Dual Effects of Angiotensin II on Calcium Currents in Neonatal Rat Nodose Neurons

K. Bacal and D. L. Kunze

Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030

Angiotensin II (AII) reversibly modulates calcium current in isolated neonatal rat nodose ganglion cells by two different pathways. A maximum inhibitory effect of 43 ± 6% ($n = 25$) of the peak calcium current at −10 mV was observed at 10 nM AII. The IC_{50} of the inhibitory response was 100 pM. Losartan, a specific antagonist for the AT_1 type of AII receptor, abolished the AII-induced inhibition, as did preincubation with pertussis toxin (PTX). When ω-conotoxin GVIA (CTX) was added to the bath solution, AII produced no inhibition of the remaining calcium current, indicating that the AII inhibition was mediated through CTX-sensitive calcium channels. Reversible facilitation of calcium current was seen more rarely. The AII-induced facilitation was unaffected by losartan and PTX, indicating that the effect is mediated by a non-AT_1 receptor and does not depend upon a PTX-sensitive G-protein. The facilitation is present when the CTX-sensitive current has been blocked and involves activation of a reserve pool of dihydropyridine (DHP)-sensitive channels. In general, a particular neuron exhibited either inhibition or facilitation. However, in some neurons both inhibition and facilitation could be demonstrated in the presence of the appropriate blocking agents.

[Key words: calcium, baroreceptor, nodose, angiotensin II, ω-conotoxin, losartan]

Angiotensin II (AII) is present in many regions of the brain, but levels are particularly high in cardiovascular control centers such as the nucleus of the tractus solitarius (NTS) (Fuxe et al., 1988). An immunohistochemical study has reported the presence of AII-containing terminals specifically in the medial division of the NTS (mNTS), the site of the first synapse of the baroreflex (Lind et al., 1985). Receptors for the peptide are also present in this area, as indicated by the finding that microinjection of AII into the mNTS provokes a dose-dependent change in blood pressure (Casto and Phillips, 1986) and by studies using radio-labeled AII and AII antagonists that reveal a high number of binding sites in the mNTS (Gutkind et al., 1988).

There is indirect evidence to suggest that at least a portion of the binding sites in the mNTS are located on the central terminals and/or axons of sensory afferent fibers. The number of AII binding sites in the medulla significantly decreases following ligation of the vagus or the sinoaortic depressor nerve (Diz et al., 1986;

Speth et al., 1987). A. M. Allen et al. (1988) have demonstrated AII receptor binding in both neuronal cell bodies of the nodose ganglion and the fibers of the sensory nodose neurons. These studies suggest that AII receptors are located on presynaptic sensory terminals and that AII may exert its influence in the mNTS by presynaptic modulation of neurotransmitter release. The nodose ganglia contain the cell bodies of peripheral receptors of various sensory modalities, including the aortic baroreceptors, and as such provide us with access to the presynaptic cells of the first synapse of the reflex pathway. We chose to investigate the effects of AII on the whole-cell calcium currents because of the important role played by this presynaptic conductance in neurotransmitter release. The work was facilitated by recent characterization of calcium currents in the aortic baroreceptors (Mendelowitz and Kunze, 1992).

Preliminary reports have appeared earlier (Bacal et al., 1991; Bacal and Kunze, 1993).

Materials and Methods

Neonatal Sprague–Dawley rats (1 d old) of both sexes were sacrificed, and the nodose ganglia were excised and enzymatically digested in a solution containing Earle's Balanced Salt Solution with 5 mg/ml trypsin (Worthington Biochemical), 12 mM cysteine, 0.5 mM EDTA, and 1.5 mM $CaCl_2$.

After a 30 min digestion at 37°C, cells were mechanically dispersed and plated onto poly-D-lysine–coated coverslips at an approximate density of 100,000 cells/dish. Cells were maintained in nodose complete media [Dulbecco's Modified Eagle Medium with 5% fetal bovine serum (JRH Biosciences), 0.1% serum extender (MITO, Collaborative Research), 1% penicillin/streptomycin, and 8 ng/ml nerve growth factor (CR 7S, Collaborative Research)] at 37°C and 5% CO_2 for 15–48 hr before use. At this time, cell bodies were spherical (diameter ~ 20–30 µm), with minimal processes.

