AMRP Peptides Modulate a Novel K\(^+\) Current in Pleural Sensory Neurons of *Aplysia*

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**AMRP Peptides Modulate a Novel K\(^{+}\) Current in Pleural Sensory Neurons of *Aplysia***

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Modulation of *Aplysia* mechanosensory neurons is thought to underlie plasticity of defensive behaviors that are mediated by these neurons. In the past, identification of modulators that act on the sensory neurons and characterization of their actions has been instrumental in providing insight into the functional role of the sensory neurons in the defensive behaviors. Motivated by this precedent and a recent report of the presence of *Aplysia* *Mytilus* inhibitory peptide-related (AMRP) neuropeptides in the neuropile and neurons of the pleural ganglia, we sought to determine whether and how pleural sensory neurons respond to the AMRPs. In cultured pleural sensory neurons under voltage clamp, AMRPs elicited a relatively rapidly developing, then partially desensitizing, outward current. The current exhibited outward rectification; in normal 10 mM K\(^{+}\), it was outward at membrane potentials more positive than \(-80\) mV but disappeared without reversing at more negative potentials. When external K\(^{+}\) was raised to 100 mM, the AMRP-elicited current reversed around \(-25\) mV; the shift in reversal potential was as expected for a current carried primarily by K\(^{+}\). In the high-K\(^{+}\) solution, the reversed current began to decrease at potentials more negative than \(-60\) mV, creating a region of negative slope resistance in the *I*-*V* relationship. The AMRP-elicited K\(^{+}\) current was blocked by extremely low concentrations of 4-aminopyridine (4-AP; IC\(_{50}\) = 1.7 \(\times\) 10\(^{-7}\) M) but was not very sensitive to TEA. In cell-attached patches, AMRPs applied outside the patch—thus presumably through a diffusible messenger—increased the open probability of the channel decreased with hyperpolarization; together, these two factors accounted for the outward rectification of the macroscopic current. Submicromolar 4-AP included in the patch pipette blocked the channel by reducing its open probability without altering the single-channel current. Based on the characteristics of the AMRP-modulated K\(^{+}\) current, we conclude that it is a novel current that has not been previously described in *Aplysia* mechanosensory neurons. In addition to this current, two other AMRP-elicited currents, a slow, 4-AP-resistant outward current and a Na\(^{+}\)-dependent inward current, were occasionally observed in the cultured sensory neurons. Responses consistent with all three currents were observed in sensory neurons in situ in intact pleural ganglia.

**INTRODUCTION**

In many systems, the search for the cellular mechanisms of behavioral plasticity has critically implicated neuromodulators that alter key cellular properties of the neurons involved. Along with the identification of novel modulators, these studies have helped reveal novel cellular properties, for instance previously unknown ion currents, on which the modulators act. Classic work of this kind has been done with simple invertebrate model preparations. Perhaps best known are the siphan/gill- and tail-withdrawal reflexes of *Aplysia* (for review, see Byrne and Kandel 1996; Byrne et al. 1991; Kandel and Schwartz 1982). As in many other modulated systems, these reflexes are modulated by multiple modulators. The known modulators of the *Aplysia* withdrawal reflexes include serotonin, dopamine, FMRFamide, the small cardioactive peptides (SCPs), and bag-cell peptides. Studies of the cellular actions of these modulators have revealed, in the mechanosensory neurons that mediate the reflexes, a novel ion current, the “S” K\(^{+}\) current, which is suppressed by serotonin and the SCPs and enhanced by FMRFamide (reviewed by Belardetti and Siegelbaum 1988).

Recent work has characterized a new family of *Aplysia* neuropeptides, the *Aplysia* *Mytilus* inhibitory peptide-related peptides, or AMRPs (Fujisawa et al. 1999). The AMRP precursor was cloned; all 14 AMRP peptides predicted by it were found in *Aplysia* neurons and ganglia biochemically or by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry; their physiological actions were demonstrated on several *Aplysia* muscles; and the AMRPs’ widespread expression in the central ganglia and in peripheral tissues of *Aplysia* was mapped by Northern analysis, in situ hybridization, and immunohistochemistry. In particular, the AMRPs were found to be abundantly expressed in the pleural neuropile and in neurons in the anterior part of the pleural ganglia, where FMRFamide-containing neurons that mediate the tail-withdrawal reflex are also located (Xu et al. 1994). This raised the possibility that the AMRPs, too, might be modulators of the *Aplysia* tail-withdrawal reflex. Here we use current-, voltage-, and patch-clamp techniques to examine actions of the AMRPs on electrophysiological properties of the pleural mechanosensory neurons that mediate the tail-withdrawal reflex. We find that, indeed, the AMRPs modulate the neurons’ electrophysiological properties. In particular, they activate a distinctive K\(^{+}\) current that has not been described previously in these neurons.
METHODS

