, P < 0.05; before vs. washout, t = 0, P > 0.05). Thus both CP2 and FCAP peptides lowered the plateau-potential threshold and increased the excitability of B64.

We next sought to determine whether HFS of CBI-2 can also affect the excitability of B64. B64 was injected every 30 s with a subthreshold current pulse (I_threshold = I_threshold − (0.15 × I_threshold)) of 0.5-s duration. In all the preparations tested (n = 10), after HFS of CBI-2 (13 Hz for 30 s) the subthreshold current pulses elicited plateau potentials in B64 to evoke action potential spikes, indicating that the threshold for eliciting plateau potentials was lowered by HFS of CBI-2 (Fig. 2A, top). This effect persisted for 11.2 ± 1.5 min.

In a separate series of experiments, we measured the effects of HFS of CBI-2 on the number of spikes elicited by injections of constant current pulses into B64 in a high-divalent ASW (Fig. 2A, bottom). HFS increased the number of spikes from 5.3 ± 1.3 to 17.4 ± 2.9 (overall, P < 0.05, F = 7.99, df = 2,8, n = 5: pre-HFS vs. post-HFS, t = 3.4, P < 0.05; post-HFS vs. recovery, t = 3.5, P < 0.05; pre-HFS vs. recovery, t = 0.08, P > 0.05). The mean time for the recovery to control values was 7.9 ± 1.53 min post-HFS.

Because both HFS and exogenous peptides exerted a similar action on B64, we reasoned that FCAP and CP2 released from CBI-2 during HFS may contribute to the lowering of the plateau-potential threshold observed in the aftermath of HFS of CBI-2. To explore this possibility, we used the occlusion paradigm (Koh and Weiss 2005; Swensen and Marder 2000) and tested the ability of exogenous peptides to occlude the effects of HFS on the plateau-potential threshold in B64. Ganglia were superfused with 3 × Ca2+ ASW to suppress polysynaptic actions. B64 was injected every 30 s with current pulses of 80, 90, and 100% of the threshold current that elicited plateau potentials. In the absence of FCAP + CP2, in all five preparations used, the subthreshold current pulses (80 and 90% of the pre-HFS threshold) evoked plateau potentials in B64 after HFS of CBI-2 (Fig. 2B2, black bar, grouped data), thus indicating a decrease of threshold. A representative recording is shown in Fig. 2B1, left panel. In contrast, there was no significant lowering of threshold in the five preparations in which HFS of CBI-2 was performed in the presence of FCAP + CP2 (χ2 = 8.4, P > 0.05; 80 vs. 90%, P = 1; 90 vs. 100%, P > 0.05; 80 vs. 100%, P > 0.05) (Fig. 2B2, gray bar, grouped data). A representative recording is shown in Fig. 2B1, right panel.

Although FCAP + CP2 occluded the effect of HFS on the plateau-potential threshold, we could not exclude the possibility that this was not a true occlusion and instead that the threshold was maximally lowered by FCAP + CP2, making it impossible to lower it any further. We thus designed a positive control experiment, i.e., an experiment that sought to identify a CBI-2–independent means of shortening the protraction phase. Esophageal nerve stimulation is known to produce motor programs of short duration (Proekt et al. 2004). Thus we sought to determine whether esophageal nerve stimulation also lowers plateau-potential threshold and, if so, whether such a threshold-lowering action would possibly not be occluded by superfusion of FCAP + CP2. We used the same paradigm as in Fig. 2B and found that, similar to HFS of CBI-2, esophageal nerve stimulation (5 ms, 5 Hz, 2 min, 4–6 V) decreased the plateau-potential threshold in B64 in all five preparations we tested (Fig. 2, C1, left and C2, black bar). We therefore sought to determine whether FCAP + CP2 would occlude the ability of esophageal nerve stimulation to lower the plateau threshold in B64. Unlike the case of HFS of CBI-2, esophageal nerve stimulation in the presence of FCAP + CP2 was able to lower the plateau-potential threshold in B64 in the five preparations tested (Fig. 2, C1, FCAP + CP2 and C2, gray bar). Thus occlusion by FCAP + CP2 of the ability of CBI-2 HFS to lower the plateau-potential threshold cannot be attributed to the threshold having reached its lowest possible value.