To isolate the calcium currents in these cells, the bath solution consisted of (in mM) 139 tetraethylammonium chloride, 2 $CaCl_2$, 2 glucose, 10 hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES), and 5 4-aminopyridine, pH 7.4. The intracellular pipette solution contained (in mM) 124 CsCl and 10 HEPES with free calcium buffered to approximately 100 nM by 11 EGTA, 1 $CaCl_2$, and 2 $MgCl_2$, pH 7.2.

For most experiments, the perforated patch technique using 50 µg/ml nystatin added to the pipette solution was used to prevent washout of the cell interior. Cell-attached patches were made on the nodose cell bodies using polished electrodes pulled from 8161- or 7052-type glass (Garner Glass). Tip resistances were 1–3 MΩ and junction potentials were 1–3 mV. Access to the intracellular compartment was indicated by a change in capacitative transients and input resistance (usually 5–20 min). When whole-cell open patches were used, nystatin was omitted from the intracellular solution.

All experiments were carried out at room temperature. The indifferent electrode was a silver–silver chloride plug connected to the bath solution via a 150 mM potassium chloride bridge. Calcium current recordings were obtained during constant perfusion with either bath or experimental solutions from a large-bore multibarrel pipette positioned close to the cell.

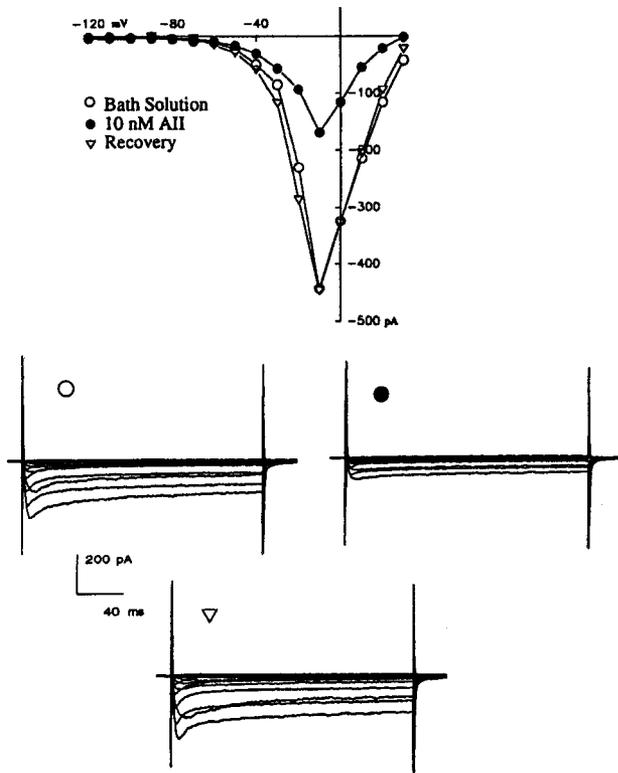Angiotensin II was obtained from Bachem. ω-conotoxin GVIA (CTX)

Figure 1. Current traces and resulting current–voltage relationship demonstrating the reversible effect of 10 nM AII on whole-cell calcium current in an individual neuron. The pulse protocol stepped from −120 mV to +30 mV for 300 msec in intervals of +10 mV.

was purchased from Sigma Chemical, while nimodipine and Bay K 8644 [2,6-dimethyl-3-carbomethoxy-5-nitro-4-(2-trifluoromethyl-phenyl)-1,4-dihydropyridine; "Bay K"] were kindly provided by Dr. A. Scriabne, Miles Laboratories, Inc. (West Haven, CT). Losartan [Du-P753; 2-butyl-4-chloro-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-l)-methyl]-5-(hydroxymethyl)imidazol] was kindly provided by the DuPont Merck Pharmaceutical Company (Wilmington, DE); CGP 42112B [nicotinyl-Tyr-(N-benzyl-oxycarbonyl-Arg)-Lys-His-Pro-Ile-OH], by Dr. M. de Gasparo, CIBA-GEIGY, Switzerland; and PD 123319 (1-[4-(dimethy-lamino)-3-methylphenyl]-5-(diphenylacetyl-4,5,6,7-tetrahydro-1H-im-idazo[4,5-c]pyridine-6-carboxylic acid), by Dr. J. Keiser (Research Division, Parke-Davis Pharmaceutical Company, Ann Arbor, MI).