Experiments were performed on pleural sensory neurons in dissociated cell culture as well as in situ in the intact ganglion.

Cell culture

Cell cultures were prepared essentially as described by Schacher and Proshansky (1983). Briefly, pleural ganglia from 100 to 150 g Aplysia were incubated in Type IX protease (Sigma) for 2.5 h at 34°C. The ganglia were then desheathed in isotonic L-15 medium (Sigma L-15 medium supplemented with salts to marine concentrations). Sensory neurons located in the pleural ventrocaudal cluster (Walters et al. 1983) were individually extracted with fine-tipped glass microelectrodes and plated into poly-l-lysine-coated culture dishes containing isotonic L-15 medium. The cultures were maintained at 17°C for 12–36 h before experimentation. We saw no obvious change in the responses of the neurons over this time. For experiments, the L-15 medium in the culture dish was replaced with an artificial sea water (ASW; see following text).

To record whole cell currents, we used discontinuous single-electrode voltage clamp with sharp intracellular microelectrodes. Electrodes were filled with 3 M K-acetate plus 30 mM KCl to minimize drift and had resistances of 10–20 MΩ. To eliminate uncontrolled currents in regrown cell processes, the cultured neurons were routinely axonimized close to the cell body before recording. Whole cell currents were low-pass filtered at 30 Hz.

To record single-channel currents, we used the cell-attached patch-clamp mode. Patch pipettes were pulled from borosilicate glass (PG-52150–4, WPI) and fire-polished. Pipettes were filled with normal ASW or in some experiments with 100 mM K⁺ ASW or normal ASW containing 2 × 10⁻⁷ M 4-aminopyridine (4-AP). All solutions were sterile-filtered before use. Single-channel records were low-pass filtered at 2 kHz.

The intracellular voltage, and thus the absolute voltage difference across the patch, was unknown in the cell-attached mode. However, in the intracellular experiments, we found the resting potential of the neurons under the same conditions to be −49.6 ± 6.4 mV (mean ± SE of 70 neurons). We therefore routinely used the value of −50 mV to estimate the absolute trans-patch voltage; all patch-clamp voltages given in this paper already incorporate this correction.

Intact ganglion

The pleural and pedal ganglia were desheathed in a 1:1 mixture of isotonic MgCl₂ and normal ASW, then superfused with normal ASW. Sensory neurons were identified by their position, size, and electrophysiological properties (Walters et al. 1983). The neurons were impaled with single intracellular microelectrodes containing 3 M K-acetate, with resistances of 3–8 MΩ, for simple voltage recording.

Solutions and peptide application

Normal ASW contained (in mM) 460 Na⁺, 10 K⁺, 55 Mg²⁺, 11 Ca²⁺, 602 Cl⁻, and 10 HEPES buffer (pH 7.6); 0 Na⁺ ASW was made by replacing all Na⁺ with equimolar N-methyl-d-glucamine; 100 mM K⁺ ASW was made by replacing 90 mM Na⁺ with equimolar K⁺. TEA and 4-AP were added without substitution.

AMRP peptides (custom-synthesized by AnaSpec, San Jose, CA) were reconstituted in normal ASW or 0 Na⁺ ASW as required and applied by pressure ejection. The pressure-ejection pipette, with a tip diameter of 5–10 μm, was positioned −100 μm from the neuron. In most experiments, brief 50- to 200-nsec trains of peptide were used; these elicited responses that could be reproduced every 20–30 s without marked desensitization. Where desensitization was studied, longer (20–90 s) puffs of peptide were applied.

