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Structure and action of buccalin: A modulatory neuropeptide localized to an identified small cardioactive peptide-containing cholinergic motor neuron of *Aplysia californica*

(arousal/cotransmitter/invertebrate/modulation/muscle contractility)

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ABSTRACT **A model system that consists of a muscle utilized in biting, the accessory radula closer (ARC), and the two cholinergic motor neurons innervating this muscle, neurons B15 and B16, has been used to study the expression of food-induced arousal in the marine mollusk *Aplysia*. The ARC muscle receives modulatory input from an extrinsic source, the serotonergic metacerebral cells, which partially accounts for the progressive increase in the strength of biting seen in aroused animals. Another source of modulation may arise from the ARC motor neurons themselves, which synthesize neuropeptides that can potentiate ARC contractions. Neuron B15 synthesizes the two homologous peptides, small cardioactive peptides A and B, whereas neuron B16 synthesizes the structurally unrelated peptide myomodulin. Here we report the purification and sequencing of a neuropeptide termed buccalin and show that it is colocalized with the small cardioactive peptides to neuron B15. Buccalin is also bioactive at the ARC neuromuscular junction but, in contrast to the small cardioactive peptides, when exogenously applied, it decreases rather than increases the size of muscle contractions elicited by firing of the motor neurons. Also unlike the small cardioactive peptides, which exert postsynaptic actions, buccalin seems to act only presynaptically. It has no effect on muscle relaxation rate and decreases motor neuron-elicited excitatory junction potentials in the ARC without affecting contractions produced by direct application of acetylcholine to the muscle. Neuron B15, therefore, appears to contain three modulatory neurotransmitters, two of which may act postsynaptically on the muscle to potentiate the action of the primary neurotransmitter acetylcholine and one of which may act presynaptically on nerve terminals to inhibit acetylcholine release.**

We have been studying a motivational state, food-induced arousal, that is manifested in the consummatory biting response of the marine mollusk *Aplysia californica* as progressive increases in both the speed and strength of biting (1, 2). Work in a model system (3) that consists of one of the muscles utilized in biting, the accessory radula closer (ARC), and the two cholinergic motor neurons that innervate this muscle, buccal motor neurons B15 and B16, has led us to conclude that arousal in the *Aplysia* biting response is partially mediated by activity of the serotonergic metacerebral cells (4–6).

We have also demonstrated that motor neurons B15 and B16 synthesize neuropeptides that have bioactivity at the ARC muscle (7, 8). Neuron B15 contains the two structurally homologous (9–11) peptides, small cardioactive peptides A and B (SCP_A and SCP_B) (7), whereas neuron B16 contains the unrelated peptide myomodulin (8). When exogenously applied, all three peptides produce increases in the size and the relaxation rate of muscle contractions elicited by motor neuron stimulation (7, 8, 12). Therefore, we have hypothesized that the ARC neuromuscular system is modulated both extrinsically (by means of the metacerebral cell) as well as intrinsically [by means of the peptides released from the motor neurons themselves (7)].

In the present study, we report the purification and sequencing of a neuropeptide termed buccalin and colocalize buccalin with SCP_A and SCP_B to the ARC motor neuron B15. We also demonstrate that exogenously applied buccalin depresses ARC contractions by means of a presynaptic mechanism. We have thus localized three modulatory neuropeptides to a single cholinergic motor neuron. Two of the peptides potentiate contractions, whereas a third depresses contractions. These peptides provide an experimentally advantageous opportunity for studying cotransmission in a behaviorally relevant context.

METHODS

Radiolabeling of B15 Neurons. Physiologically identified B15 neurons (3) were marked by intracellular iontophoresis of fast green dye and were radiolabeled (7, 13). Buccal ganglia were incubated in [^{35}S]methionine and colchicine for 24 hr and were rinsed in 50% hemolymph/50% L15 medium (Flow Laboratories, McLean, VA) for 4–6 hr. Individual B15 neurons were dissected (14) and then extracted by heating for 10 min in 100 μl of 0.1 M trifluoroacetic acid containing nanomolar quantities of synthetic peptides.

The B15 extract was fractionated by reverse-phase HPLC (RP-HPLC). Fractions containing radioactivity were identified by scintillation counting, and fractions containing synthetic peptides were identified by absorbance measurements at 215 nm.