Voltage protocols were applied, and currents measured, with pCLAMP software (Axon Instruments). Data were acquired using the CLAMPEX program and analyzed with CLAMPAN and/or CLAMPFIT. A normalization procedure was necessary for grouped data because the amplitude of the calcium current varied considerably from cell to cell. In some figures the currents are therefore expressed as percentages of the maximum current.

Results

The effect of AII on the calcium currents of individual neurons was one of three types: solely inhibitory, solely facilitory, or a mixed facilitation–inhibition. All neurons cultured from animals of the same litter behaved similarly. The data are presented below for each of the two individual effects and then for cells in which the dual effect was observed.

Inhibitory effects of AII

The effects of 10 and 100 nM AII on calcium current were studied using the perforated patch technique to reduce the washout of second messenger substances. In this set of experiments the voltage of the neuron was routinely clamped at a holding po-
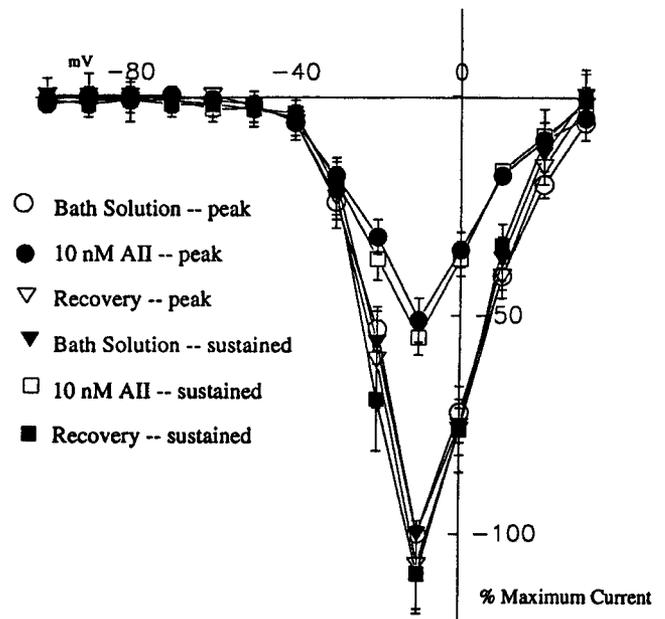

Figure 2. The effect of AII on the peak and steady-state (at 275–295 msec) calcium current (n = 12) utilizing protocol described in Figure 1. Current was normalized to peak value (at −10 mV).

tential of −80 mV. A pulse protocol consisting of steps in 10 mV increments from −120 to +30 mV was applied to obtain a current–voltage relationship. In the control records, a low-threshold "T-type" current of modest magnitude was observed in approximately half the cells studied. This current can be seen in the current–voltage relationship (Fig. 1) as the small current present at −60 to −40 mV. Even at maximum amplitude, the T-type current was less than 10% of the maximum amplitude of the high-threshold current observed at −10 mV. The high-threshold current was activated at potentials more positive than −40 mV and at least two kinetic components were evident during a 300 msec voltage step: a transient current that quickly inactivates and a longer lasting current that persists through the end of the voltage pulse (for detailed description, see Mende-lowitz and Kunze, 1993).

As can be seen in Figure 1, 10 nM AII caused a net reduction in the current elicited at all voltages positive to −40 mV, voltages that activate the high-threshold calcium currents. AII at 10 nM (n = 25) or 100 nM (n = 14; not shown) induced a net inhibition of the calcium current of 43 ± 4% and 40 ± 6%, respectively, at the peak current. The AII effect was reversible within a few seconds of reperfusion with bath solution.

