Peptide Cotransmitter Release from Motorneuron B16 in Aplysia californica: Costorage, Corelease, and Functional Implications

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Many neurons contain multiple peptide cotransmitters in addition to their classical transmitters. We are using the accessory radula closer neuromuscular system of Aplysia, which participates in feeding in these animals, to define the possible consequences of multiple modulators converging on single targets. How these modulators are released onto their targets is of critical importance in understanding the outcomes of their modulatory actions and their physiological role. Here we provide direct evidence that the partially antagonistic families of modulatory peptides, the myomodulins and buccalins, synthesized by motorneuron B16 are costored and coreleased in fixed ratios. We show that this release is calcium-dependent and independent of muscle contraction. Furthermore, we show that peptide release is initiated at the low end of the physiological range of motorneuron firing frequency and that it increases with increasing motorneuron firing frequency. The coordination of peptide release with the normal operating range of a neuron may be a general phenomenon and suggests that the release of peptide cotransmitters may exhibit similar types of regulation and plasticity as have been observed for classical transmitters. Stimulation paradigms that increase muscle contraction amplitude or frequency also increase peptide release from motor neuron B16. The net effect of the modulatory peptide cotransmitters released from motorneuron B16 would be to increase relaxation rate and therefore allow more frequent and/or larger contractions to occur without increased resistance to antagonist muscles. The end result of this modulation could be to maximize the efficiency of feeding.

Key words: Aplysia; neuropeptide; cotransmitter; buccalin; myomodulin; immunolocalization; RIA; release; motorneuron

Neuropeptides, the most diverse class of signaling molecules in the nervous system, are often hypothesized to function as cotransmitters. However, despite their pervasiveness and undoubtedly important function in the nervous system, most neuropeptides retain the status of putative rather than established cotransmitters. Furthermore, many neurons contain multiple, functionally distinct peptides (Kupfermann, 1991; Iversen, 1995). Without detailed knowledge of the actions and release parameters of peptide cotransmitters, it is virtually impossible to determine their physiological function. It has been suggested that the task of understanding the functional role of neuropeptides may be facilitated by studying relatively simple preparations where the biological context is well understood (Iversen, 1995).

One preparation in which both the biological context and the nature of the primary transmitters and cotransmitters have been extensively studied is the accessory radula closer (ARC) muscle of Aplysia (Weiss et al., 1992). This muscle is innervated by two cholinergic motorneurons, B15 and B16. Each of these motor neurons also contains multiple neuropeptides, which are members of two distinct neuropeptide families that have partially antagonistic effects on muscle. The cellular mechanisms of action of acetylcholine, the primary transmitter, and of the neuropeptides have been well characterized. Furthermore, because the firing of motorneurons in freely behaving animals has been characterized (Cropper et al., 1990b), previous work provides an excellent background for more advanced studies of peptide release and peptide action in this system.

Motorneuron B15 contains the SCPs and buccalins (BUCs), whereas motorneuron B16 contains the myomodulins (MMs) and BUCs (Weiss et al., 1992). The SCPs and MMs act postsynaptically to increase contraction amplitude and relaxation rate, whereas the BUCs depress contraction amplitude by reducing ACh release. How these motorneurons release these peptides critically affects the interpretation of the biological function of the neuromuscular system (Breznina et al., 1996). In a recent series of studies, we conclusively demonstrated that neuron B15 coreleases SCPs and BUCs in a fixed ratio and that release occurs within the normal range of firing frequencies of B15 (Vilim et al., 1996a,b). However, motorneuron B16 contains a different set of partially antagonistic peptide families and exhibits a different range of firing frequencies (Cropper et al., 1990b). Because motorneuron B16 is coactive with motorneuron B15, it is also important to determine the release parameters for the modulators contained in B16 if an understanding of their role in the functioning of the system is to be achieved.

In the present study we sought to achieve three goals. First, to measure the release of MMs from motorneuron B16. This is of particular importance because despite the presence of MMs and related peptides in a number of systems of a variety of species (Breznina et al., 1995; Kellett et al., 1996; Greenberg et al., 1997; Wang et al., 1998), no direct evidence exists for their release.
Second, we sought to determine if the MMs and BUCs are released in a fixed or a variable ratio from motoneuron B16. Third, we sought to determine whether or not release of the peptides is coordinated with the normal range of firing frequencies for B16.

**MATERIALS AND METHODS**

*animals.* Specimens of *Aplysia californica* were maintained at 14–16°C on a 12 hr light/dark cycle and fed every 3 d. Before dissection animals were injected with isotonic MgCl₂ (25–50% body wt).