Purification and Sequence Analysis of Buccalin. Buccalin was purified from the same 30 g of ARC muscle that was used for the purification of myomodulin (8). Muscles were heated for 10 min at 100°C in 0.1 M acetic acid (final tissue concentration of 0.1 g/ml), homogenized with a Polytron, heated again for 5 min, and centrifuged at 7000 × *g* for 20 min. The supernatant was applied to six C_{18} cartridges (Sep-Pak, Waters), in series, and peptides were eluted with 8 ml of 0.01 M trifluoroacetic acid/66% acetonitrile (CH_3CN)/34% H_2O. A sample of radiolabeled B15 peptides (see above) was added to the Sep-Pak eluate, and this mixture was subjected to four steps of sequential RP-HPLC (Table 1). Prior to the first chromatography step the eluate was diluted to <20%

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Abbreviations: AcCho, acetylcholine; ARC, accessory radula closer; EJP, excitatory junction potential; RP-HPLC, reverse-phase HPLC; SCP_A and SCP_B, small cardioactive peptides A and B.

Table 1. Protocol for analytical purification of buccalin

Step	Column	Solvent A	Solvent B	Gradient
1	Zorbax C_8	0.01 M F_7C_3COOH/ H_2O	0.01 M F_7C_3COOH/ CH_3CN	20–35% (vol/vol) B in 120 min
2	Zorbax C_8	0.01 M F_3CCOOH/ H_2O	0.01 M F_3CCOOH/ CH_3CN	20–40% (vol/vol) B in 80 min
3	Aquapore RP-300	0.1 M Et_3NOAc/ H_2O	0.1 M Et_3NOAc/ 80% (vol/vol) CH_3CN/20% (vol/vol) H_2O	15–35% (vol/vol) B in 80 min
4	Aquapore RP-300	0.01 M F_3CCOOH/ H_2O	0.01 M F_3CCOOH/ CH_3CN	10–45% (vol/vol) B in 35 min

In steps 1, 2, and 4 the pH was 2.0. In step 3 the pH was 5.5. Columns were 4.6 × 250 mm. All gradients were linear, and the flow rate in each case was 1 ml/min. Fractions were collected every 30 sec.

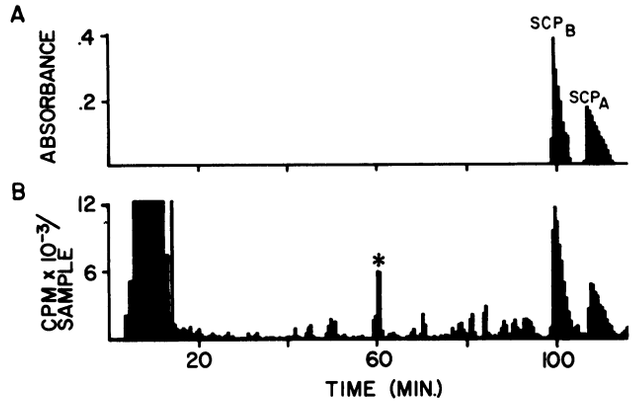

FIG. 1. RP-HPLC of 54 B15 neurons following *in vivo* radiolabeling with [^{35}S]methionine and extraction of radiolabeled peptides in the presence of 63 nmol of synthetic SCP_A and SCP_B. Chromatography was performed in the presence of heptafluorobutyric acid, and the column was developed with a linear gradient of 20–35% acetonitrile in 120 min. Samples were collected every 0.5 min. Synthetic SCP_A and SCP_B were detected by absorbance measurements at 215 nm (*A*), and 5% of each fraction was counted to detect radioactivity (*B*). B15 neurons yielded one major peak of radioactivity, buccalin (indicated by an asterisk), in addition to that which precisely coeluted with synthetic SCP_A and SCP_B.

CH_3CN, and to avoid exceeding column capacity, it was identically chromatographed in four equal portions. RP-HPLC fractions that contained buccalin were identified either by the presence of the radioactive buccalin marker (detected by scintillation counting, steps 1–3) or by their bioactivity on the ARC muscle (steps 2 and 4; see below).

Gas-phase sequence analysis of the purified material was performed at the Howard Hughes Medical Institute Protein Chemistry Core Facility of Columbia University (8). Based on the results of this analysis, a peptide was commercially synthesized (Milligen, Bedford, MA), and the hypothesized structure for buccalin was verified by comparing the bioactivity and chromatographic properties of the commercially synthesized peptide to those of the peptide purified from the ARC. The chromatographic properties of the commercially synthesized peptide were also compared to those of radiolabeled buccalin synthesized by B15. Three sequential RP-HPLC passes were used; the first was identical to step 4 used in the purification of the ARC peptide (see Table 1), the second was identical to purification step 3 (Table 1) except that the gradient was 20–50% solvent B in 30 min, and the final pass was identical to purification step 1 except that the gradient was 20–55% solvent B in 35 min.