To ascertain whether AII acts preferentially on the transient or the steady-state component of the high-threshold current, the current in 12 cells was measured at both peak current and at the end of the step (275–295 msec). No difference was found between the maximum and sustained current responses to 10 nM AII (Fig. 2).

While these results clearly indicated the inhibitory effect of AII on the high-threshold currents, an effect of the peptide on the low-threshold currents was not apparent. Because of overlap in the high-threshold and the low-threshold "T-type" current–voltage relationships and the small amplitude of the T-type current when present, an AII effect on the T-type current cannot be completely ruled out. However, examination of the grouped current–voltage relationships in the presence and absence of AII
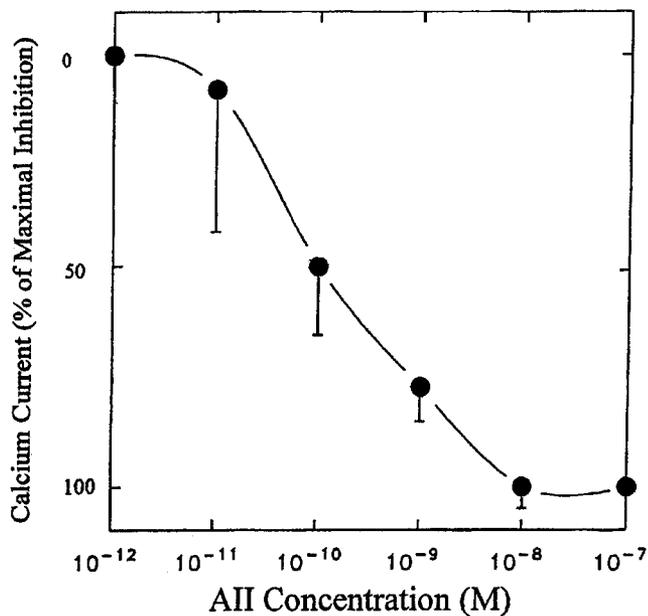
Figure 3. Concentration–response curve for AII inhibition. Calcium current of each cell was normalized to the maximum current remaining with a step to -10 mV during perfusion with 10 nm AII. Data were averaged for each point and fit by a single site binding model ($R =$ 0.97). $n = 3$ at 1 pm, 6 at 10 pm, 5 at 100 pm, 4 at 1 nm, 7 at 10 nm, and 4 at 100 nm. Nimodipine (2 μm) was present in the bath to block any facilitatory response.

(Fig. 2) suggests that there is no effect on the low-threshold current.

Threshold for inhibition occurs between 1 and 10 pm ($n = 3$, $n = 6$, respectively) AII and reaches a maximum at 10 nm ($n = 7$). The data were fit by a single site binding curve to give an IC_{50} of 100 pm (Fig. 3).

The effect of AII on voltage-dependent inactivation of the calcium current was also examined. Cells were exposed to a conditioning voltage for 2 sec, and then a test voltage step to 0 mV for 300 msec. Conditioning voltages were varied between -120 and 0 mV, and the results are displayed in Figure 4, which shows both individual and grouped responses. AII had no effect on the voltage-dependent inactivation of calcium current ($n = 7$).

Effect of losartan on the AII-induced inhibition. Losartan, previously known as DuP 753, is a specific antagonist for the AT_1 subpopulation of AII receptors. Losartan (100 nm) alone had no effect on whole-cell calcium current.

The effect of 100 nm losartan when applied simultaneously with 10 nm AII was compared to the effect when 10 nm AII was perfused alone. In these cells, losartan completely reversed the inhibition produced by AII ($n = 10$; Fig. 5). This indicates that an AT_1 receptor underlies the inhibition of calcium current.

Effect of GTPγs on the AII-induced inhibition. AT_1 receptors in other systems have been shown to be coupled to G-proteins. To determine whether this was also the case for the nodose cells, GTPγs, an hydrolysis-resistant GTP analog, was employed. GTPγs was added at 10 μm to the intracellular solution, and an open patch whole-cell recording was obtained. When 10 nm AII was applied to this system, an irreversible net inhibition of calcium current ($47 \pm 8\%$; $n = 6$; Fig. 6) was observed.

