Opioid modulation of calcium current in cultured sensory neurons: μ-modulation of baroreceptor input

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Hamra, Mary, Robert S. McNeil, Martin Runciman, and Diana L. Kunze. Opioid modulation of calcium current in cultured sensory neurons: μ-modulation of baroreceptor input. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H705–H713, 1999.—We used the whole cell open-patch or perforated-patch technique to characterize μ-opioid modulation of Ca\(^{2+}\) current (I\(_{\text{Ca}}\)) in nodose sensory neurons and in a specific subpopulation of nodose cells, aortic baroreceptor neurons. The μ-opiate receptor agonist Tyr-o-Ala-Gly-MePhe-Gly-ol enkephalin (DAGO) inhibited I\(_{\text{Ca}}\) in 95% of neonatal [postnatal day (P)1–P3] nodose neurons. To the contrary, only 64% of juvenile cells (P20–P35) and 61% of adult cells (P60–P110) responded to DAGO. DAGO-mediated inhibition of I\(_{\text{Ca}}\) was naloxone sensitive, irreversible in the presence of guanosine 5′-O-(3-thiotriphosphate), absent with guanosine 5′-O-(2-thiodiphosphate), and eliminated with pertussis toxin; DAGO's inhibition of I\(_{\text{Ca}}\) was G protein mediated. Incubation of neurons with ω-conotoxin GVIA eliminated the effect of DAGO in neonatal but not in juvenile cells. In the latter, DAGO reduced 37% of the current remaining in the presence of ω-conotoxin. In the subset of nodose neurons, aortic baroafferents, the effect of DAGO was concentration dependent, with an IC\(_{50}\) of 1.82 × 10\(^{-8}\) M. DAGO slowed activation of I\(_{\text{Ca}}\), but activation curves constructed from tail currents were the same with and without DAGO (100 nM). In summary, μ-opiate modulation of I\(_{\text{Ca}}\) in nodose neurons was demonstrated in three age groups, including specifically labeled baroafferents. The demonstration of a mechanism of action of μ-opioids on baroreceptor afferents provides a basis for the attenuation of the baroreflex that occurs at the level of the nucleus tractus solitarii.

ARTERIAL PRESSURE is modulated by the opiate peptides acting at sites within the central nervous system. Central opiate injection near the nucleus tractus solitarii (NTS), a major site of integration of cardiovascular sensory information (1), may produce pressor, depressor, or biphasic responses (9, 10). This most likely reflects multiple sites and/or mechanisms of action. The central components of the arterial baroreceptor reflex pathways originate in the NTS, and several groups have demonstrated opioid modulation of baroreflexes at this level (9, 10). Thus the purpose of this study was to identify specific sites and mechanisms of action for the opioid effects on the baroreflex pathway at the NTS.

Opiate receptors and opioid-containing neurons are abundant in the NTS region (2, 35). In addition to localization to dendrites and soma (5), the μ-opiate receptor subtype is located presynaptically on vagal afferents terminating in the NTS (7, 15). These receptors, synthesized in cell bodies of the nodose ganglion, are transported centrally where opiate activation may modulate synaptic transmission. Ca\(^{2+}\) channels are likely targets mediating modulation, and the multiple Ca\(^{2+}\) channel types that are expressed in the soma of nodose neurons have been inferred to be present at the central terminals in approximately similar proportions to those in the soma (17). Therefore, examination of opiate effects on Ca\(^{2+}\) currents of cell bodies may provide information regarding modulation at the synapse.

METHODS

General. Nodose ganglia were excised from neonatal rats [postnatal day (P)1–P3], from juvenile rats (P20–P35), and from adult rats (P60–P110). They were placed in nodose complete media (DMEM with 5% fetal bovine serum, 0.1% penicillin/streptomycin). Neuronal tissue was enzymatically treated with trypsin (5 mg/ml in Earle's balanced salt solution) for 30 min at 37°C. Nodose tissues from juvenile and adult animals were enzymatically treated with collagenase type 2 (1 mg/ml in Earle's balanced salt solution) plus 10–15 drops of papain for 1 h at 37°C. The ganglia were triturated through small-bore pipettes. The cells were plated on poly-o-lysine-coated coverslips and were maintained in nodose complete media with nerve growth factor (8 ng/ml) at 37°C and 5% CO\(_2\). Experiments were performed at 25°C on neurons 15–24 h after isolation. In the neonatal group, there were no differences in data from animals that were 1, 2, or 3 days old; therefore, these data were pooled.

