Peptidergic Contribution to Posttetanic Potentiation at a Central Synapse of *Aplysia*

Hae-Young Koh and Klaudiusz R. Weiss

*Department of Neuroscience, Mount Sinai School of Medicine, New York City, New York*

Submitted 21 January 2005; accepted in final form 1 April 2005

Koh, Hae-Young and Klaudiusz R. Weiss. Peptidergic contribution to posttetanic potentiation at a central synapse of Aplysia. *J Neurophysiol* 94: 1281–1286, 2005. First published April 7, 2005; doi:10.1152/jn.00073.2005. Posttetanic potentiation (PTP)-like phenomena appear to be mediated by a variety of mechanisms. Although neuropeptides are located in a large number of neurons and many neuropeptides, like PTP, can enhance synaptic transmission, there is a paucity of studies indicating that peptides may actually participate in PTP. Here, we utilize a single central synapse in the feeding circuit of *Aplysia* to investigate a possible peptidergic contribution to PTP in the CNS. The cholinergic command-like interneuron, cerebral-buccal interneuron 2 (CBI-2), contains two neuropeptides, feeding circuit activating peptide (FCAP) and cerebral peptide 2 (CP2). Previous studies showed that tetanic prestimulation or repeated stimulation of CBI-2, as well as perfusion of FCAP and CP2, increase the size of the cholinergic excitatory postsynaptic potentials (EPSPs) that CBI-2 evokes in the motoneurons B61/62 and shorten the latency to initiate B61/62 firing in response to CBI-2 stimulation. We used temperature-dependent suppression of peptide release and occlusion experiments to examine the possible contribution of FCAP and CP2 to PTP at the CBI-2 to B61/62 synapse. When peptide release was suppressed, perfusion of exogenous peptides increased the size of posttetanic EPSPs. In contrast, when peptide release was not suppressed, exogenous peptides did not enhance the size of posttetanic EPSPs, thus indicating occlusion. Temperature manipulation and occlusion experiments also indicated that peptides extend PTP duration. This peptide-dependent prolongation of PTP has functional consequences in that it extends the duration of time during which the latency to initiate B61/62 firing in response to CBI-2 stimulation is shortened.

**INTRODUCTION**

Posttetanic potentiation (PTP)-related phenomena may not be mediated by single mechanisms, and several types of PTP-like phenomena have been identified (Fisher et al. 1997; Zucker and Regehr 2002). An exciting possibility is that some aspects of PTP may be mediated by neuropeptides. This is suggested by the fact that a large number of neurons contain neuropeptides, and many neuropeptides can enhance synaptic transmission (Parker 2000; Svensson et al. 2001). However, there is a scarcity of studies demonstrating that peptides actually participate in PTP. A notable exception is a study that provided evidence for a peptidergic contribution to PTP at a neuromuscular junction of *Aplysia* (Fox and Lloyd 2001). Here, we investigated the possibility that within the CNS, peptidergic cotransmission may contribute to PTP and thereby affect the initiation of motor programs. We studied the synapse between the feeding-initiating cerebral-buccal interneuron 2 (CBI-2) and motoneurons B61/62, which are the first neurons activated by CBI-2 in feeding motor programs in *Aplysia*. Previous work demonstrated PTP of EPSPs that CBI-2 elicits in B61/62 and an accompanying shortening of the latency of B61/62 firing in response to CBI-2 stimulation (Proekt and Weiss 2003; Sanchez and Kirk 2002). These synaptic potentials were also increased by perfusion of cerebral peptide 2 (CP2) (Phares and Lloyd 1996) and feeding circuit activating peptide (FCAP) (Sweedler et al. 2002), two neuropeptides present in CBI-2 (Koh et al. 2003; Morgan et al. 2000). Because peptides are preferentially released in response to high-frequency or repeated stimulation (Iverfeldt et al. 1989; Vilim et al. 1996, 2000; Whim and Lloyd 1989), we sought evidence that peptides may contribute to PTP at the CBI-2 to B61/62 synapse and thereby to the shortening of the latency to initiate motor programs.

**METHODS**

*Aplysia californica* (125–175 g) were used. Preparations consisting of connected cerebral and buccal ganglia were maintained at either 13.8–14.5 or 25°C. To reduce polysynaptic responses, preparations were continuously superfused with modified artificial sea water (ASW) that contained (in mM) 460 NaCl, 25 CaCl₂, 10 KCl, 82.5 MgCl₂, and 10 HEPES buffer, pH 7.6. FCAP was perfused at 10⁻⁶ M and CP2 at 10⁻⁵ M (both peptides were obtained from SynPep, Dublin, CA). In preliminary experiments, these concentrations were found to produce a maximal enhancement of EPSP size.