The decrease in calcium current is seen immediately (seconds) after application of AII in contrast to the time course of inhi-
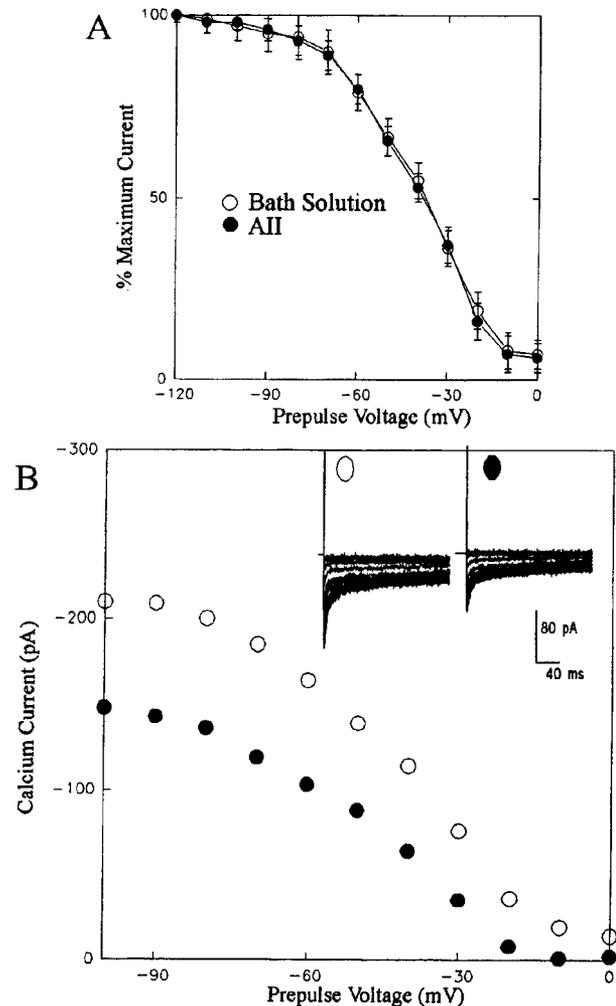

Figure 4. *A,* Effect of AII on the voltage dependence of inactivation of whole-cell calcium current in neurons demonstrating AII-induced inhibition. Cells were clamped at -100 mV and subjected to a 2 sec prepulse before stepping to the test voltage of 0 mV. The prepulse was varied from -120 to 0 mV increasing 10 mV every 8 sec. Currents were normalized to the peak current seen following a prepulse to -120 mV to show superimposition of voltage dependence; data were then averaged ($n = 7$). *B,* Current traces and inactivation curve showing effect of AII on magnitude of the current in an individual neuron.

bition (several minutes) seen when GTPγs alone is added to the intracellular solution (Dolphin and Scott, 1989).

Effect of pertussis toxin on the AII-induced inhibition. As the GTPγs results indicated that a G-protein was involved in the AII-induced inhibition, a group of nodose cells was incubated overnight in media containing pertussis toxin (200 ng/ml), while another group (obtained from the same preparation) was incubated in control media. The control cells demonstrated the typical AII-induced net inhibition (data not shown; $41 \pm 5\%$; $n = 12$) of calcium current. In the pretreated cells, however, 10 nm AII had no significant effect on calcium current (Fig. 7; $n = 10$), suggesting that a pertussis-sensitive G-protein is involved in AII-induced inhibition.