To further probe the idea that peptides released during HFS of CBI-2 are involved in HFS-elicted threshold lowering, we took advantage of the finding that peptide release in *Aplysia* is suppressed at elevated temperatures (Fox and Lloyd 2001; Koh et al. 2005; Vilim et al. 1996; Whim and Lloyd 1990). One could therefore expect that if peptides released during HFS are involved in threshold lowering, at elevated temperatures HFS should no longer lower plateau-potential thresholds. In an experiment similar to that shown in Fig. 2B, we studied the effects of CBI-2 HFS at 14 and 25°C (Fig. 3A1). Two current pulses, subthreshold before HFS, evoked plateau potentials in B64 after HFS at 14°C (χ2 = 2.0, P > 0.05, n = 6: 80 vs. 90%, P = 1; 80 vs. 100%, P = 1) (Fig. 3A2, black bar). In contrast, at 25°C there was no significant effect of HFS because the two subthreshold pulses failed to elicit plateau potentials after HFS (χ2 = 10.3, P < 0.01, n = 6: 80 vs. 90%, P = 1; 90 vs. 100%, P = 0.063; 80 vs. 100%, P = 0.031) (Fig. 3A2, gray bar).

Finally, to ensure that the absence of threshold lowering at 25°C was a result of the absence of peptide release rather than of peptide insensitivity, we also tested the effects of FCAP + CP2 on the plateau-potential threshold at 25°C. Plateau-potential threshold was decreased by superfusion with FCAP + CP2 (Fig. 3B1). Pooled data of responses to FCAP + CP2 at 25°C from the same six animals showed that the two subthreshold current pulses evoked plateau potentials in B64 in the presence
DISCUSSION

In intact animals (Kupfermann 1974; Susswein et al. 1978) and in the isolated nervous system (Proekt and Weiss 2003; Sanchez and Kirk 2002) repeated activation of feeding responses leads to a shortening of both response latency and protraction duration. Previous studies of the neural basis of response-latency shortening showed that HFS of the command-like neuron CBI-2 results in a shortening of latency to initiate CBI-2–elicited motor programs. The latency shortening was shown to be associated with posttetanic potentiation (PTP) at the synapse between CBI-2 and the protraction motoneurons B61/62 (Proekt and Weiss 2003; Sanchez and Kirk 2002). Studies also demonstrated that the two neuropeptides released from CBI-2 contribute to the PTP at the CBI-2 to B61/62 synapse (Koh and Weiss 2005; Koh et al. 2003).

In the present study, we investigated a possible contribution of peptides to the shortening of protraction-phase duration in...
the aftermath of repeated CBI-2 stimulation. We used a combination of peptide superfusion, peptidergic occlusion of HFS effects, and temperature manipulations of peptide release. Overall, the results of our experiments are consistent with the hypothesis that peptides released by HFS of CBI-2 lower the plateau-potential threshold in B64. In CBI-2–elicited motor programs, B64 gradually depolarizes during protraction and, on reaching threshold, B64 generates a plateau potential that produces inhibition of protraction neurons and excitation of retraction neurons. Thus peptide-mediated lowering of the plateau-potential threshold may contribute to the HFS-induced temporal advancement of plateau-potential generation and thereby contribute to a shortening of the protraction-phase duration; however, we cannot exclude the possibility that additional mechanisms may also act to shorten protraction duration.

Importantly, because the HFS of CBI-2 was within the normal range of firing of this neuron, the results of the present study may be relevant for feeding behavior of intact animals. Combined with previous studies, our results suggest that two distinct, yet peptidergic, mechanisms may participate in mediating the two manifestations of activity-dependent network states: shortening of response latency and shortening of protraction duration. Shortening of response latency is mediated by peptidergic homosynaptic potentiation of fast cholinergic EPSPs that CBI-2 elicits in motoneurons B61/62. The effects of this homosynaptic facilitation are manifested only when CBI-2 fires action potentials, i.e., when the network is activated through CBI-2 activity. In contrast, because modulation of plateau-potential threshold modifies an intrinsic biophysical characteristic of B64, the effects of this modulation could manifest themselves independently of whether B64 is brought to its threshold by an input from CBI-2 or by a different source. Previous work showed that repeated activation of different inputs to the central pattern generator leads to development of different network states (Proekt et al. 2004). Importantly, a network state established through activation of one input can subsequently affect how this network responds to another input. It is attractive to hypothesize that in contrast to the homosynaptic facilitation of CBI-2 synapses, the CBI-2–mediated modulation of intrinsic properties of CPG elements may allow peptides contained in CBI-2 to extend CBI-2’s actions to responses elicited by non-CBI-2 inputs.

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References