Where not stated otherwise in the figure legends, experiments were done with the brief, 100- to 200-ms puffs of 10⁻⁵ or 10⁻⁴ M peptide, onto neurons voltage-clamped at −40 mV, bathed in normal ASW. All experiments were done at room temperature (20–24°C). The results in this paper were collected from >100 sensory neurons.

RESULTS

AMRP peptides elicit membrane ion currents in cultured pleural sensory neurons

In isolated, cultured pleural sensory neurons voltage-clamped at −40 mV, brief puffs of the AMRP peptide GSPRFFa typically elicited a large outward current, between 0.5 and 4 nA in peak amplitude (Figs. 1A and 2A). When the application of the peptide was prolonged, the current gradually declined, presumably due to desensitization (Fig. 1A). The desensitization did not readily reverse and the response remained strongly depressed even after a 20-min wash.

In most of this work, we used the peptide GSPRFFa because, of all the AMRP peptides, it occurs in by far the largest number of copies on the cloned AMRP precursor (Fujisawa et al. 1999). However, we also tested a second AMRP peptide, GAPRFVa. This also elicited the gradually desensitizing outward current (see following text).

In a small proportion of the sensory neurons (9%), the outward current elicited by GSPRFFa was preceded by a small transient inward current (Fig. 1B, left). The full inward current was unmasked when the bath contained 10⁻³ M 4-AP, which

$$\text{4-AP}$$

FIG. 1. Aplysia Mytilus inhibitory peptide-related peptide (AMRP)-elicited ion currents in cultured pleural sensory neurons. A: desensitizing outward current elicited by prolonged application of GSPRFFa. B: biphasic current elicited by puff of GSPRFFa. Bath application of 10⁻³ M 4-aminopyridine (4-AP) blocked the outward-current component of the response, unmasking the full inward current. C: pure inward current elicited by puff of GSPRFFa. The current was abolished by removal of Na⁺ from the bath.
the experiment in Fig. 2 around −26 mV. This represents a positive shift in reversal potential of ≥50 mV, close to the Nernst value of 58 mV for a purely K⁺-selective current and similar to the shifts observed for bona fide K⁺ currents in these and other Aplysia neurons (e.g., Brezina et al. 1987; Ichinose et al. 1989; Pollock et al. 1985). Similar I-V relationships were obtained with Na⁺-free solutions containing 10 and 100 mM K⁺. Altogether, these data indicated that the AMRP-elicited outward current is carried primarily by K⁺ ions.

The outward rectification of the AMRP-elicited current was considerably greater than that predicted simply from the asymmetry between the external and internal K⁺ concentrations by the Goldman-Hodgkin-Katz (GHK) constant-field equation (see GHK fit to the 10 mM K⁺ GSPRFFa values in Fig. 2B). Furthermore, in the 100 mM K⁺, the current still exhibited the outward rectification. Indeed, as the voltage was made more negative than about −60 mV, the reversed inward current began to decrease, creating a region of negative slope resistance in the I-V relationship (● in Fig. 2B).

Dose dependence of the AMRP K⁺-current response

To study the dose dependence of the AMRP K⁺-current response, we applied brief puffs of different peptide concentrations at the single holding potential of −40 mV (Fig. 3). As Fig. 3A illustrates, ≥10⁻⁶ M GSPRFFa was required to activate a significant current; the amplitude of the current then increased with increasing concentration of GSPRFFa until the response saturated above ~10⁻⁴ M. The concentration of GSPRFFa that gave half of the maximal current amplitude (EC₅₀) was 7.8 × 10⁻⁵ M (mean of 5 neurons; ● in Fig. 3C).

We compared the dose dependence of GSPRFFa with that of the second AMRP peptide, GAPRFVa. When equivalent concentrations (10⁻⁵ M) of the two peptides were applied in parallel to the same neuron, GAPRFVa elicited significantly more current than GSPRFFa (Fig. 3B). Comparison of the complete dose-response relationships for the two peptides (Fig. 3C) showed that GAPRFVa was an order of magnitude more potent, with an EC₅₀ of 7.9 × 10⁻⁷ M (mean of 4 neurons), than GSPRFFa. GAPRFVa was also more efficacious, eliciting a maximal current ~30% larger than that elicited by GSPRFFa. Similar data for both peptides were obtained in Na⁺-free solution.