Effect of ω-conotoxin on the AII-induced inhibition. As described previously by Mendelowitz and Kunze (1992), the majority of the high-threshold current (65%), both transient and sustained components, was inhibited by 1 μm ω-conotoxin GVIA. Although most of this current is inhibited quickly (<5 min),
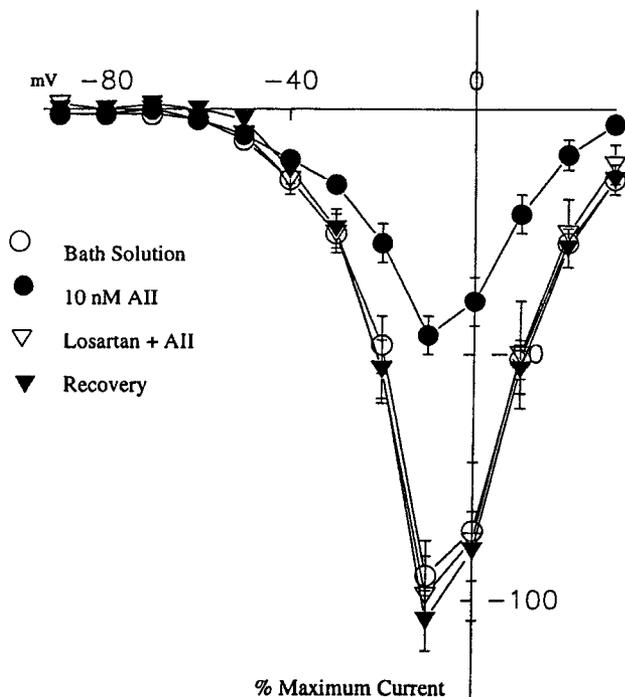
Figure 5. The effect of the AT_1 receptor antagonist losartan on AII-induced inhibition. Coperfusion of 100 nM losartan and 10 nM AII wholly abolished the inhibition of calcium current observed during perfusion of 10 nM AII alone ($n = 10$).

Figure 6. The effect of GTPγS on AII-induced inhibition. In these experiments, nystatin was omitted from the intracellular solution, 100 μM GTPγS was added, and open patch recordings were obtained. Current traces and the current–voltage relationship of a representative cell are shown.
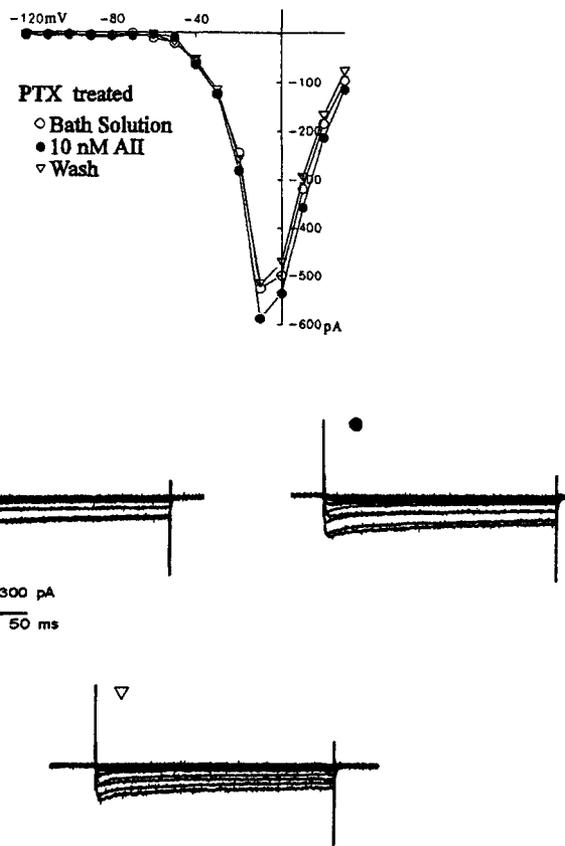