Actions of RP-HPLC Fractions and Synthetic Peptides on ARC Contractions. Testing of RP-HPLC fractions and synthetic peptides for bioactivity was done as described (7). Briefly, the ARC muscle was placed in a subchamber isolated from the buccal ganglion. Muscle contractions were produced by trains of motor neuron action potentials and were monitored with an isotonic force transducer. Peptides and lyophilized RP-HPLC fractions were made up in artificial seawater and were injected as concentrated boluses into the ARC subchamber. To study peptide effects on excitatory junction potentials (EJPs), ARC muscle fibers were impaled with single barrel electrodes (10–20 mΩ), and motor neurons were fired by single current pulses. Experiments in which

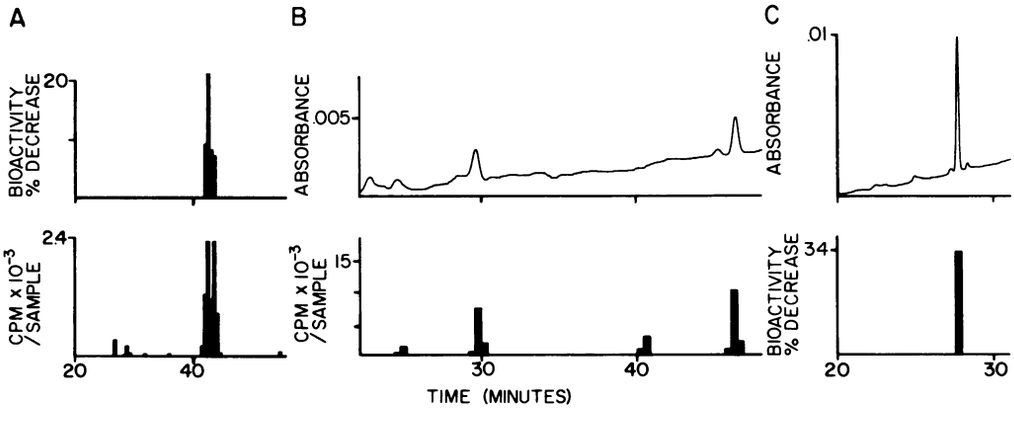

FIG. 2. Analytical purification of buccalin. The remaining 95% of the major non-SCP peak of B15 radioactivity (Fig. 1) was added to ARC extract, which was then subjected to four sequential steps of RP-HPLC (Table 1). The first purification step (step 1 in Table 1) was done under conditions identical to those used to fractionate B15 neurons to obtain radiolabeled buccalin (see Fig. 1). Aliquots of the resulting fractions were counted, and a peak of radioactivity with a retention time similar to that of the B15 peptide was observed (not shown). (*A*) Rechromatography (step 2 in Table 1) of this peak of radioactivity yielded two overlapping peaks of radioactivity (*Lower*), only one of which, the first peak, was bioactive (*Upper*). Bioactivity is expressed as the percent decrease in the amplitude of muscle contractions elicited by stimulation of motor neuron B15 or B16 3 min after fraction application directly on the ARC muscle. (*B*) Further chromatography (step 3 in Table 1) of the bioactive peak from the second step of purification yielded two major UV-absorbing peaks (*Upper*) that both contained radioactivity (*Lower*). Chromatography of peak I yielded several UV-absorbant peaks, none of which was bioactive (not shown). (*C*) Chromatography (step 4 in Table 1) of peak II. It eluted as a single bioactive (*Lower*), UV absorbant (*Upper*) peak.

ARC contractions were directly elicited by application of acetylcholine (AcCho) were performed as described (12).

Immunocytology. B15 neurons were physiologically identified and were then marked by iontophoretic injection of Lucifer yellow dye. Immunocytology on whole mounts was performed (15) with a rabbit primary antibody raised against buccalin conjugated to bovine serum albumin by using the carbodiimide method (Berkeley Antibody, Richmond, CA). When this antiserum was preabsorbed with synthetic buccalin (100 μM for 24 hr at 4°C), no specific staining was observed. The second antibody was rhodamine-conjugated goat anti-rabbit IgG, Fab fragment (Cappel Laboratories, Malvern, PA). Ganglia were viewed with a Leitz microscope equipped with epifluorescence and were photographed with Tri-X (ASA 400) film.