**Antibodies**

The rabbit antibody to buccalin used for immunocytochemistry and RIA was raised against BUCa coupled to bovine serum albumin (BSA), as has been described previously (Miller et al., 1991, 1992; Vilim et al., 1996a,b). The rat antibody to MMc described previously (Miller et al., 1991) was not sufficiently sensitive for RIA. Consequently, a new antibody was made. Because we had previously obtained the best sensitivity with BSA-coupled antigens, we decided to make two new antibodies, one against MMA coupled to BSA and the other against MMA coupled to BGG. RIA tests of these antibodies indicated that the titer (and sensitivity) of the BSA-coupled antigen increased with additional boosts, whereas the titer of the BGG-coupled antigen leveled off after about the third boost. In the end, the tenth and final bleed of the BSA-coupled MMA antigen had the best sensitivity and was used in all the relevant experiments. The peptides were coupled to the carrier protein with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). All the antibodies were prepared by BABCO (Richmond, CA), and the peptides were synthesized by Applied Biosystems (Foster City, CA).

**Immunocytochemistry**

Immunocytochemistry was performed as previously described (Vilim et al., 1996a). Tissues were fixed in freshly prepared fixative (4% paraformaldehyde, 0.2% picric acid, 25% sucrose, and 0.1 M NaH₂PO₄, pH 7.6) for either 3 hr at room temperature (RT) or overnight at 4°C. After washes with PBS to remove the fixative, ganglia from larger animals were desheathed to expose the neurons whereas ganglia from smaller animals (10–15 gm) were processed without desheathing. All subsequent incubations were done at RT with rocking. Tissue was permeabilized and blocked by overnight incubation in blocking buffer (BB: 10% normal donkey serum, 2% Triton X-100, 1% BSA, 154 mM NaCl, 10 mM Na₂HPO₄, 50 mM EDTA, and 0.01% thimerosal, pH 7.4). The primary antibody was diluted 1:250 in BB and incubated with the tissue for 4–7 d. The tissue was then washed twice a day for 2–3 d with washing buffer (WB: 2% Triton X-100, 1% BSA, 154 mM NaCl, 10 mM Na₂HPO₄, 50 mM EDTA, and 0.01% thimerosal, pH 7.4). After the washes, the tissue was incubated with 1:500 dilution of secondary antibody (lissamine–rhodamine donkey anti-rat; Jackson ImmunoResearch, West Grove, PA) for 2–3 d. The tissue was then washed twice with WB for 1 d and four times with storage buffer (SB: 1% BSA, 154 mM NaCl, 10 mM Na₂HPO₄, 50 mM EDTA, and 0.01% thimerosal, pH 7.4) for 1 d. The tissues were then stored at 4°C or viewed and photographed on a Nikon microscope equipped with epifluorescence.

**EM immunocytochemistry.** Procedures used for these experiments were adapted from those of others (Reed et al., 1988; Merighe et al., 1989), as has been described (Vilim et al., 1996a). Briefly, tissue was fixed (4% glutaraldehyde, 10% sucrose, 11 mM magnesium chloride, and 0.2 M Na HEPES, pH 7.6) for 3 hr (RT), post-fixed with 1% osmium tetroxide in buffer at 4°C for 1 hr, and embedded in EMbed 812 (EM Sciences). Ultrathin sections were etched with saturated sodium metaperiodate, washed with TBS, then blocked with 8% normal goat serum in TBS for 1 hr. The sections were stained overnight with a 1:100 dilution of primary antibody, washed, then stained for 1 hr in a 1:4 dilution of gold-labeled secondary antibody (Amersham, Arlington Heights, IL). The sections were labeled with uranyl acetate and lead citrate, then examined and photographed with a JEOl 100 CX at 80 kV. Electron microscopy supplies and reagents were obtained from EMS (Fort Washington, PA).