Figure 7. The effect of pertussis toxin (PTX) on AII-induced inhibition. A representative example from a group of nodose cells ($n = 10$) was incubated overnight in media containing PTX (200 ng/ml) while another group (obtained from the same preparation) was incubated in control media.
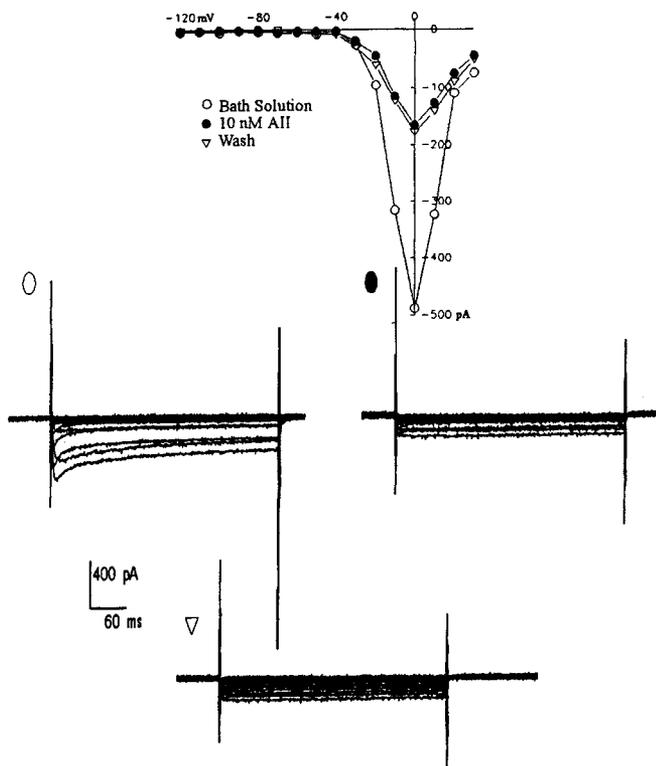
block increases progressively until a stable plateau is reached approximately 15 min after CTX application. In order to ensure that the observed decreases in calcium currents were the result of AII application and not further CTX block, cells were incubated for 15–45 min in 1 μM CTX before study. As expected, a CTX-resistant current was present when the recording was obtained; this current was unaffected by application of AII ($n = 6$; Fig. 8), while matched controls demonstrated an AII-induced inhibition of calcium current (data not shown; 44 ± 7%; $n = 6$). CTX-sensitive channels thus appear to underlie the AII-induced inhibition.

Facilitory effects of AII

Recordings were also obtained from neurons of litters in which the effect of AII was a reversible enhancement of the whole-cell calcium current. The amplitude of the facilitation was highly variable although it was similar among all neurons from the same litter. The composite current–voltage relationship for 10 of these cells is presented in Figure 9. In this set of cells the current increased by 76 ± 11% of the peak control current elicited by a step to −10 mV (Fig. 9). Like inhibition, the facilitation was reversible. The high-threshold current was clearly increased; however, no facilitation of the T-type current was apparent.

The voltage dependence of inactivation was examined in one group of cells that demonstrated facilitation ($n = 4$). As can be seen by the comparison of the inactivation curves in the presence
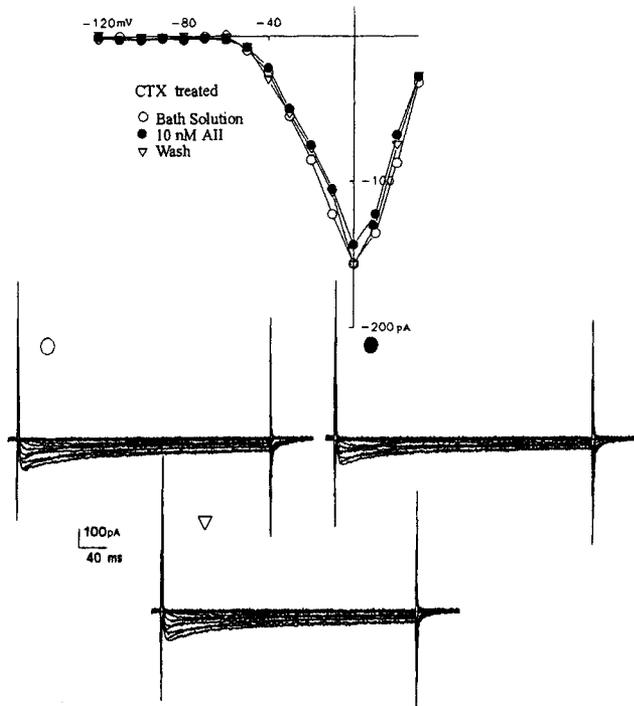
Figure 8. Current–voltage relationship and representative traces from a neuron following incubation in 1 μm CTX; the CTX-resistant current was unaffected by AII (n = 6).