RESULTS

Purification and Sequence Analysis of Buccalin. B15 neurons radiolabeled *in vivo* with [^{35}S]methionine and fractionated by using RP-HPLC (Table 1, step 1) yielded three major peaks of radioactivity (Fig. 1). The two late peaks were identified as SCP_A and SCP_B. Subsequent analysis showed that the early peak (eluting at ≈60 min) contained a bioactive peptide that we have termed buccalin. This peak could be observed from fractionation of extracts of single B15 neurons as well as from pooled B15 neurons.

Large-scale purification of buccalin was accomplished by subjecting material extracted from ARC muscles to four steps of sequential RP-HPLC (Table 1). An internal tracer [i.e., the radiolabeled B15 peak that was not SCP_A or SCP_B (see above)] was added to the ARC extract. Chromatographic conditions used for the first purification step (Table 1, step 1) were identical to those used to fractionate the B15 neurons illustrated in Fig. 1. Buccalin was therefore recognized by its elution time (≈60 min). Fractions of the ARC extract that contained radiolabeled buccalin were rechromatographed (Table 1, step 2), which resulted in two major peaks of radioactivity that overlapped (Fig. 2*A Lower*). Only one of these peaks, the first, was bioactive on the ARC muscle (Fig. 2*A Upper*). It decreased the size of contractions produced by stimulation of either motor neuron B15 or B16 (see Fig. 3*A Top*). When the bioactive peak of radioactivity was rechromatographed (Table 1, step 3), two major UV-absorbing peaks (Fig. 2*B Upper*), which also contained radioactivity (Fig. 2*B Lower*), became apparent. Both peaks were rechromatographed (Table 1, step 4); peak I split into a number of UV-absorbant peaks, none of which were bioactive (data not shown). Peak II, however, eluted as a single, bioactive (Fig. 2*C Lower*), UV-absorbant peak (Fig. 2*C Upper*) that, from its shape, appeared to contain a single peptide. Peak II, therefore, was subjected to gas-phase sequence analysis. The sequence and amount (in picomoles) of amino acids detected were as follows: Gly(100)-Met(80)-Asp(33)-Ser(2)-Leu(53)-Ala(54)-Phe(44)-Ser(2)-Gly(27)-Gly(25)-Leu(6).

All of the peptides described in the ARC muscle to date have an amidated carboxyl terminal (7, 8, 16). Since a carboxyl-terminal amide would not have been detected by the sequencing method used, an amidated peptide with the above amino acid sequence was commercially synthesized. When this synthetic peptide was chromatographed, under conditions identical to those used in the final step of purification of the ARC extract (Table 1, step 4), it eluted with the same retention time. Under these conditions, nonamidated peptides have a retention time that is ≈2 min longer than amidated peptides (8, 17). When the synthetic peptide was tested for bioactivity on the ARC muscle, it produced a dose-dependent decrease in the size of motor neuron-elicited contractions, as did the peptide purified from the ARC extract (Fig. 3*A*). These results suggest, therefore, that the amino acid sequence obtained from our analysis was complete and that the peptide purified from the ARC extract was amidated at its carboxyl terminal.