4-AP, but not TEA, is a potent blocker of the AMRP-elicited K⁺ current

We next examined the sensitivity of the AMRP-elicited K⁺ current to the classical K⁺-channel blockers 4-AP and TEA. 4-AP (Fig. 4) was an extremely potent blocker of the current. As Fig. 4A shows, even 10⁻⁶ M 4-AP decreased the current to some extent; 10⁻⁴ to 10⁻³ M often blocked it completely. The concentration of 4-AP that blocked half of the current (IC₅₀) was 1.7 × 10⁻³ M (mean of 4 neurons; Fig. 4B). Similar results were obtained with GAPRFVa.

However, in a proportion of the neurons (28%), even 10⁻³ M 4-AP did not block the GSPRFFa-elicited current completely. A small outward current could still be elicited by the peptide. This is discussed further in the next section.

In contrast to 4-AP, TEA (Fig. 5) was not a very potent blocker of the AMRP-elicited K⁺ current. Very high concen-
Concentrations of TEA, \( >10^{-2} \) M, were required for noticeable block (Fig. 5A). IC\(_{50}\) for TEA was 2.35 \( \times 10^{-2} \) M (mean of 3 neurons; Fig. 5B).

Possible second component of AMRP-elicited outward current

As just mentioned, in some neurons a residual AMRP-elicited current persisted even in the presence of high 4-AP. This component of the current was much smaller, and appeared to have slower activation and desensitization kinetics (Fig. 6A, right), than the 4-AP-sensitive component of the current (Fig. 6A, left). In several neurons, the AMRP-elicited current was small and slow even in the absence of 4-AP (Fig. 6B, left), and in these neurons 4-AP had no effect (Fig. 6B, right). Altogether, this suggested that, in addition to the large, relatively fast, 4-AP-sensitive K\(^+\) current, the AMRPs may also activate a second component of small, slow, 4-AP-resistant outward current in some sensory neurons. However, because this component was small and infrequently seen, we did not attempt to characterize it further.

Single channels modulated by AMRP

To study the single-channel basis of the AMRP response, we recorded from cell-attached patches on the soma of the cultured sensory neurons. The patch was held substantially depolarized, usually at approximately +50 mV (see METHODS), while GSPRFFa was applied to the rest of the neuron. Figure 7 shows a typical result. GSPRFFa dramatically increased channel activity in the patch. The main responding channel type, of which at least three were present in the patch in Fig. 7, carried outward current of \( \sim 4.8 \) pA at +50 mV, although it also appeared to have a much less frequent, \( \sim 35\% \) lower subconductance level (Fig. 7B, left, *). Infrequent openings of the channel were observed even in the absence of the peptide (e.g., in Fig. 7B, left). Thus it appeared that AMRP modulates, rather than absolutely activates, the channel.

As the channels inside the patch responded to AMRP applied outside the patch, it further appeared that the modulation of the channel involves some type of diffusible intracellular messenger.

i-V relationship and K\(^+\) dependence of the AMRP-modulated channel

We measured the single-channel current amplitude, \( i \), of the AMRP-modulated channel as a function of the patch potential (Fig. 8). When the patch pipette contained normal 10 mM K\(^+\), only outward openings of the channel could be resolved, at voltages more positive than about \( \sim 60 \) mV. The single-channel current never reversed in any of the patches tested (Fig. 8A, 8B).

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**Fig. 3.** Dose dependence of the AMRP K\(^+\)-current response. A: representative currents elicited by puffs of 10\(^{-2}\), 10\(^{-3}\), and 10\(^{-3}\) M GSPRFFa. B: representative currents elicited by puffs of 10\(^{-3}\) M GSPRFFa and 10\(^{-5}\) M GAPRFVa. C: complete dose-response relationships for the AMRP K\(^+\)-current response. Means \( \pm \) SE of peak-current measurements for GSPRFFa from 5 neurons and for GAPRFVa from 4 neurons. All measurements were first normalized to the maximal GSPRFFa-elicited current amplitude in that particular neuron. The solid curves, _, with the indicated EC\(_{50}\) values, are the best symmetrical sigmoid fits to the data.