**Radioimmunoassays.** Desaminotyrosinated MMA and BUCa were iodinated (¹²⁵I) using the chloramine T method. Iodinated stocks were repurified using reverse-phase HPLC and diluted in RIA buffer (154 mM NaCl, 10 mM Na₂HPO₄, 50 mM EDTA, 0.01% thimerosal, and 1% BSA, pH 7.5) to a final activity of 10,000–15,000 cpm/100 μl. Antibodies were diluted in RIA buffer so that 100 μl bound <50% of the counts in 100 μl of iodinated peptide trace (with a sample volume of 50 μl). The reaction was performed for 2–3 d at 4°C and terminated by addition of 2 ml of RIA charcoal (10 mM Na₂HPO₄, 0.25 mM thimerosal, 0.25% activated charcoal, 0.025% 70,000 kDa dextran, pH 7.5). The samples were spun and the supernatant, containing the bound peptide, was decanted and counted in a gamma counter. Standard curves were generated using serial dilutions of peptide in 50 μl of artificial sea water containing 1% BSA to prevent sticking. A spreadsheet program (Kaleidagraph 2.1) was used to plot the standard curves, to convert counts bound to femtomoles of peptide in the unknowns, and display the data as graphs. Because the RIAs are probably measuring the release of more than one peptide cotransmitter within a family (Price, 1990), the release of the family is referenced (i.e., MM = MMA, b, etc. and BUC = BUCa, b, etc.; Miller et al., 1993a,b). Because different preparations released differing amounts of peptide, the data were normalized to the average release for all thestimulation periods in a single experiment. The percentage of average release was calculated by adding all the released peptide detected in the experiment and dividing by the total number of stimulation periods. The release for each stimulation period was then divided by the average release for that experiment and multiplied by 100. The normalized data were then combined for the same experimental conditions across different preparations, and statistical analysis was performed. A statistical analysis program (StatView 4.5) was
Figure 2. Effect of extracellular calcium and hexamethonium on peptide release from B16 in the ARC. **A1:** Results from a single experiment in which MM release is measured with and without normal calcium in the perfusate. Motorneuron B16 was stimulated during four 5 min periods (indicated by the black bars) with a physiologically relevant frequency and pattern (20 Hz for 3.5 sec every 7 sec). **A2:** The average of four such experiments for each peptide. For each peptide, the mean ± SE of four separate experiments is plotted at each calcium concentration. The results show that release of both peptides is dependent on extracellular calcium (p < 0.0001). **B1:** Results from a single experiment in which BUC release is measured without and with 0.1 mM hexamethonium (which completely abolished the contraction of the muscle) in the perfusate. Motorneuron B16 was stimulated during four 5 min periods (indicated by the black bars) with a physiologically relevant frequency and pattern (20 Hz for 3.5 sec every 7 sec). **B2:** The mean ± SE of the normalized release from four such experiments for each peptide. The results show that release of both peptides is not dependent on contraction of the muscle (p > 0.5).

used to perform a within-subject repeated-measures ANOVA on relevant data to assess the overall level of statistical significance. Individual comparisons were performed using paired t tests.

**Results** The ARC buccal ganglion preparation was isolated as described elsewhere (Vilim et al., 1996a). Briefly, the buccal ganglion was pinned in a dish containing 25% isotonic MgCl₂ to prevent lated as described elsewhere (Vilim et al., 1996a). Briefly, the buccal ganglion was pinned in a dish containing 25% isotonic MgCl₂ to prevent spontaneous activity, and the nerve was passed through a slit in the side of the dish. The ARC was suspended outside the dish and encased with a combination of silicone grease (Dow Corning, Corning, NY) and paraffin. The ARC was perfused through an artery, and the perfusate was collected (drops, every 2.5 min) directly into the RIA tubes. The motorneuron was impaled with two independent glass microelectrodes, one for recording voltage, and one for injecting current. The temperature of the ARC was maintained at 15 ± 0.5°C by cooling the room with an air conditioner. For the experiments examining the effects of calcium and hexamethonium on peptide release, the agents were introduced into the ARC via the perfusion line. The perfusing solutions were exchanged simply by turning a peristaltic pump off and transferring the perfusion line to another solution, and turning the perfusion line on again. This method maintained a constant rate of perfusion for the different perfusing solutions, and the small dead volume (<100 μl) enabled fairly rapid (<5 min) exchange of solutions. All reagents were obtained from Sigma (St. Louis, MO) except where otherwise noted.