AxoClamp 2A was used to amplify intracellular signals that were obtained using glass microelectrodes filled with 2 M K acetate and 100 mM KCl. Experiments were performed only if neurons B61/62 had a resting potential more negative than −60 mV, and the data were analyzed only if the resting potential did not change by >3 mV. CBI-2 was stimulated intracellularly with 17- to 20-ms current pulses. Tetanic stimulation of CBI-2 used throughout this study was a 10-Hz train lasting 30 s. Our pilot experiments showed that this produced a maximal potentiation of the EPSPs. This stimulation frequency is within the normal physiological range of CBI-2 activity (Rosen et al. 1991).

All statistical tests involving more than two conditions were performed using one-way ANOVA, t-test with the Bonferroni correction were used for post hoc comparisons. Paired t-test were used for repeated-measure paradigms. GraphPad (San Diego, CA) was used for statistical comparisons. Sigma Plot was used to obtain exponential fits and linear regression.

**RESULTS**

To seek evidence that peptides contained in CBI-2 contribute to PTP and to the shortening of the latency of B61/62 firing...
in response to CBI-2 stimulation, we performed experiments that involved occlusion of actions of endogenous peptides (Swensen and Marder 2000) and temperature-dependent suppression of peptide release (Fox and Lloyd 2001).

In principle, PTP could be entirely mediated by peptide actions. If this was the case, PTP should not be observed when peptide release is completely suppressed. To test this hypothesis, we conducted our first experiment at 25°C, a temperature at which peptide release in *Aplysia* is suppressed (Fox and Lloyd 2001; Vilim et al. 1996; Whim and Lloyd 1990). Although we were not able to measure peptide release at the CBI-2 to B61/62 synapse, we reasoned that peptide release at this synapse may also show temperature dependence.

CBI-2 was stimulated every 3 min with 5-Hz trains (3- to 4-s duration). No PTP was observed with this interstimulation interval. Three stimulations were performed before a single tetanic stimulation (10 Hz for 30 s), which was followed within 10 s by another 5-Hz stimulation. The size of the 10th EPSP in each series was measured. We used this method because at 25°C continuous low-frequency stimulation of CBI-2 elicited very small EPSPs that could not be measured reliably. Within-facilitation, which was produced by the 5-Hz stimulation, allowed us to reliably measure the size of EPSPs. Inspection of data showed that the 10th EPSP was easy to measure, and its size was approximately in the middle of the size range thereby allowing us to detect both increases and decreases of EPSP sizes. Importantly, the facilitation ratio (FR) measured as the size of the 10th EPSP divided by the 3rd EPSP was not significantly affected by either tetanic stimulation (pretetanus FR = 1.46 ± 0.08 and posttetanus FR = 1.383 ± 0.04; paired t-test: $P > 0.3$, $t = 1.11$, df = 4) or FCAP+CP2 perfusion (prepeptide FR = 1.34 ± 0.06 and postpeptide FR = 1.39 ± 0.04; paired t-test: $P > 0.5$, $t = 0.66$, df = 4).

All measurements were normalized to the mean of the three pretetanetic values. The size of EPSPs increased to 2.82 ± 0.17 times the pretetanetic values (Fig. 1A, left, pre vs. post). We found that PTP was present at 25°C, thus suggesting the existence of peptide-independent PTP.

If endogenous peptides contribute to PTP, their effects should be mimicked by addition of exogenous peptides to the preparation in which peptide release is suppressed (i.e., at 25°C). It follows that addition of exogenous peptides should increase the size of posttetanetic EPSPs compared with those recorded in the absence of exogenous peptides. We found that this is exactly what happened at 25°C. Specifically, joint application of both peptides increased the PSP size to 3.42 ± 0.23 times the control values [Fig. 1A, pre* vs. pre(P)], and the tetanus increased it further to 8.17 ± 0.44 times the control values measured in the absence of peptides [Fig. 1A, pre* vs. post(P)]. The posttetanic EPSP size in the presence of FCAP+CP2 [post(P)] was significantly larger than that in ASW (post). In the presence of peptides, tetanic stimulation increased the size of the EPSP to 2.44 ± 0.11 times the pretetanetic value [pre(P) vs. post(P)], which is not significantly different from the potentiation in ASW (2.82 ± 0.17). Thus when release of endogenous peptides was suppressed, the absolute size of the EPSP following tetanic stimulation was larger in the presence of exogenous peptides than in their absence, but the degree of tetanus-evoked PTP was similar. The fact that the degree of PTP was similar in the presence and absence of peptides is indicative of the existence of a peptide-independent PTP.