**Fig. 4.** 4-AP block of the AMRP-elicited K\(^+\) current. A: representative currents elicited by puffs of GSPRFFa in the presence of increasing concentrations of 4-AP in the bath. B: complete dose dependence of the 4-AP block. Means \( \pm \) SE of peak currents elicited by GSPRFFa in 4 neurons. All values were first normalized to the control current amplitude in that particular neuron. The solid curve, _, with the indicated IC\(_{50}\) value, is the best symmetrical sigmoid fit to the data.
The slope conductance of the channel, measured over the quasi-linear portion of the $i-V$ relationship at positive voltages, was $48 \text{ pS}$ (mean of 10 patches).

When the patch pipette contained 100 mM K$^+$, the single-channel $i-V$ relationship shifted so that the current now clearly reversed, on average between 20 and 10 mV (Fig. 8A, right; ○ in B; Fig. 9B). The $i-V$ relationship became more linear, but still retained a degree of outward rectification. Altogether, these data indicated that the AMRP-modulated channel is a K$^+$ channel.

**Voltage dependence of gating of the AMRP-modulated K$^+$ channel**

The close resemblance of Figs. 8B and 2B made the AMRP-modulated K$^+$ channel a good candidate to underlie part of the macroscopic AMRP-elicited K$^+$ current. There is, however, one significant difference between Figs. 8B and 2B: the outward rectification, in particular the curvature of the $I-V$ relationship at negative voltages in 100 mM K$^+$, is considerably stronger for the macroscopic than for the microscopic current. This could be explained if the other determinant of the macroscopic $I-V$ relationship, the open probability of the channel, were also voltage dependent.

To examine this, we measured, from records like those in Fig. 8A, right, the probability that the AMRP-modulated K$^+$ channel was open, $P_o$, as a function of the patch potential, in 100 mM K$^+$. In practice, because each patch contained an indeterminate number of the channels, $N$, we measured the product $NP_o$. The data are shown in Fig. 9A. Indeed, $NP_o$ was voltage dependent: the open probability of the channel gradually decreased with hyperpolarization.
Could this decrease in open probability, together with the rectification in the open-channel \( i-V \) relationship, fully explain the rectification in the macroscopic \( i-V \) relationship?

Guided by the equation \( I = N P_o i \), we multiplied the voltage dependence of \( NP_o \) (Fig. 9A) by the voltage dependence of \( i \) (Fig. 9B) to predict the voltage dependence of \( I \) (Fig. 9C). This macroscopic \( i-V \) relationship predicted from the microscopic measurements indeed closely resembled the macroscopic \( i-V \) relationship actually found in 100 mM K\(^+\) (Fig. 2B). This was confirmed by statistical analysis. While there was no significant effect of 4-AP in the patch pipette on \( i \) (Fig. 10C), there was a highly significant (\( P < 0.05 \), t-test) decrease in \( NP_o \) (Fig. 10D).

Thus we concluded that the AMRP-modulated K\(^+\) channel is highly 4-AP sensitive, indeed to about the degree that it should be if it underlies the bulk of the macroscopic AMRP-elicited K\(^+\) current. Furthermore, it appeared that 4-AP does not block the response by interacting with the AMRP receptor; it may very well act on the channel itself.

**4-AP blocks the AMRP-modulated K\(^+\) channel**

Another signal characteristic of the bulk of the macroscopic AMRP-elicited K\(^+\) current is its 4-AP sensitivity. We therefore examined the 4-AP sensitivity of the AMRP-modulated K\(^+\) channel (Fig. 10). We added the 4-AP either to the bath, where (neglecting any slow passage of 4-AP across the membrane and through the interior of the cell) it could presumably interact with the AMRP-activated receptors but not with the AMRP-modulated channels in the patch, or included it inside the patch pipette, where it could interact with the channels but not with the receptors.

When even very high (10\(^{-3}\) M) 4-AP was added to the bath, there was no obvious effect on the appearance of the AMRP-modulated K\(^+\) channels during the response (Fig. 10A), and no statistically significant effect either on \( i \) (Fig. 10C) or on \( NP_o \) (Fig. 10D).