**RESULTS** The accessory radula closer muscle is innervated by two cholinergic motorneurons, B15 and B16 (Cohen et al., 1978). Motorneuron B15 contains SCPs (Cropper et al., 1987a), motoneuron B16 contains MMs (Cropper et al., 1987b, 1990a), and both contain BUCs (Cropper et al., 1988; Cropper et al., 1990a; Vilim et al., 1994). This is illustrated by the LM immunocytochemistry in Figure 1. Here you can see processes immunostaining for buccalin and myomodulin that are indicative of B16 processes. (In addition, there are processes immunostaining for buccalin but not myomodulin that are indicative of B15 processes.) To subcellularly localize the B16 peptides, we performed EM immunocytochemistry. We found that MM is present in dense core vesicles (DCVs), which had not been shown. In fact we found that vesicles that are myomodulin-immunoreactive are also buccalin-immunoreactive. Data from three preparations with 13 terminals containing a total of 122 DCVs showed that 97 (79.5%) were costained with myomodulin and buccalin. In contrast, 10 B15 terminals from the same sections contained 220 DCVs staining for buccalin, and only four of those vesicles had gold particles corresponding to myomodulin staining. These results demonstrate that the overall background levels were low and that the immunostaining is specific. Thus, LM and EM evidence indicates that the BUCs and MMs are not differentially packaged and targeted. However, this evidence cannot determine whether the relative amounts of MMs and BUCs are fixed in all DCVs.

To approach this issue we first sought to determine whether peptides were released from B16 constitutively or in an activity-dependent manner. Both peptides were in fact released in a calcium-dependent manner when B16 was stimulated (Fig. 2A) and B15 (intraburst firing frequency, 20 Hz; burst duration, 3.5 sec; interburst interval, 3.5 sec). Because low calcium solutions not only block release but also prevent muscle contractions, the above results could be obtained if the peptides were constitutively released and squeezed into the eluate by muscle contractions. We therefore used hexamethonium, a cholinergic antagonist that blocks muscle contractions but does not inhibit the release process (Vilim et al., 1996a). Under these conditions we again observed that the release of both peptides depended on motorneuron firing (Fig. 2B) (n = 4) (firing rate, 20 Hz; burst duration, 3.5 sec; interburst interval, 3.5 sec). Because the motorneuron in these experiments was stimulated within its normal firing rate and pattern, this experiment indicates that peptide release is physiologically relevant. Furthermore, it is the first direct demonstration that MMs are released in any system.

We next wanted to determine whether or not the ratio was fixed during release. To accomplish this, we stimulated for a longer period of time and analyzed alternate samples for MM or BUC content. By comparing the kinetics of BUC and MM release, we
can determine if the ratio is fixed or not. When the motorneuron is stimulated at a constant frequency over time, even though the amount of peptides released changes, the ratio of the two peptides remains constant (Fig. 3) \((n = 4)\) (firing rate, 20 Hz; burst duration, 3.5 sec; interburst interval, 3.5 sec; total period of stimulation, 1 hr). This observation was confirmed by further experiments in which we manipulated the parameters of motorneuron stimulation and found that although the total amount of peptides released from B16 changed as a function of the parameters of stimulation, the ratio of the two peptides remained constant (see below).

Because the two peptides are released together and they are released in a constant ratio, the functional role of corelease in this system requires that the action of the two peptides be conceptualized jointly. As indicated before, the joint action of these two peptides is to preferentially accelerate the relaxation rate and thus to shorten the duration of the relaxation of the contraction. Accelerated relaxation rate promotes unopposed action of antagonistic muscles, especially when muscle contractions occur frequently and/or are large (Fig. 4A1,B1). We therefore investigated the relationship between peptide release and those parameters of motorneuron stimulation that produce frequent or large contractions. Different frequencies of muscle contractions were produced by changing the interburst interval of motorneuron firing while maintaining a constant intraburst frequency and burst duration. All of these parameters used were within the physiological range of motorneuron firing, i.e., the intraburst firing frequency was 20 Hz, the burst duration was 3.5 sec, and interburst intervals were 3.5, 5.0, and 7.5 sec (Cropper et al., 1990b). Data from an individual experiment in which we measured the release of buccalin are shown in Figure 4A2. We observed that indeed peptide release was greater when a motorneuron was stimulated to produce more frequent contractions. Because there are more action potentials per unit of time when the interburst interval is shorter, we expressed peptide release as femtomoles per action potential. The plot of these data is shown in Figure 4A3, and it indicates that indeed release per action potential increased as the frequency of contractions increased \((n = 4)\). The second set of conditions under which faster relaxation rate is advantageous occurs when muscle contractions are large (Fig. 4B1). We varied the size of muscle contractions by changing the intraburst frequencies while maintaining a constant burst duration and interburst interval. Again all of these parameters were maintained within the physiological range (Cropper et al., 1990b), i.e., intraburst firing frequencies were 10, 15, and 20 Hz, the burst duration was 3.5 sec, and the interburst interval was 3.5 sec. We found that indeed peptide release was largest when motorneurons were stimulated at frequencies that produced large contractions \((n = 4)\) (Fig. 4B2,B3). Importantly, in experiments in which we varied either the interburst interval or the intraburst frequency, peptide release occurred not only at the high end but also at the low end of our stimulation parameters. These parameters were selected to mimic the range of motorneuron activity recorded in freely behaving animals. Larger amounts of peptides were released under conditions that produced large or frequent contractions—conditions in which appropriate shortening of the relaxation has advantageous behavioral consequences.