If at 14°C released peptides contribute to PTP, the posttetanic EPSP size should be the same in the presence and absence of exogenous peptides. This is because preapplied exogenous peptides would have replaced and occluded the actions of endogenous peptides released by subsequent tetanic stimulation. This is in contrast to the effects of exogenous peptides at 25°C where the size of EPSP after tetanic stimulation was significantly larger in the presence than in the absence of exogenous peptides.

PTP was examined at 14°C, a temperature at which peptide release is not suppressed (Vilim et al. 1996; Whim and Lloyd 1990). CBI-2 was stimulated continuously at 1 Hz. After 3 min of recording pretetanic EPSPs at 1 Hz, a tetanus was given. Stimulation of CBI-2 was returned to the 1-Hz frequency and continued until the amplitude of posttetanetic EPSPs was considered to be stationary and equal to pretetanic EPSPs. When the effects of tetanus were studied in the presence of peptides, peptide perfusion started ~10 min before the onset of tetanic stimulation. The average amplitudes of 10 EPSPs were obtained for each 10-s bin after tetanus in each preparation. For data analysis the PSP amplitudes were normalized to the size of control EPSPs (~300 EPSPs for 5 min) that were measured in the absence of peptides and before tetanus.

Perfusion of FCAP or CP2, or FCAP+CP2 increased the EPSP size to 2.6 ± 0.04, 3.94 ± 0.28, 6.02 ± 0.43 times the control, respectively (gray bars in FCAP, CP2, FCAP+CP2, Fig. 1B) and tetanic stimulation further increased the EPSP size to 9.21 ± 0.31, 10.95 ± 1.05, 11.01 ± 0.85 times the control (0 peptide), respectively (corresponding white bars, Fig. 1B). In ASW (0 peptide), tetanic stimulation increased the EPSP size to 9.86 ± 0.93 times the pretetanic values (Fig. 1B, ASW). The posttetanetic EPSP sizes in the four conditions (ASW, FCAP, CP2, FCAP +CP2) were not significantly different from each other. Thus consistent with occurrence of occlusion, when release of endogenous peptides was not suppressed, the size of the EPSP after tetanic stimulation was similar independent of whether exogenous peptides were present or absent. This is in contrast to the results obtained when peptide release was suppressed.

Together, the results shown in Fig. 1, A and B, are consistent with the hypothesis that tetanic stimulation increases the size of the EPSP through both peptide-independent and -dependent mechanisms. We further probed this hypothesis by examining the time course of decay of the PTP. The time course of PTP decay reflects the time course of waning of both peptide-dependent and -independent components. When exogenous peptides were continuously perfused, their actions did not wane and for ≥40 min, there was no evidence of receptor desensitization (unpublished observations). Therefore in the presence of exogenous peptides, the time course of decay reflects only the decay of the peptide-independent component. If the two components have different time courses of decay, then occlusion experiments could be used as an indicator of a peptidergic contribution to the duration of PTP.

Because the size of the EPSP was already increased and the PTP decayed to this increased level in the presence of peptides, we used scaled data to compare the decay time courses. The scaling brought all our data into the same range of values, i.e., 1 to 0. Briefly, for each time point after tetanic stimulation, we calculated the difference in the size of the EPSP from the pretetanic value. Each of these calculated values was then divided by...
the maximum difference. The maximum occurred immediately after the tetanus. The following formula was used for scaling

$$\text{posttetanus}(t_i) - \text{pretetanus}$$

$$\text{posttetanus}(t_i) - \text{pretetanus}$$

where $\text{posttetanus}(t_i)$ = mean of posttetanus EPSP sizes in each $i$th 10-s bin; $\text{pretetanus}$ = mean of pretetanus EPSP sizes; $\text{posttetanus}(t_i)$ = mean of posttetanus EPSP sizes in the first 10-s bin. The resulting plots of the decay time course in the absence and presence of peptide(s) are shown in Fig. 1C. These plots suggested that in the presence of FCAP+CP2 the decay of PTP was much faster than in ASW or in the presence of single peptide. For quantification, the same data were plotted on a log scale in Fig. 1D. In the presence of CP2+FCAP, the rate constant of decay was significantly greater than in ASW. There was no significant difference between the rate constants in ASW and FCAP alone or CP2 alone. The longer time course that was observed when a single peptide was perfused likely represents the slow time course of waning of the action of the endogenously released peptide whose actions were not oc-