Neurons were voltage clamped using the whole cell open-patch or perforated-patch technique. The data from the two methods were not different and were grouped for analysis. We used List EPC-5 and Axopatch 200A amplifiers and pCLAMP software (Axon Instruments) to apply voltage protocols and measure whole cell currents. Whole cell current data was filtered at 1 kHz, and the sampling interval was 500 μs except in tail current experiments where data was sampled at 16- to 20-μs intervals and filtered at 5–10 kHz. The resistance of the patch electrodes ranged from 0.5 to 4 MΩ, and the series resistance was typically 2–15 MΩ. The data presented include only those experiments in which the access resistance did not change during the course of the experiment. Neurons without processes were studied so that adequate space clamp could be achieved. During experiments, drug protocols were...
not initiated until control $\mathrm{Ca}^{2+}$ current during bath superfusion was stabilized. The effects of Tyr-$\beta$-Ala-Gly-MePhe-Gly-ol enkephalin (DAGO) were determined only in those cells in which full recovery followed opiate withdrawal. $\mathrm{Ca}^{2+}$ currents ($I_{\mathrm{Ca}}$) were isolated with the following solutions: pipette solution consisted of (in mM) 124 CsCl, 11 EGTA, 1 CaCl$_2$, 2 MgCl$_2$, and 10 HEPES, pH 7.2; bath solution consisted of (in mM) 139 TEA chloride, 2 CaCl$_2$, 2 glucose, 10 HEPES, and 5 4-aminopyridine, pH 7.4. For perforated-patch experiments, 50 µg/ml nystatin were added to the pipette solution. Superfusion of cells with test solutions was effected through a large-bore catheter positioned close to the neuron. Flow rate was 500 µl/min. To obtain the concentration-response curves, DAGO was applied to the same cell in incremental concentrations with intervening recovery in control solution before subsequent applications. If recovery was not obtained, the data from the previous concentrations were used, and the experiment was terminated. Four cells received only the highest concentration of 1 µM.

Protocols. $I_{\mathrm{Ca}}$ was elicited with voltage steps (ranging from -100 to +40 mV in +10-mV increments, 400-ms duration) from a holding potential of -80 mV. Voltage dependence of activation was determined from analysis of tail currents present when the voltage was returned to -80 mV after test pulses varying from -80 to 0 mV. The test pulse was 25 ms in duration.

In one series of experiments, the DAGO effect was demonstrated in specific aortic baroreceptor neurons labeled with the fluorescent anterograde tracer 4-(4-(dihexadecylamino)styryl)-N-methylpyridinium iodide (DiA; Molecular Probes, Eugene, OR). Juvenile rats were anesthetized with pentobarbital sodium (30 mg/kg). The left aortic depressor nerve was identified where it joins the superior laryngeal nerve before entering the nodose ganglion. The fluorescent tracer was applied to the uncut nerve (16), the surgical area was closed, and the animal was allowed to recover for 3–4 days. Subsequently, the left nodose ganglia were isolated, and the cells dissociated as described previously. Aortic baroreceptors were identified before electrophysiological recording by the presence of the fluorescent dye with a microscope equipped with appropriate fluorescent filters.

Data analysis. Data were analyzed using pCLAMP software (Axon Instruments). Standard current-voltage ($I-V$) relationships were constructed for both peak currents (those obtained at the point of maximal activation) and sustained currents (those present at the end of the 400-ms depolarizing pulse). Currents were leak subtracted using the currents over the range of 90–70 mV. In Figs. 1–9, the DAGO effects shown

![Fig. 1. Tyr-$\beta$-Ala-Gly-MePhe-Gly-ol enkephalin (DAGO)-mediated inhibition of $\mathrm{Ca}^{2+}$ current ($I_{\mathrm{Ca}}$) in a neonatal nodose neuron.](http://ajpheart.physiology.org/)
cells found in nodose ganglia. DAGO concentration was 100 nM. Responses in both age groups and may reflect the mixed population of symbols represent a different cell. Figure illustrates the wide range of maximum (max) current in both neonates and juveniles. Each averaged data are expressed as means ± SE. Any contribution of rundown was marginal or non-existent in these experiments, agonist-induced inhibition was so rapid that any contribution of rundown was marginal or nonexistent. Averaged data are expressed as means ± SE.