**DISCUSSION**

Our results provide direct evidence that the BUCs and MM are costored and coreleased from motorneuron B16 terminals in the ARC muscle. This release is calcium-dependent and contraction-independent, demonstrating that it is not artifactual. In addition, several observations suggest that the amounts of peptides that are...
recovered and measured are a good reflection of the amounts released. Specifically, we are measuring the release of two different peptide families, and peptides from each of these families differ in their length and chemical structure. Despite these differences, the myomodulins and buccalins are recovered in constant amounts of peptides detected, the error bars of the combined normalized data from several preparations are all very small.

We demonstrate that peptide cotransmitter release from B16 increases in response to changes in firing patterns that result in contraction amplitude increases or decreases in the interval between contractions. Furthermore, we show that the peptide release is initiated at the low end of the physiological range of B16 firing frequency and then increases with increasing B16 firing frequency in the range recorded in vivo during feeding. Previously, we reported (Vilim et al., 1996b) that release of SCPs and BUCs from motorneuron B15 is coordinated with the range of firing frequencies observed in vivo when the animal feeds. However, Cropper et al. (1990b) reported that the range of firing frequencies for B15 and B16 are somewhat different (7–12 Hz for B15 and 10–20 Hz for B16). In Figure 5 we compare the release per spike from B15 and B16 as a function of the overall average frequency of motorneuron firing. It is clear that the release as a function of frequency is different for the two motorneurons, but increases for both as the frequency increases (above a certain threshold frequency, which appears to be different for the two neurons). Release of SCPs from neurons B1 and B2 in culture suggests that release from these neurons also appear to be coordinated with their in vivo firing (Whim and Lloyd, 1994). However, these neurons fire single action potentials in vivo (Lloyd et al., 1988) and also release peptide in response to single action potentials. These results support the general conclusion that neuropeptide release properties appear to be neuron-specific (as opposed to peptide-specific). Moreover, as hypothesized (e.g., Vilim et al., 1996a,b) peptide cotransmitter release is coordinated with the normal range of firing frequencies for neurons in Aplysia, and probably in other species as well. This is a more attractive
described previously (Vilim et al., 1996b). The data for B16 were recal-
vals of stimulation (3.5, 5, and 7 sec). The data for B15 have been
motorneuron was averaged and plotted (in all experiments. The results from eight separate experiments for each
and intraburst frequencies (12 Hz for B15, 20 Hz for B16) were identical
corrected to give the release per action potential. Burst duration (3.5 sec)
and interburst interval (3.5 sec) were identical in all experiments. The results from eight separate experiments for
each motorneuron were averaged and plotted (± SE) at three different
intraburst frequencies of stimulation. The physiological range of firing
frequencies for B15 varies between 7.5 and 12 Hz, and B16 varies between
10 and 20 Hz. Peptide release appears to be coordinated with the normal
operating range of frequencies of each motorneuron. B, Normalized
release per action potential at each of the three interburst intervals
corrected to give the release per action potential. Burst duration (3.5 sec)
and intraburst frequencies (12 Hz for B15, 20 Hz for B16) were identical
in all experiments. The results from eight separate experiments for each
motorneuron was averaged and plotted (± SE) at three interburst inter-
vals of stimulation (3.5, 5, and 7 sec). The data for B15 have been
described previously (Vilim et al., 1996b). The data for B16 were recal-
culated from Figure 4.

model of peptide release than one that postulates that peptide
release only occurs at high frequencies (Hökfelt, 1991) because it
allows for greater flexibility in output.