In contrast, when \( 2 \times 10^{-7} \) M 4-AP (a concentration close to the IC\(_{50}\) for block of the macroscopic current) was included in the patch pipette, the AMRP-modulated K\(^+\) channels opened much less frequently during the response, although, when open, their single-channel current amplitude appeared normal (Fig. 10B). This was confirmed by statistical analysis. While there was no significant effect of 4-AP in the patch pipette on \( i \) (Fig. 10C), there was a highly significant (\( P < 0.05 \), t-test) decrease in \( NP_o \) (Fig. 10D).

Thus we concluded that the AMRP-modulated K\(^+\) channel is highly 4-AP sensitive, indeed to about the degree that it should be if it underlies the bulk of the macroscopic AMRP-elicited K\(^+\) current. Furthermore, it appeared that 4-AP does not block the response by interacting with the AMRP receptor; it may very well act on the channel itself.
AMRPs depolarize and hyperpolarize pleural sensory neurons in intact pleural ganglia

All of the results so far were obtained in sensory neurons in dissociated cell culture. Can similar AMRP responses be found in sensory neurons in situ in the intact ganglia?

In these experiments, we simply recorded the membrane voltage of pleural sensory neurons in intact ganglia in response to brief puffs (Fig. 11, A and B) or longer applications (Fig. 11C) of GSPRFFa. We found three distinct types of response. The first was a rapid hyperpolarization, of 13.5 ± 1.5 mV (mean ± SE from 2 neurons) with 10⁻⁵ M GSPRFFa, that was reversibly blocked by low concentrations of 4-AP (Fig. 11A). Second, we found a rapid depolarization, of 12.3 ± 3.1 mV (4 neurons), that was abolished when Na⁺ was removed from the bath (Fig. 11B). Finally, we found a slow hyperpolarization, of 8.6 ± 3.4 mV (6 neurons), that was insensitive to low concentrations of 4-AP (Fig. 11C).

DISCUSSION

The mechanosensory neurons located in the abdominal and pleural ganglia of Aplysia are among the most extensively studied neurons in the animal (reviewed by Byrne and Kandel 1996; Byrne et al. 1991; Kandel and Schwartz 1982). These neurons constitute a major link in the generation of defensive withdrawal behaviors, and modulation of the neurons alters the strength of the behaviors. Several modulators, in particular serotonin and FMRFamide, have been extensively studied and in each case found to exert multiple cellular effects on the sensory neurons (see, e.g., reviews by Byrne and Kandel 1996; Byrne et al. 1993). For instance, each modulator alters the activity of several K⁺ currents. Serotonin suppresses the "S" K⁺ current (I_{K,s}), a current that indeed was first identified and characterized by means of this serotonergic modulation (Baxter and Byrne 1989; Klein et al. 1982; Pollock et al. 1985; Siegelbaum et al. 1982; reviewed by Belardetti and
the neuropeptides. However, physiological actions were shown to be the most functionally significant. Actions will probably be common. Aplysia sensory neurons to the AMRPs. We have in this paper begun a characterization of the responses of the pleural sensory neurons to the AMRPs. This previous work has made it clear that functional understanding requires first a characterization of the various individual effects, which can then provide the basis for subsequent integrative studies of their roles in the modulation of the withdrawal behavior. We reasoned that the same approach should be applied to new classes of modulators. The recent finding that peptides of a newly characterized family, the AMRPs, are abundant in the neuropile and neurons in the anterior part of the pleural ganglia (Fujisawa et al. 1999) raised the possibility that the AMRPs, too, may modulate the pleural sensory neurons that mediate the tail-withdrawal reflex. As a first step toward elucidating the role of the AMRPs, therefore, we have in this paper begun a characterization of the responses of the pleural sensory neurons to the AMRPs. The work of Fujisawa et al. (1999) left no doubt that the AMRPs are an important, fully functional family of Aplysia neuropeptides. However, physiological actions were shown only on the peripheral musculature. We have now described the first actions on central neurons. Judging by the widespread expression of the AMRPs in the Aplysia CNS, their central actions will probably be common.