DAGO appears to slow activation in some cells so that "peak current" during DAGO superfusion instead represents the more sustained portions of the current profile. To determine the effect of DAGO on the initial transient portion early in the depolarizing pulse, we measured current amplitude at the point of peak current during control superfusion with bath solution and then measured current at the same point during DAGO superfusion. These are the measurements represented in all figures of "peak" current. This method was chosen to assess the effect of DAGO at a point in time that was relevant to opening of Ca\(^{2+}\) channels during an action potential and gave a value that was equivalent to the steady-state component if the true peak of the current was evaluated. For voltage dependence of activation, the true peak of the current (wherever that occurred) was used to calculate test current-to-maximum current ratios.

**RESULTS**

Effect of DAGO on \(I_{\text{Ca}}\) in neonatal nodose neurons. The \(I_{\text{Ca}}\) in nodose sensory neurons, dissected pharmacologically, includes a small component of low-threshold T-type current and a more substantial higher-threshold current consisting of primarily N- but also P-, Q-, R- and L-type channels (16, 17). An example from a neonatal neuron is shown in Fig. 1A. The µ-opiate-receptor agonist DAGO caused a reversible concentration-dependent inhibition of \(I_{\text{Ca}}\) in these neurons. Figure 1A shows currents recorded (from top to bottom, respectively) during control superfusion with bath solution, during test superfusion with DAGO (100 nM), and again with bath solution to wash. The I-V relationships for both peak of the transient and the sustained \(I_{\text{Ca}}\) in this neuron are shown in Fig. 1B. DAGO inhibited both peak and sustained \(I_{\text{Ca}}\) at membrane potentials more positive than −40 mV. DAGO-induced inhibition taken at the time of the peak of the control current was greater than that for sustained current, and both effects were fully reversible with wash. The T-type low-threshold current observed at potentials more negative than −30 mV was not modified. DAGO decreased Ca\(^{2+}\) current in 95% of neonatal cells studied.

In some cells, DAGO also appeared to slow the activation of \(I_{\text{Ca}}\) (especially at more depolarized pulses and at higher DAGO concentrations); in others, it did not, similar to observations in dorsal root ganglion neurons (8, 18). Both effects could be seen in different cells from the same preparation, and both effects were seen in both open and perforated patches. We analyzed tail currents after conditioning prepulses (from −80 to 0 mV) from a holding potential of −80 mV, with a return to −80 mV, and constructed activation curves (Fig. 2). Activation of a small T-type current is evident at −70 to −40 mV. The high-threshold currents activate about −30 mV. DAGO did not shift the activation curve, change the slope factor, or alter the voltage of half-maximal activation.

Effect of DAGO on \(I_{\text{Ca}}\) in juvenile neurons. Neurons isolated from juvenile rats (P20–P34) and from adults (P60–P110) also responded to DAGO. However, there was a developmental difference in the percentage of cells that responded to DAGO. For all cells studied with DAGO (≥10 nM), inhibition was seen in 91/96 neonates (95%), in 51/80 juveniles (64%), and in 17/28 adults (61%). It was difficult to compare the magnitude of DAGO-mediated inhibition between the neonate and juvenile age groups because of the variability of responses within each group. That variability is seen in the scatter plot of responses from both groups to DAGO at 100 nM, a near-saturating concentration (Fig. 3). This suggested that the nodose neurons were not homogenous in response to DAGO and led to the next set of experiments.
Effect of DAGO on I_{Ca} of baroreceptor neurons. Nodose ganglia contain the cell bodies of a number of visceral sensory afferents. The variability in the response to DAGO may reflect the multiplicity of cell types within the ganglion. We were particularly interested in that subpopulation of nodose neurons that represents aortic baroreceptors. Because 95% of all neonatal cells responded to DAGO, we can reasonably assume that aortic baroafferents in this age group are modulated by µ-receptor stimulation. This assumption could not be made for juvenile cells, since almost 40% were found to be unresponsive to DAGO. To study baroafferents specifically, these sensory neurons (from juveniles, P29–P35) were labeled by application of the fluorescent tracer, DiA, to the aortic depressor nerve before study (see METHODS). Figure 4 illustrates the current profile from one such neuron and the peak I-V relationship for all labeled cells studied with a maximum inhibitory concentration of 1 µM DAGO (Fig. 4B). DAGO decreased I_{Ca} at all voltages more positive than −40 mV. In contrast to the general population of neonatal neurons, the response to the opioid was less varied as is shown in the concentration-response curve for DAGO-mediated inhibition of I_{Ca} in labeled baroafferenents (Fig. 5). In these experiments, DAGO was applied in increasing 10-fold increments with recovery during a control wash between increments or with a single concentration. The group data gave a concentration-dependent effect with an IC_{50} of 1.82 × 10^{-8} M when fit with a single binding site model. DAGO-mediated inhibition was seen in 14/15 labeled neurons (93%). There was no indication of desensitization of the response when repeated applications at the same concentration were applied at the concentrations used in the present experiments. This is consistent with previous studies (24, 27, 34).
Naloxone blocks the inhibition of $I_{\text{Ca}}$ by DAGO. We next characterized the $\mu$-mediated inhibition of $I_{\text{Ca}}$. The effect of naloxone, 100 nM, on the DAGO response in neonatal cells is seen in Fig. 6. In this experiment, neonatal cells were first tested for the response to DAGO, 100 nM, and subsequently (after recovery) for the response to naloxone alone and then DAGO in the continued presence of naloxone. Naloxone by itself had no effect on the currents but completely antagonized the effect of DAGO. Naloxone also completely antagonized DAGO-mediated inhibition of $I_{\text{Ca}}$ in juvenile cells ($n = 3$, data not shown).