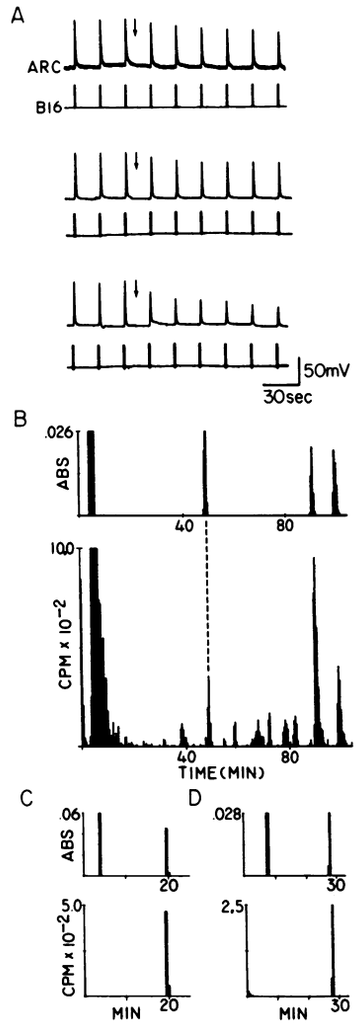

FIG. 3. Verification of the putative structure of buccalin. (*A*) Comparison of bioactivity of native material extracted from the ARC muscle and amidated synthetic buccalin. The top trace in each pair is the record of ARC contractions; the bottom trace is the record of fixed bursts of spikes in B16. Arrows indicate injection of RP-HPLC fractions containing material extracted from ARC muscle (*Top*) and of amidated synthetic buccalin at 1.7×10^{-8} M (*Middle*) and 3.4×10^{-8} M (*Bottom*). Both native and synthetic material decreased the size of motor neuron-elicited muscle contractions. (*B–D*) Comparison of chromatographic properties of radiolabeled buccalin synthesized by B15 and amidated synthetic buccalin. Methionine-containing peptides in eight B15 neurons were radiolabeled *in vivo* and extracted in the presence of 10 nmol of amidated synthetic buccalin, 6 nmol of synthetic SCP_A, and 6 nmol of SCP_B. Extracted material was sequentially chromatographed through three RP-HPLC passes (*B–D*). Synthetic material was detected by absorbance measurements at 215 nm (*Upper* in *B–D*), and B15 radioactivity was detected by counting aliquots of fractions (*Lower* in *B–D*). (Counts per whole fraction are plotted). The major non-SCP peak of B15 radioactivity and synthetic amidated buccalin precisely coeluted through all three passes.

To confirm that we had indeed obtained the correct structure for a peptide normally present in B15, the chromatographic properties of radiolabeled B15 peptides and our synthetic peptide were compared. B15 peptides were radiolabeled *in vivo* with [^{35}S]methionine and were extracted in the presence of synthetic material. Extracted material was then subjected to three sequential RP-HPLC passes. In all three passes, synthetic peptide and the major non-SCP peak of radioactivity precisely coeluted (Fig. 3). We conclude, therefore, that we have obtained the correct structure of buccalin,

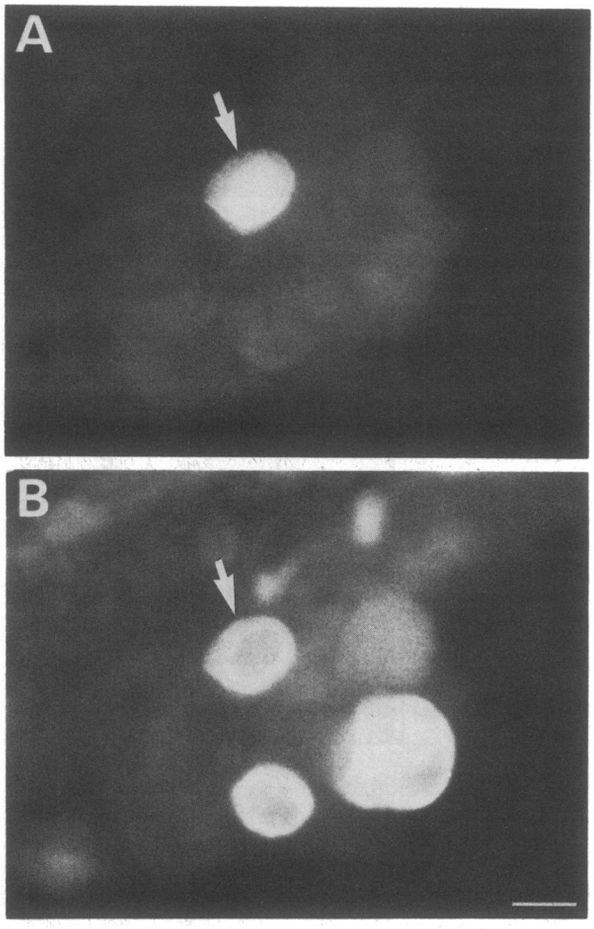

FIG. 4. Buccalin immunoreactivity of neuron B15. (*A*) Neuron B15 (arrow) was physiologically identified and injected with Lucifer yellow dye. (*B*) Whole mount immunocytology (15) was then performed by using a rabbit primary antiserum raised against a buccalin–bovine serum albumin conjugate and a rhodamine-conjugated goat anti-rabbit secondary antiserum. On the rostal surface of the ganglion, several large neurons of the ventral motor neuron cluster, and a group of 6–8 small cells were buccalin-immunoreactive as well as neuron B15 (arrow). (Bar = 100 μm.)

the B15 peptide, and that the structure is Gly-Met-Asp-Ser-Leu-Ala-Phe-Ser-Gly-Gly-Leu-NH_2.