The AMRP precursor cloned by Fujisawa et al. (1999) predicts 11 copies of GSPRFFa but only one or two copies of each of the other AMRPs. Because GSPRFFa is thus likely to be the most functionally significant AMRP, we used it in most experiments here. However, we also tested a second AMRP peptide, GAPRFVa, which, although probably less abundant, was found to be more potent than GSPRFFa on the peripheral musculature (Fujisawa et al. 1999). We found the same here centrally.

**Novel AMRP-modulated K⁺ current**

The most prominent and robust response of the pleural sensory neurons to the AMRPs was a relatively rapidly activating and desensitizing outward-current response. Voltage-clamp examination showed the bulk of the response to be mediated by a distinctive AMRP-elicited K⁺ current. This current has, in particular, two characteristic features. First, it exhibits considerable outward rectification that persists and indeed is revealed more obviously as a negative slope resistance region at negative voltages in high, 100 mM external K⁺. Second, the current is extremely potently blocked by 4-AP, with an IC₅₀ of 1.7 × 10⁻³ M. In contrast, TEA begins to block the current only at >10 mM.

In cell-attached patches, we observed an AMRP-modulated K⁺ channel that is likely to underlie the macroscopic AMRP-elicited K⁺ current. The channel has a conductance of ~48 pS at positive voltages in normal 10 mM external K⁺. The single-channel current exhibits some outward rectification, and in addition the open probability of the channel is voltage dependent, decreasing with hyperpolarization. Together, these two factors account for the outward rectification of the macroscopic current. The channel also has the second characteristic feature of the macroscopic current, the extremely high 4-AP sensitivity. At submicromolar concentrations, 4-AP acts as a slow blocker of the channel, decreasing its open probability but not the single-channel current. 4-AP does this only when it is included in the patch pipette, consistent with the idea that it acts, from the outside, directly on the channel itself rather than on, for instance, the AMRP receptor.

The channel opens with a low probability even in the absence of the AMRPs, which therefore modulate the channel rather than absolutely activate it. As the AMRPs are able to modulate channels in the cell-attached patch even when they are applied outside the patch, they presumably act through some type of diffusible intracellular messenger.

Can the AMRP-modulated K⁺ current be identified with any of the other K⁺ currents, modulated or unmodulated, that have been described in the sensory neurons or in other Aplysia neurons? The AMRP-modulated current appears to differ from each of these other currents in at least one significant characteristic. It differs from the various types of early-transient, A-type K⁺ current (Iₖ,A) found in Aplysia neurons (e.g., Furukawa et al. 1992) in its lack of voltage-dependent inactivation at positive voltages, and—even though Iₖ,A too is usually considered to be a 4-AP-sensitive current—in its much higher 4-AP sensitivity. It differs from Iₖ,S in its voltage dependence and from both Iₖ,V and Iₖ,Ca in its higher 4-AP sensitivity and lower TEA sensitivity (cf. Baxter and Byrne 1989; Hermann and Gorman 1981a,b). Finally, the AMRP-modulated K⁺ current is not Iₖ,S. The AMRP-modulated current is somewhat more sensitive to TEA, and much more sensitive to 4-AP, than Iₖ,S. Furthermore, the AMRP-modulated K⁺ channels exhibit a single-channel current rectification and voltage dependence of open probability that, in most reports, single S K⁺ channels lack (cf. Baxter and Byrne 1989; Brezina et al. 1987; Klein et al. 1982; Pollock et al. 1985; Shuster and Siegelbaum 1987; Shuster et al. 1991; Siegelbaum et al. 1982).