$\mu$-Opioid inhibition of $I_{\text{Ca}}$ is $G$ protein mediated. It has been reported in dorsal root ganglia sensory afferents that $\mu$-stimulated inhibition of $I_{\text{Ca}}$ is $G$ protein mediated. We also found this to be the case for nodose sensory afferents. We tested the effect of DAGO on $I_{\text{Ca}}$ recorded in the presence of guanosine 5′-O-(3-thiotriphosphate) (GTP-S) or guanosine 5′-O-(2-thiodiphosphate) (GDP-S) or after incubation with pertussis toxin (Table 1). When the nonhydrolyzable GTP-S was included in the patch pipette, the reduction in current by DAGO became irreversible. With GDP-S present in four of five cells, the DAGO-induced inhibition of $I_{\text{Ca}}$ was abolished. Finally, after cells were incubated 20–24 h in pertussis toxin, 200 ng/ml, superfusion with DAGO failed to decrease $I_{\text{Ca}}$. Control cells from the same preparation that were not incubated with the toxin still responded to DAGO.

Comparison of DAGO-mediated $Ca^{2+}$ channel inhibition in neonatal and juvenile neurons. To identify the channel type inhibited by DAGO, we examined the effect of DAGO in cells incubated with $\omega$-conotoxin GVIA to block the major component of the current, the N-type channel (Fig. 7). Because nearly all neonatal cells respond to DAGO, it was not necessary to use each cell as its own control. Control neonatal cells in Fig. 7A were from the same preparation as those in Fig. 7B. This method allowed for prolonged $\omega$-conotoxin block without rundown of the currents or deterioration of the patch imposed by the electrode. The control cells showed a large peak $Ca^{2+}$ current and the characteristic DAGO-mediated inhibition of $I_{\text{Ca}}$. In Fig. 7B, neonatal cells were incubated with $\omega$-conotoxin, 1 $\mu$M, for 30 min before treatment with DAGO (100 nM). High-threshold control currents in these cells were greatly reduced, and DAGO-mediated inhibition of $I_{\text{Ca}}$ was absent. Toxins that inhibit other components of the $I_{\text{Ca}}$ were not used in neonates since there was no residual DAGO-mediated effect after N-channel blockade.

In the initial $\omega$-conotoxin experiments in juveniles, each cell had to serve as its own control; absence of DAGO-mediated inhibition in the presence of $\omega$-conotoxin could not otherwise be interpreted, since almost 40% of this population are nonresponders. In these experiments, DAGO was applied to the same cell in the absence and then in the presence of $\omega$-conotoxin. As discussed earlier, we had no evidence of desensitization at the concentrations used in the present studies.