FIG. 1. The posttetanic potentiation (PTP) at cerebral-buccal interneuron 2 (CBI-2) to B61/62 synapse in the absence and presence of peptides. A, Left: at 25°C, tetanic stimulation of CBI-2 significantly increased excitatory postsynaptic potential (EPSP) size in the absence of feeding circuit activating peptide (FCAP) + cerebral peptide 2 (CP2; paired $t$-test: $P < 0.05; t = 5.73, df = 4$). Right: addition of FCAP+CP2 increased EPSP size (gray bar) and tetanic stimulation increased it further (paired $t$-test: $P < 0.05; t = 9.12, df = 4$). Posttetanic EPSPs (white bar) normalized to controls in artificial sea water (ASW; black bar) were significantly larger in the presence of FCAP+CP2 than in their absence (post-tetanus: $P < 0.01, t = 12.86, df = 8$). There was a significant difference in the degree of PTP in the presence of peptides (pre(P) vs. post(P)) and in their absence (pre vs. post; $t$-test: $P > 0.05, t = 1.395, df = 8$). All values are means $\pm$ SE ($n = 5$). B: at 14°C, there was no significant difference between posttetanic EPSP sizes in the 4 conditions (white bar: $P > 0.3, F = 1.2, df = 3.16$). Note that there is no distinction between “control” and “pretetanus” in the condition without peptide (ASW). The ratio of posttetanic to pretetanic EPSP size in the presence of peptides (white vs. gray bars in the 3 peptide groups) was calculated. This ratio was: $4.43 \pm 0.14$ for the FCAP group, $2.77 \pm 0.26$ for the CP2 group, $1.84 \pm 0.14$ for the FCAP+CP2 group. Statistical analyses showed a significant overall difference among the 4 groups ($P < 0.0001, F = 39.5, df = 3.16$). Compared with the ASW the values for FCAP, CP2, and FCAP+CP2 groups were significantly smaller (ASW vs. FCAP, $t = 7.691, P < 0.001$; ASW vs. CP2, $t = 8.611, P < 0.001$; ASW vs. CP2: $t = 9.849, P < 0.001$). All values are means $\pm$ SE ($n = 5$). C: decay time course of PTP in the presence and absence of peptides at 14°C. Scaled data are plotted. Red circles, in the absence of peptide (ASW); open circles, in the presence of both peptides (FCAP+CP2); green diamonds, CP2 only; blue squares, FCAP only. The exponential fits for the ASW and FCAP+CP2 plots are drawn as 2 solid line curves over the plots to help distinguish between these 2 plots. D: PTP decay plots on log scale. The data shown in C were plotted on log scale for quantification of the decay time course. The solid lines are linear regressions of the plots in the absence and presence of single peptide or both peptides. The slope of the linear regression is the rate constant of PTP decay. The overall ANOVA of rate constants of decay in the different groups (ASW, FCAP, CP2, and FCAP+CP2) showed that there was no significant difference between groups ($F = 10.220.33$.2 on October 14, 2017 http://jn.physiology.org/ Downloaded from
included. Notice that these data suggest that the actions of both peptides waned with a similar time course. Joint application seemed to occlude the slowly decaying component of the PTP. The component of the PTP that is putatively mediated by peptides decayed slowly with a lingering tail until ~1,000 s (Fig. 1C), whereas the peptide-independent component lasted <300 s.

Summation to action potential threshold in B61/62 is more rapid when the size of EPSPs is increased in the aftermath of tetanic stimulation (Proekt and Weiss 2003; Sanchez and Kirk 2002). To test the ability of the slow component of PTP to shorten the latency of B61/62 firing, we examined the persistence of latency shortening after tetanic stimulation. CBI-2 was stimulated at ~6 Hz for ~15 s (5-min interstimulation interval) to elicit B61/62 firing. No PTP was observed at the 5-min interstimulation interval. The latency of B61/62 firing is defined by the period between the first spike of CBI-2 and the appearance of the first action potential in B61/62 (double-headed arrows in Fig. 2, A1 and B1). After obtaining three pretetanic measurements of latency, CBI-2 was given a tetanus, and then the latency was measured at 400, 700, 1,000 and 1,300 s after the end of tetanic stimulation. (Fig. 2A, I and 2). Compared with the pretetanus latency, there was a significant shortening of the latency at 400 and 700 s, the times at which the EPSP size was still increased. There was no shortening at 1,000 and 1,300 s, the times when the EPSP size was no longer increased. Thus the slow component of PTP appears to be functionally significant.