There are other modulator-elicited K⁺ currents in molluscan neurons and muscle that the AMRP-elicited current also, in some respects, resembles. These include an Ach-elicited K⁺ current in Aplysia sensory neurons (Ichinose et al. 1989), K⁺ currents elicited by FMRFamide and other modulators in Lymnaea neurons (Kits et al. 1997; van Tol-Steye et al. 1997), and K⁺ currents elicited by Mytilus inhibitory peptides, the homologues of the AMRPs (see Fujisawa et al. 1999), in Achataina and Helix neurons (Kiss et al. 1999; Yongsiri et al. 1989). Some of these currents clearly lack either the rectification or the very high 4-AP sensitivity of the AMRP-modulated current. In other cases, no definitive comparison can be made for lack of data, in particular about the degree of 4-AP sensitivity of the other current. Somewhat similar, also, are the K⁺ channels activated by cGMP and closed by light in Onchidium extra-ocular photoreceptor neurons (Gotow et al. 1997). These, however, have a higher single-channel conductance, are blocked by 4-AP less potently, and furthermore only when the 4-AP is added to the bath rather than to the patch pipette, the converse of what we found here. The AMRP-modulated current is overall perhaps most similar to the K⁺ current elicited by several modulators in Aplysia muscles (Brezina et al. 1994; Cropper et al. 1994; Scott et al. 1997), and, especially, the K⁺ current activated by light in the hyperpolarizing ciliary photo-
receptors of *Lima* and *Pecten* (Gomez and Nasi 1994a,b). The former, however, has a somewhat more moderate rectification and 4-AP sensitivity than the AMRP-modulated current, while the latter has a clearly different single-channel conductance (26 vs. 48 pS).

Neither can the AMRP-modulated K+ current be obviously identified with any current known in other invertebrates or vertebrates. For K+ currents in vertebrates, the lowest IC_{50} values for 4-AP block are of the order of 10–20 μM (see e.g., Rudy 1988; Storm 1993), two orders of magnitude higher than the IC_{50} of the AMRP-modulated current. [Although some part of the difference might be accounted for by the different pH values in the two cases, as discussed by Gomez and Nasi (1994b), a very significant difference remains.]

Altogether, we conclude that the AMRP-elicited K+ current is a novel current that has not previously been described in *Aplysia* sensory neurons. Its relationship, if any, to other molluscan modulator-elicited K+ currents, as indeed their mutual relationships (see e.g., Kits et al. 1997), remain to be clarified.

In addition to the fast, 4-AP-sensitive AMRP-modulated K+ current, we also observed in some sensory neurons an apparent small component of slower, 4-AP-resistant outward current, as well as an inward Na+ current.

As most of our work was done on sensory neurons in dissociated cell culture, we carried out an additional series of experiments to confirm that sensory neurons in situ in the intact pleural ganglia respond to the AMRPs similarly. Indeed, we observed responses consistent with each of the three types of AMRP-elicited current, including, in particular, a substantial AMRP-elicited hyperpolarization that was blocked by 4-AP.

Comparison of the relative incidence of the three responses reveals, however, a possible discrepancy between the cultured neurons and the ganglia. In particular, the fast K+-current response appeared to be considerably rarer in the ganglia than in culture. Changes in the relative expression of the responses in culture cannot be ruled out, the most likely explanation has to do with the differential penetration of exogenously applied agents to receptors differentially distributed on the neuron that in intact ganglia has been shown to lead to the appearance and disappearance of responses—especially those that, like the AMRP K+-current response, desensitize—depending on the exact point of application (see e.g., Ascher 1972; Levitan and Tauc 1972; Sun et al. 1996). Knowing these problems, we wished here simply to confirm that the three responses can, in fact, be found in the intact ganglia.

As yet, it has not been demonstrated that the AMRP-containing neurons in the pleural ganglia make functional connections to the pleural mechanosensory neurons or that they release the AMRPs in the behavioral circumstances when the tail-withdrawal reflex is known to be modulated. However, the precedent provided by the well-established modulators of the tail- and siphon/gill-withdrawal reflexes, especially by FMRFamide with its activation of the broadly similar S K+ current, suggests the functional contributions that the AMRP-activated K+ current might make. They will likely include decreased excitability of the sensory neurons and increased spike accommodation (see e.g., Critz et al. 1991), increased mechanosensory threshold (Billy and Walters 1989), and, under some circumstances, spike narrowing leading to decreased release of transmitter at the sensory-motor synapse (Critz et al. 1991; Klein 1995; Mackey et al. 1987; Pieroni and Byrne 1992). The precedents also strongly suggest that, besides the responses we have already identified, the AMRPs will be found to modulate also other, voltage-dependent currents and a host of other processes generally tending to depress the sensory-motor synapse and inhibit the tail-withdrawal reflex (Byrne and Kandel 1996; Klein 1995). Much work clearly remains to be done to establish the AMRPs as modulators of *Aplysia* withdrawal behavior, but we believe that our results here provide a solid start.

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**REFERENCES**