### Table 1. DAGO effect on $I_{\text{Ca}}$ is $G$ protein mediated

<table>
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<td>$-203 \pm 32$</td>
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<td>$-537 \pm 109$</td>
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<td>$-315 \pm 84$</td>
<td>$-677 \pm 169$</td>
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<tr>
<td>Cells incubated</td>
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<td>$-749 \pm 89$</td>
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Values are means ± SE; n, no. of experiments. Units are pA.

DAGO, Tyr-$\alpha$-Ala-Gly-MePhe-Gly-$\alpha$ enkephalin; $I_{\text{Ca}}$, $Ca^{2+}$ current; GTP-S, guanosine 5′-O-(3-thiotriphosphate); GDP-S, guanosine 5′-O-(2-thiodiphosphate); PTX, pertussis toxin; NA, not applicable.

Fig. 5. Grouped concentration-response curve for DAGO-mediated inhibition in labeled baroafferents. All labeled cells came from juvenile rats. Figure depicts percent inhibition of max current (ordinate) vs. the log molar concentration of DAGO. Curve was fit with a single-site binding model; $IC_{50}$ was $1.82 \times 10^{-8}$ M. No. of experiments is in parentheses.

Fig. 6. Naloxone antagonism of DAGO-mediated inhibition of $I_{\text{Ca}}$ in neonatal cells. Figure depicts the I-V relationships during control condition (●), DAGO at 100 nM (◆), recovery (▲), naloxone (▼), and DAGO in the presence of naloxone at 100 nM (●).
Figure 8 shows the effect of DAGO (100 nM) in a juvenile cell with and without prior antagonism with \(\omega\)-conotoxin (1 \(\mu\)M). The current traces in Fig. 8A show the characteristic DAGO-mediated inhibition, and those in Fig. 8B show the effect of DAGO during \(\omega\)-conotoxin blockade. DAGO-mediated inhibition was still present, although greatly reduced, during \(\omega\)-conotoxin block of the N-type channel in this juvenile cell. Similar responses were seen in three additional responder cells (cells that served as their own controls), even with \(\omega\)-conotoxin block extending to 15 min. In these four cells, DAGO decreased \(I_{\text{Ca}}\) by 57 \pm 5\% in the absence of \(\omega\)-conotoxin. The \(I_{\text{Ca}}\) was reduced by 42 \pm 11\% by \(\omega\)-conotoxin, and DAGO reduced this remaining current by 37 \pm 7\%.

Figure 9 shows the effect of \(\omega\)-conotoxin in the specific subset of labeled baroafferents in juveniles. Figure 9 demonstrates that DAGO-mediated inhibition of \(I_{\text{Ca}}\) in this subset is still present during \(\omega\)-conotoxin blockade. This result contrasts with the absence of a residual response in neonatal neurons after \(\omega\)-conotoxin block (see Fig. 7B) and suggests a developmental difference in the \(Ca^{2+}\) channel type(s) targeted by this \(\mu\)-specific agonist in baroafferents.

DISCUSSION

Our primary interest in this study was the opiate modulation of baroreceptor input. Comparison of the response of the neonatal neuron with those from older animals provided several interesting observations. DAGO-mediated inhibition of \(I_{\text{Ca}}\) was present in essentially all neonates (95\%). Therefore, although we were unable to obtain labeled baroreceptor neurons at the early age of P0–P1, we could be reasonably certain that the baroafferent population in neonates responded to \(\mu\)-receptor stimulation. The inhibition is naloxone sensitive and \(G\) protein mediated. The effect was present when using the perforated-patch or whole cell recordings, and it was rapid in onset. Data obtained from open-patch whole cell recordings indicate that a diffusible second messenger may not be necessary in this reaction and are consistent with direct \(G\) protein coupling between the \(\mu\)-receptor and high-threshold \(Ca^{2+}\) channels in a variety of cell types (4, 19, 27, 37). Within the same culture, there were neurons that responded to DAGO with a slowing of activation and others that did not. The slowing was described by Bean (3) as a change in voltage dependence that could be relieved by strong depolarization. Formenti et al. (8) have attributed similar mixed results to the presence of two distinct modulatory effects with, perhaps, different functions in the transmission of nociceptive information.