Immunocytology. Five B15 neurons were physiologically identified and iontophoretically injected with Lucifer yellow dye. Buccal ganglia were then incubated with a buccalin-bovine serum albumin primary antibody and a rhodamine-conjugated goat secondary antibody. As expected, in all cases neuron B15 was buccalin immunoreactive (Fig. 4).

Action of Buccalin vs. SCP on the ARC. The SCPs appear to exert postsynaptic actions on the ARC muscle since they increase ARC cAMP levels and potentiate contractions elicited by direct application of AcCho to the muscle (12). Furthermore, consistent with a postsynaptic action is the observation that in addition to increasing the size of motor neuron-elicited muscle contractions, the SCPs also increase the muscle relaxation rate (Fig. 5 *A* and *B*). By contrast, buccalin, when exogenously applied alone or in the presence of SCP, has no effect on the muscle relaxation rate and only affects the size of muscle contractions ($n = 5$; Fig. 5 *A* and *B*). This suggests that buccalin might exert its actions primarily presynaptically. Consistent with this hypothesis, we found that buccalin decreased EJPs recorded from ARC muscle fibers ($n = 4$; Fig. 6) and had no effect on the size of ARC contractions produced by direct application of AcCho to the muscle ($n = 2$; Fig. 5*C*).

DISCUSSION

Previously we demonstrated that the cholinergic ARC motor neuron B15 contains the two structurally homologous (9–11) neuropeptides SCP_A and SCP_B (7, 8). Available evidence indicates that the SCPs are encoded for by a single mRNA and are present on the same precursor protein (10). In this study, we have shown that in addition to the SCPs neuron B15 contains a neuropeptide termed buccalin that is not present on the characterized SCP precursor protein. When exogenously applied, the SCPs increase both the relaxation rate and the size of ARC contractions elicited by firing of motor neurons B15 and B16 (7, 8, 12). The SCPs act postsynaptically, most probably by increasing cAMP levels in the muscle (12). Exogenously applied buccalin, however, decreases the size of muscle contractions and has no effect on the muscle relaxation rate. Our evidence indicates that the inhibitory effects of buccalin are exerted presynaptically. The cholinergic motor neuron B15, therefore, contains at least three peptides that may function as modulatory neurotransmitters. Two of the peptides (SCP_A and SCP_B) are capable of acting postsynaptically on the muscle to potentiate