Because we observed that the slowly decaying component of PTP was occluded by the joint application of FCAP and CP2, we sought to determine whether the decay of latency shortening is similarly affected. Exogenous peptides increase the size of EPSPs and therefore we expected a shortening of latency in their presence (Koh et al. 2003). To assure that peptides did not

---

**FIG. 2.** The decay of the effect of tetanus on B61/62 firing latency in the absence and presence of FCAP+CP2 at 14°C. A1: a representative recording of B61/62 firing elicited by CBI-2 stimulation before and 400, 700, and 1,000 s after the tetanus in the absence of peptides. A2: pooled data (n = 5). The pretetanus value in this bar chart is the average of 3 latency measurements before tetanus. There was a significant overall difference between different time points (P < 0.0001, F = 24.1, df = 4,16). Compared with the pretetanus latency there was a significant shortening of the latency at 400 s (paired t-test: P < 0.001, t = 7.67, df = 4) and 700 s (paired t-test: P < 0.001, t = 5.35, df = 4). B1: a representative recording of CBI-2 elicited B61/62 firing before and 60 and 240 s after tetanus in the presence of both peptides. B2: pooled data (n = 5). Application of FCAP+CP2 before tetanus significantly shortened the latency of B61/62 firing (paired t-test: P < 0.01, t = 8.11, df = 4; not shown in the bar graph). In the presence of FCAP+CP2, there was an overall significant difference between different time points (P < 0.0001, F = 83.4, df = 4.16). The latency was significantly shortened at 60 s (paired t-test: P < 0.001, t = 14.6, df = 4). There was no significant shortening at 240, 420, and 600 s (P > 0.05). The pretetanus value in this figure is the average of 3 latency measurements before tetanus. *, a significant difference. All values in the bar charts are means ± SE.
produce a maximum shortening, which would prevent further shortening as a result of tetanic stimulation, CBI-2 was stimulated at a lower frequency (4 Hz for 13 s) to obtain latency measurements. No PTP was observed with the 3-min interstimulation interval used in this experiment. Perfusion of FCAP and CP2 shortened the latency to B61/62 firing (pre-tetanus, Fig. 2B1). Due to the faster time course of PTP decay in the presence of peptides, the posttetanus latency was measured at shorter intervals (240, 420, and 600 s after tetanus). Because we expected that in the presence of peptides the slowly decaying component would be occluded, we also included a 60-s group to determine whether at least the rapidly decaying component of potentiation was able to shorten the latency. The latency was significantly shortened at 60 s. There was no significant shortening at 240, 420, and 600 s (Fig. 2B, 1 and 2).

We next sought to determine whether at 25°C, the temperature at which peptide release is suppressed, the decay of the posttetanic shortening of the latency also lacks the slowly decaying component. To elicit B61/62 firing, CBI-2 was stimulated with 6-Hz trains of 13-s duration every 3 min. After obtaining three measurements for pretetanus, CBI-2 was given a tetanus, and then the latency was measured at 60, 240, 420, and 600 s after the tetanus. We expected that at this temperature the slowly decaying component would be absent. The latency was shortened significantly at 60 s, but no significant shortening was observed at 240, 420, and 600 s (Fig. 3A, 1 and 2). To ensure that this was due to the absence of peptide release rather than insensitivity to peptides, we also tested the effects of exogenous peptides after the latency was fully recovered to the pre-tetanus value. Because exogenous peptides significantly shortened the latency (Fig. 3A2), the absence of a slowly decaying component was not due to insensitivity to peptides.