Interestingly, in the general population of neurons from juvenile or adult animals, the response to DAGO was less frequent (60–65\%). We examined the subpopulation of aortic baroreceptor afferents in juvenile animals and found that almost all (93\%) demonstrated DAGO-mediated modulation of \(I_{\text{Ca}}\). Therefore, the population of nodose neurons that loses sensitivity to opioids appears not to include the baroreceptors. The second difference between the two populations was that, while \(\omega\)-conotoxin eliminated the inhibition in neonatal cells, this was not true for juveniles. Thus DAGO acts via only the N-type channel in neonates but modulates \(I_{\text{Ca}}\) through multiple channel types in older animals. This is consistent with studies in adult dorsal root ganglia neurons (23), a neuroblastoma cell line (28), and expression systems (4) where opioids have been shown to modulate \(N\), \(P\), and \(Q\) high-threshold currents. In a recent study, Rusin and Moises (24) have shown that DAGO modulates \(N\), \(P/Q\), and \(R\) but not \(L\) \(Ca^{2+}\) channels in nodose neurons of animals 10–30 days old. The difference in the response at the two ages may reflect differences in the relative expression of the specific \(Ca^{2+}\) channels or age-dependent changes in the
coupling of the receptor to the Ca$^{2+}$ channels and G protein. Although \(\omega\)-conotoxin-, agatoxin-sensitive currents, and a residual current are present in the neonatal neurons, we are unaware of studies comparing the relative age-dependent expression of Ca$^{2+}$ channels in nodose neurons. In our studies, the T-type current in neonatal or juvenile neurons appeared not to be altered by DAGO, although the overlap in part of the activation range for the low- and high-threshold currents as well as the small size of the T-type current precluded a definite conclusion. Both an inhibition (27) and no effect (19) of opiates on T-type current in other preparations have been reported.

Role of \(\mu\)-opioid receptors at central baroreceptor terminals. If the \(\mu\)-receptor is located on presynaptic terminals of the sensory afferents, then the opiate-mediated inhibition of \(I_{Ca}\) in both younger and older animals has implications for baroreflex modulation at the level of the first synapse of the reflex pathway. In a recent detailed electron microscopy study, Cheng and colleagues (5) clearly defined locations of the \(\mu\)-receptor in the medial NTS, a site of termination of baroreceptor afferents. Using an antibody against a peptide corresponding to a COOH terminal region of the \(\mu\)-receptor, they localized receptors at the extrasynaptic subplasmalemmal regions of unmyelinated axons and terminals. They reported that some of the labeling occurred near asymmetric synapses indicative of the sensory afferent synapses in the NTS, leading these authors to suggest, as others before, presynaptic modulation of the release of neurotransmitter at the synapse. The fact that the immunoreactivity could be seen on fine unmyelinated axons may indicate that the modulation is primarily on the C-fiber terminals. This is consistent with the assumption that most of our recordings were from C-type neurons that are 8–10 times more abundant than A-type neurons in our cultures (unpublished observations based on presence of TTX-resistant sodium current) and in the baroreceptor nerves in vivo. However, we cannot rule out an effect on A-type neurons.

Fig. 8. Effect of \(\omega\)-conotoxin (CTX) on DAGO-mediated inhibition of \(I_{Ca}\) in a juvenile cell. Effect of DAGO (100 nM) in a juvenile cell with and without prior antagonism with \(\omega\)-conotoxin (1 \(\mu\)M). A: current traces represent (from top to bottom) control with bath superfusion, \(I_{Ca}\) inhibition with DAGO (100 nM), and subsequent washout of the DAGO-mediated effect. B: traces of (from top to bottom) \(I_{Ca}\) during \(\omega\)-conotoxin block, DAGO inhibition of \(I_{Ca}\) in the continued presence of \(\omega\)-conotoxin, and subsequent wash of the DAGO-mediated effect.
Activation of μ-opiate receptors located presynaptically on baroafferent terminals at the central synapse could be expected to reduce neurotransmitter release through the inhibition of Ca\(^{2+}\) influx (13, 20). The validity of this hypothesis depends on whether the \(I_{Ca}\) we record in the soma are the same as those at the presynaptic terminal. Mendelowitz et al. (17) demonstrated a similarity between the pharmacological profile of Ca\(^{2+}\) channels on the soma and those responsible for glutamate neurotransmission at the synapse between the nodose sensory neurons and the postsynaptic NTS neuron. This group also demonstrated that inhibition of the N-type Ca\(^{2+}\) channel was the major factor in reducing the size of the postsynaptic potential at the synapse between sensory afferents and NTS neurons and that P/Q channels are involved but to a lesser extent. The effect on transmitter release could be particularly pronounced in C-type neurons where the duration of the action potential (and therefore the period of Ca\(^{2+}\) influx) is lengthened by the presence of a TTX-resistant sodium current that is lacking in the A-type neurons (26). Previous experiments in mouse dorsal root ganglia neurons (29, 36) and in rabbit nodose C-type neurons (11) showed an opiate effect on action potential duration that was biphasic and concentration dependent. The respective excitatory and inhibitory effects were linked to decreases in potassium conductance and/or increases in \(I_{Ca}\) in the former and to opposite changes in the two conductances in the latter (6). In our study, the excitatory response was not seen, even with the lowest concentration of DAGO (10\(^{-10}\) M), nor did incubation with pertussis toxin unmask an agonist-induced excitatory effect. Thus μ-receptor stimulation in nodose neurons, under conditions where \(K^+\) currents are eliminated, is limited to an inhibitory effect via depression of \(I_{Ca}\). Because we did not study DAGO’s effect on \(K^+\) currents, we can not rule out a μ-receptor-mediated excitatory effect, since one may occur through inhibition of a \(K^+\) channel (29, 30).