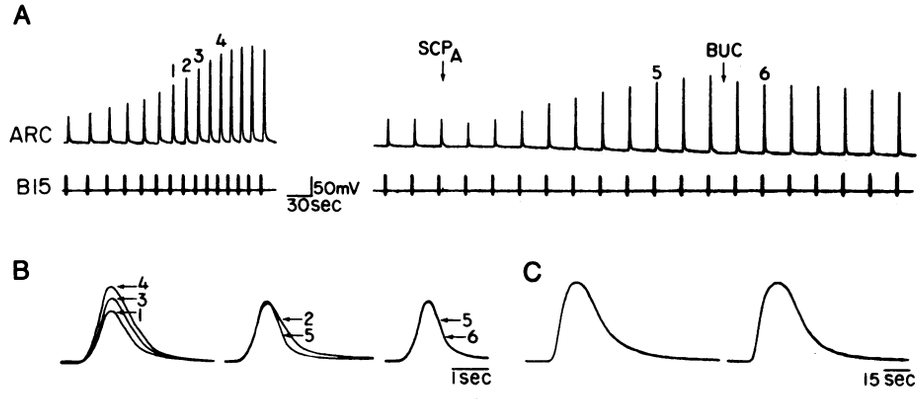

FIG. 5. Effects of synthetic buccalin (BUC) and SCP_A on the ARC. (*A*) The top traces are records of ARC contractions; the bottom traces are records of fixed bursts in B15. (*B*) Contractions 1–6 in *A* at a faster speed. When the size of muscle contractions is changed by decreasing interburst interval (*A Left*) (i.e., increasing posttetanic potentiation), muscle contractions take longer to relax as shown in *B Left*, which is a superimposition of contractions 1, 3, and 4 in *A*. Relaxation rate of contractions potentiated by synthetic SCP_A at 10^{-9} M (*A Right*) is however increased as shown in *B Center*, where for purposes of comparison a contraction potentiated by posttetanic potentiation (contraction 2) is superimposed on a contraction potentiated by SCP_A (contraction 5). Addition of synthetic buccalin (10^{-8} M) reduces the contraction size but does not produce a change in relaxation rate as shown in *B Right*, where a contraction potentiated by SCP_A contraction (5) is superimposed on a contraction reduced by buccalin (contraction 6). (*C*) Records of ARC contractions produced by direct application of 20 pmol of AcCho to the ARC made before (*Left*) addition of synthetic buccalin (10^{-6} M) and 7 min after addition of buccalin (*Right*). Buccalin produces no change in contraction size.

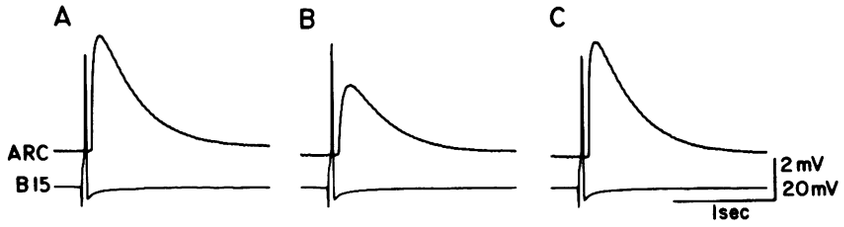

FIG. 6. Effects of synthetic buccalin on ARC EJPs. Top traces are EJPs recorded intracellularly from an ARC muscle fiber and elicited by stimulation of motor neuron B15 (bottom traces). (*A*) Before buccalin application. (*B*) In the presence of 10^{-6} M buccalin. (*C*) After buccalin washout. A higher concentration of buccalin was used to illustrate its effects on EJP size than was used to show effects on contraction size because of the properties of the ARC neuromuscular junction. The ARC is a nonspiking muscle; its contraction size is directly dependent on its degree of depolarization (3). Since small changes in ARC EJPs produce large changes in the size of muscle contractions (3), concentrations of buccalin (e.g., 10^{-8} M) that produce significant decreases in contraction size produce less striking effects on EJPs. Higher concentrations of buccalin (e.g., 10^{-6} M), which produce larger effects on EJPs, virtually eliminate detectable muscle contractions.

the action of the primary neurotransmitter, AcCho. However, the third peptide, buccalin, can depress contractions and may act presynaptically on the nerve terminals to inhibit AcCho (and perhaps peptide) release.

Buccalin is present in neuron B15, but it presynaptically decreases the size of muscle contractions elicited by stimulation of either motor neuron B15 or B16. It is possible, therefore, that buccalin is actually present in both motor neurons. Since neuron B16 contains myomodulin, which increases the size of motor neuron-elicited muscle contractions (8), this neuron may be similar to neuron B15 in that it also contains apparently antagonistic neuromodulators.

In neuron B15 the fact that buccalin and the SCPs are not encoded by the same precursor protein raises the possibility that they are packaged in different synaptic vesicles and may be differentially released or that their synthesis may be differentially controlled. It is interesting to note that the effects of the SCPs and buccalin are actually only partially antagonistic. SCP application increases the size of motor neuron-elicited muscle contractions, and buccalin decreases muscle contraction size, but the SCPs also affect another parameter of muscle contraction unaffected by buccalin—relaxation rate. If the opposite actions of the SCPs and buccalin on contraction size were to be equal in magnitude, the resulting contractions would not change in size, but they would differ in rate of relaxation. Ordinarily the time to relax is closely linked to the magnitude of contraction. When muscle contractions are increased in size by increasing posttetanic potentiation [i.e., presumably by increasing the AcCho released (3)], the time it takes for the muscle to relax increases (Fig. 5). Changes in the ratio of the amounts of buccalin vs. SCP released or selective modulation of SCP and buccalin receptors by other neuromodulators [e.g., serotonin (4), Phe-Met-Arg-Phe-NH_2 (16), and myomodulin (8)] acting at the muscle could function to alter the coupling that exists between the magnitude of contraction size and the time for the muscle to relax. Since buccal muscles are involved in the execution of several different behaviors, the multiple peptides in the buccal motor neurons may function to appropriately alter the detailed nature of the muscle contractions. Our data, together with growing evidence for cotransmission at other invertebrate (e.g., refs. 18–23) as well as vertebrate (e.g., refs. 24–26) muscles, reinforce the idea that synapses can transmit complex chemical signals that may fine tune the response of the postsynaptic cell.