To determine whether the faster time course of decay of latency shortening at 25°C versus 14°C might be related to a faster decay of the tetanus effects on EPSP size at 25°C, we analyzed the EPSP size at different times after tetanic stimulation. The data used for this analysis were obtained from the experiments illustrated in Fig. 3A, 1 and 2. We measured the size of the 10th EPSP in each train of stimulation (for explanation of this measurement, see the description of the experi-

![Figure 3](http://jn.physiology.org/)

**Fig. 3.** The decay of the tetanus effect on B61/62 firing latency and PTP decay at 25°C. A1: a representative recording of CBI-2 elicited B61/62 firing before and 60 sfn 240 s after tetanus, and in the presence of both peptides, at 25°C. A2: pooled data ($n = 5$). The pretetanus value in this bar graph is the mean of 3 latency measurements before tetanus. There was an overall difference between groups ($P < 0.0001$, $F = 66.9$, df = 5, 20). The latency was shortened significantly at 60 s (paired $t$-test: $P < 0.001$, $t = 10.3$, df = 4), but no significant shortening was observed at 240, 420, and 600 s ($P > 0.05$). The exogenous peptides shortened the latency significantly (paired $t$-test: $P < 0.001$, $t = 11.9$, df = 4). B: to examine the PTP decay time course at 25°C, we took advantage of the recordings from the latency experiments (A, 1 and 2) and measured the 10th EPSP in each stimulation train. B1: a representative recording of the 10th EPSP before and 60, 240, 420, and 600 s after the tetanus, and also in the presence of FCAP + CP2 (without tetanus) at 25°C. B2: the pooled data taken from the latency experiments used in A. The pretetanus value in this bar graph is the mean of 3 measurements before tetanus. There was a significant overall difference between different time points ($P < 0.0001$, $F = 30.1$, df = 5, 20). The EPSP size was significantly increased at 60 s (paired $t$-test: $P < 0.001$, $t = 7.954$, df = 4). There were no significant ($P > 0.05$) differences at subsequent time points. Addition of FCAP + CP2 significantly increased EPSP size (paired $t$-test: $P < 0.01$, $t = 5.03$, df = 4). * a significant difference. All values in the bar charts are means ± SE.

*J Neurophysiol* • VOL 94 • AUGUST 2005 • [www.jn.org](http://jn.physiology.org/)
ment that is illustrated in Fig. 1A). The EPSP size was significantly increased at 60 s. The EPSP sizes at subsequent time points were not significantly different from pretetanus (Fig. 3B, I and 2). Addition of FCAP+CP2 increased the EPSP size showing that the EPSP size was sensitive to peptides (Fig. 3B2). These results suggest that the fast decay of latency shortening at 25°C may be related to a fast decay of PTP at this temperature.

**DISCUSSION**

Repeated activation of feeding responses in intact *Aplysia* leads to a progressive shortening of the latency of feeding responses (Kupfermann 1974). The shortening of latency to the initiation of CBI-2-elicted motor programs also occurs when the command-like neuron CBI-2, which triggers motor programs, is prestimulated at higher frequencies or is stimulated repeatedly (Proekt and Weiss 2003; Sanchez and Kirk 2002). This is associated with PTP at the CBI-2 to B61/62 synapse (Sanchez and Kirk 2002). The effects of high-frequency stimulation on the size of EPSPs and the B61/62 firing latency are mimicked by perfusion of exogenous peptides that are localized in CBI-2 (Koh et al. 2003). Because peptides are preferentially released under conditions of sustained high-frequency activity of neurons, we hypothesized that peptides may contribute to the PTP at the CBI-2 to B61/62 synapse. Taken together, our experiments support this hypothesis and suggest that both peptide-independent and -dependent processes contribute to PTP at the CBI-2 to B61/62 synapse.

The decay rate of the peptide-dependent component of PTP is significantly slower than that of the peptide-independent component. Furthermore, the slowly decaying peptide-dependent component of PTP contributes to the persistent shortening of latency to initiate B61/62 firing in response to CBI-2 stimulation. Because the frequency of tetanic stimulation we used was within the range of normal CBI-2 activity (Rosen et al. 1991), our findings are likely to be relevant to normal feeding behaviors.

Our data in the CNS, taken together with the findings from the neuromuscular junction (Fox and Lloyd 2001) and combined with the widespread presence of peptides in neurons, suggest that peptidergic contribution to PTP may not be a rare phenomenon. Interestingly, PTP-related phenomena may not be mediated by single mechanisms, and several types of PTP-like phenomena have been identified based on their time course (Fisher et al. 1997). Because our study implicates peptidergic involvement in the time course of PTP, it is possible that some of the differences in the time course of PTP, which have been observed in other preparations, may also be attributable to peptidergic actions.

**ACKNOWLEDGMENTS**

We thank Drs. V. Brezina and E. C. Cropper for comments on an earlier version of this manuscript.

**GRANTS**

Funding for this research was provided by the National Institute of Mental Health Grant MH-36730.

**REFERENCES**