Implications for the baroreflexes. The consequences of opioid inhibition of the \(I_{Ca}\) at a presynaptic site would be to reduce the effectiveness of the reflex, since the baroreceptor restraint on the sympathetic outflow would be minimized (12). Whole animal experiments that address this question make use of the injection of μ-agonists or -antagonists into the NTS region while monitoring the baroreflexes. Because the NTS is a complex integrative region, receptor activation may occur at any number of sites. Nonetheless, it can be noted that NTS injection of DAGO has been reported to reduce the effectiveness of the baroreflex pathway as measured by the arterial pressure and heart rate response to carotid occlusion (10) or to stimulation of the aortic depressor nerve (9). Inhibition of the baroreflex pathways may also indirectly modify other pathways with which they intersect. For instance, when A or C fibers in the tibial nerve were electrically stimulated, they elicited a reflex increase in the cardiac sympathetic discharge that was enhanced when done after injection of morphine into the intermediate and caudal NTS (14). The authors suggested that this may be the result of an inhibition of the baroreceptor pathway that then allowed the somatosensory reflex pathway to be more effective. A second potential site of action for opioids on the baroreflex pathway has been proposed. \(I_{Ca}\) in a subpopulation of acutely dissociated NTS neurons from rats 7–21 days old is also inhibited by μ-receptor agonists (22). The general population of NTS neurons was examined, and approximately one-half did not respond, so it is not clear whether the population of neurons receiving the baroreceptor primary afferents is included in the responding group.

Numerous studies in normotensive animals, including humans, have used opioid antagonists to provide evidence that endogenous opioids influence the reflex response to pharmacological or mechanical activation of baroreceptors, although they may not exert tonic regulation of blood pressure (9, 10, 32). There is also evidence for the involvement of opioids in pathological states. Endogenous opioids are increased in some forms of hypertension (33) and may influence the development of hypertension (21), and opiate antagonists may restore the blunted baroreflex associated with chronic heart failure (25).

In summary, this study provides strong support for a mechanism of action of μ-receptor active opioids in suppressing the baroreflex at the level of the NTS. In light of these results, a number of questions should be addressed. Do only the C-type baroreceptors respond to the opiates and, if so, what are the functional consequences? Does the central neuron receiving the primary afferent input also respond directly to μ-agonists? What pathways modulate the release of endogenous opiates in the NTS and under what conditions? Neurons containing enkephalin immunoreactivity are present in the NTS and in many other central areas.

Fig. 9. Grouped data for DAGO-mediated effect in labeled juvenile baroaffersent blocked with ω-conotoxin. I-V relationship for 5 labeled juvenile baroaffersents. Cells were incubated with ω-conotoxin GVIA (1 µM) for 30 min before study. ●, Control \(I_{Ca}\) remaining after ω-conotoxin block; ■, \(I_{Ca}\) during superfusion with DAGO (100 nM) after ω-conotoxin blockade; ▲, washout of the DAGO-mediated effect.
involved in cardiovascular control such as the hypothalamus, parabrachial nuclei, ventrolateral medulla, etc. (31), but how these functionally integrate with the baroreflex pathway is presently unknown.

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