In conclusion, we have purified and sequenced a modulatory neuropeptide that coexists with two other bioactive peptides in a single cholinergic motor neuron. This neuron is part of a well-characterized neuromuscular system that should provide an opportunity to study peptide cotransmission in a behavioral context (i.e., food-induced arousal).

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1. Kupfermann, I. (1974) *Behav. Biol.* **10,** 1–26.
2. Weiss, K. R. & Kupfermann, I. (1977) in *Aspects of Behavioral Neurobiology,* Soc. Neurosci. Symposia, ed. Ferrendelli, J. A. (Soc. Neurosci., Bethesda, MD), Vol. 3, pp. 66–89.
3. Cohen, J. L., Weiss, K. R. & Kupfermann, I. (1978) *J. Neurophysiol.* **41,** 157–180.
4. Weiss, K. R., Cohen, J. L. & Kupfermann, I. (1978) *J. Neurophysiol.* **41,** 181–203.
5. Kupfermann, I. & Weiss, K. R. (1982) *Brain Res.* **241,** 334–337.
6. Rosen, S. C., Kupfermann, I., Goldstein, R. S. & Weiss, K. R. (1983) *Brain Res.* **260,** 151–155.
7. Cropper, E. C., Lloyd, P. E., Reed, W., Tenenbaum, R., Kupfermann, I. & Weiss, K. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3486–3490.
8. Cropper, E. C., Tenenbaum, R., Kolks, M. A. G., Kupfermann, I. & Weiss, K. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5483–5486.
9. Morris, H. R., Panico, M., Karplus, A., Lloyd, P. E. & Riniker B. (1982) *Nature (London)* **300,** 643–645.
10. Mahon, A. C., Lloyd, P. E., Weiss, K. R., Kupfermann, I. & Scheller, R. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3925–3929.
11. Lloyd, P. E., Kupfermann, I. & Weiss, K. R. (1987) *Peptides* **8,** 179–184.
12. Lloyd, P. E., Kupfermann, I. & Weiss, K. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2934–2937.
13. Lloyd, P. E., Frankfurt, M., Stevens, P., Kupfermann, I. & Weiss, K. R. (1987) *J. Neurosci.* **7,** 1123–1132.
14. Ono, J. K. & McCaman, R. E. (1980) *Neuroscience* **5,** 835–840.
15. Longley, R. D. & Longley, A. J. (1986) *J. Neurobiol.* **17,** 339–358.
16. Weiss, K. R., Lloyd, P. E., Cropper, E. C., Frankfurt, M. & Kupfermann, I. (1986) *Soc. Neurosci. Abstr.* **12,** 947.
17. Meek, J. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1632–1636.
18. Adams, M. E. & O'Shea, M. (1983) *Science* **221,** 286–289.
19. Kuhlman, J. R., Li, C. & Calabrese, R. L. (1985) *J. Neurosci.* **5,** 2310–2317.
20. Worden, M. K., Witten, J. L. & O'Shea, M. (1985) *Soc. Neurosci. Abstr.* **11,** 327.
21. Lange, A. B., Orchard, I. & Adams, M. E. (1986) *J. Comp. Neurol.* **254,** 279–286.
22. Orchard, I. & Lange, A. B. (1986) *J. Neurobiol.* **17,** 359–372.
23. Bishop, C. A., Wine, J. J., Nagy, F. & O'Shea, M.R. (1987) *J. Neurosci.* **7,** 1769–1779.
24. Lundberg, J. M., Hedlund, B., Änggård, A., Fahrenkrug, J., Hökfelt, T., Tatemoto, K. & Bartfai, T. (1982) in *Systemic Role of Regulatory Peptides,* eds. Bloom, S. R., Polak, J. M. & Lindenlaub, E. (Schattauer, Stuttgart, F.R.G.), pp. 145–168.
25. Stjärne, L., Lundberg, J. M. & Åstrand, P. (1986) *Neuroscience* **18,** 151–166.
26. Maggi, C. A., Santicioli, P., Theodorsson-Norheim, E. & Meli, A. (1987) *Neurosci. Lett.* **78,** 63–68.