The peptide cotransmitters present in motorneuron B15 and
B16 produce dose-dependent responses when applied exog-
enuously to the preparation (Weiss et al., 1992; Brezina et al., 1995,
1996). They do not operate in an all or none manner. The peptide
cotransmitters from these motorneurons are also released in a
frequency-dependent rather than in an all or none manner within
the normal range of firing frequencies for these two motorneu-
rons. Furthermore, the release also changes gradually in response
to changes in the interval between bursts or contractions (Fig. 5).
Thus, the peptide cotransmitters in this system can alter the
characteristics of muscle contractions, amplitude and relaxation
rate, in a motorneuron firing-dependent manner. This can allow
the coordination of the amplitude and relaxation rate of the
muscle contractions to maximize the efficiency of the output of
the system (Weiss et al., 1992). By altering the relaxation rate in
response to the changing contraction amplitude and interval,
inappropriate actions of antagonist muscles can be minimized,
and the proper force can be maintained throughout a given phase
of the cycle. This could be a built-in mechanism to adjust the
response characteristics of the muscle to a wide range of amplitu-
de and interval combinations.

The ARC is a nonspiking muscle in which the contraction
amplitude is a function of the amount of ACh released (Cohen et
al., 1978). Thus, release of classical transmitter correlates with
contraction amplitude that in turn correlates with peptide co-
transmitter release. In addition, release of ACh appears to be
frequency-dependent (Lloyd and Church, 1994) as is peptide
cotransmitter release. Hence, peptide cotransmitter and classical
transmitter release appear to be functionally coupled in this
system. Therefore, it is likely that there are key shared mecha-
nisms for the release of classical and peptide cotransmitters. For
example, both release processes are calcium-dependent. The cal-
cium requirements for peptide and classical transmitter release,
however, are different (Verhage et al., 1991; Linas et al., 1992;
Peng and Zucker, 1993), and the two can be differentially released
(Matteoli et al., 1988), so some important differences in their
mechanisms of release must exist.

In summary, our findings have several general implications for
the understanding of peptidergic cotransmission. Peptide cotran-
smitters have been suggested to function as amplifiers of the
actions of conventional transmitters, being released only at very
high levels of neural firing (Hökfelt, 1991; Kupfermann, 1991). In
many ways our findings are not consistent with this view and
instead support the suggestions we have reached in our studies of
peptide release from motorneuron B15 (Vilim et al., 1996a,b).
Thus, our findings demonstrate that activity-dependent release of
two modulatory peptide cotransmitters occurs throughout the
normal firing range of a neuron, and although release becomes
larger as the frequency of motorneuron firing increases, the
release does not require extreme conditions in this system. Sec-
ond, in this system the actions of the two peptides on the size of
muscle contractions, the parameter regulated by the primary
transmitter, tend to cancel each other and therefore the actions of
these peptides are not easily conceptualized as simply amplifying
the actions of the primary transmitter. Instead, the effect that
emerges from the joint effect of these peptides is to modify the
relaxation rate of contraction, an action that is not regulated by
the primary transmitter. In view of the differences in the infor-
mation transmitted by peptide cotransmitters and primary trans-
mitters, these two classes of molecules are best conceptualized as
constituting parallel but functionally distinct pathways of infor-
mation transmission. Third, we found that peptides were prefer-
entially released when motorneurons were stimulated to produce
strong and frequent contractions, conditions that prevent full
relaxation of the muscle before its antagonist muscle begins to
contract. An incompletely relaxed muscle offers resistance to its
antagonist, which may result in a disruption of the functional
integrity of behavior. Furthermore, at higher rates of contraction
a slow relaxation rate can result in inefficient stepwise summation
and resultant tonic contractions. Because the amount of peptides
that are released is an automatic consequence of the firing pattern
of the motorneuron that produces the contraction, this arrange-
ment obviates the need for complex sensory functions that would
monitor on-line the state of muscle contraction to alter its rate of
relaxation by engaging other neuronal input to the muscle. In
more general terms, the use of peptide cotransmitters appears to

Figure 5. Comparison of the peptide release per action potential from
motorneurons B15 and B16 as a function of intraburst frequency and
interburst interval. A, Normalized release per action potential at each of
the three intraburst frequencies corrected to give the release per action
potential. Burst duration (3.5 sec) and interburst interval (3.5 sec) were
identical in all experiments. The results from eight separate experiments for
each motorneuron were averaged and plotted (± SE) at three different
intraburst frequencies of stimulation. The physiological range of firing
frequencies for B15 varies between 7.5 and 12 Hz, and B16 varies between
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motorneuron was averaged and plotted (± SE) at three interburst inter-
vals of stimulation (3.5, 5, and 7 sec). The data for B15 have been
described previously (Vilim et al., 1996b). The data for B16 were recal-
culated from Figure 4.
be a simple and elegant way to automatically modify the characteristics of the response to the primary transmitter without the added computational complexity that would result from the incorporation of additional neuronal elements.

REFERENCES