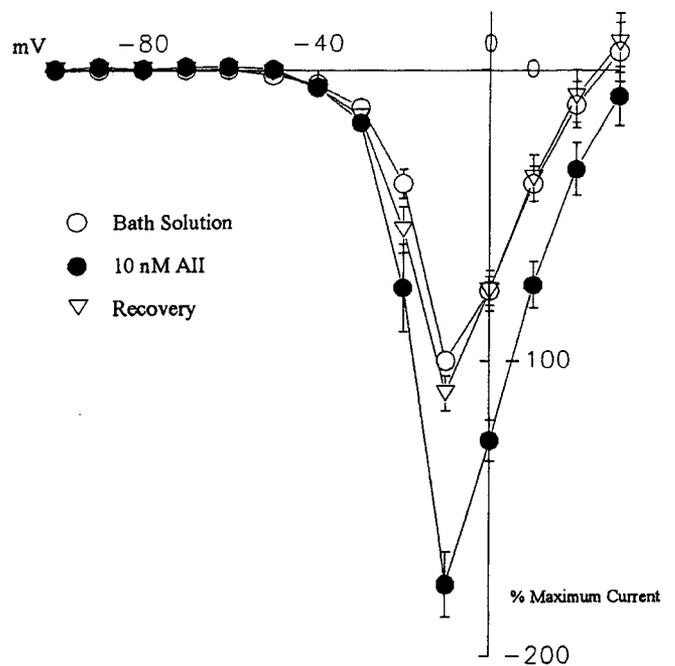

Figure 9. Current–voltage relationship demonstrating the magnitude and reversibility of AII-induced facilitation of calcium current (n = 10). Currents were normalized to the peak current (−10 mV) during control conditions.

and absence of AII (Fig. 10), AII produced no significant change in the voltage dependence of inactivation.

Effect of losartan on the AII-induced facilitation. Unlike inhibition, facilitation of calcium current by AII was not affected by coperfusion with 100 nM losartan (n = 4), as demonstrated in Figure 11. Reperfusion with bath solution, however, caused current to return to baseline levels. In an attempt to determine whether the AT_2 receptor type was responsible for the AII-induced facilitation, both CGP 42112B and PD 123319 were employed. However, even at concentrations below 50 nM, these compounds displayed agonist activity on nodose calcium currents when perfused alone.

Effect of pertussis toxin on the AII-induced facilitation. Nodose cells were divided into pertussis and control groups as described above. The control cells (n = 6) responded to AII with a 21% (±2%) increase in calcium current (data not shown). Pertussis pretreatment did not alter this effect (Fig. 12; 27 ± 4%; n = 6), thus indicating that a pertussis toxin–sensitive G-protein is not associated with the AII-induced facilitation.

Facilitation occurs in the presence of CTX. In four preparations, 10 nM AII was applied after exposure of the cells to 1 μM CTX for 15–30 min. In these cells the AII produced facilitation as illustrated in Figure 13, demonstrating that CTX-sensitive channels were not essential for the facilitory effect on calcium current.

DHP-sensitive channels and the AII-induced facilitation. Previous work had demonstrated, and we confirmed, that 2 μM nimodipine, a dihydropyridine (DHP) antagonist, had little or no effect on calcium current in nodose cells (Mendelowitz and Kunze, 1993). However, application of 1 μM Bay K 8644, a DHP agonist, caused an increase in whole-cell calcium current (n = 6 of 6; Fig. 14) that was abolished by 2 μM nimodipine (not shown). To determine whether AII may recruit the ni-

modipine-inhibitable, Bay K 8644–activatable pool of channels, a current–voltage relationship in the presence of AII was obtained. Following recovery, Bay K 8644 was added to the perfusate and calcium current increased. When AII was added again, this time in the presence of Bay K, there was no additional increase in the current (n = 2; Fig. 14), suggesting that Bay K and AII activate the same pool of channels.

Mixed responses to AII. Further support for the involvement of DHP-sensitive channels came from studies of neurons in which a dual response was present. As shown in Figure 15, perfusion of nimodipine had no effect on the baseline calcium current, and AII alone caused a small increase in calcium current (12 ± 2%; n = 4). However, when AII and nimodipine were perfused together, a reduction (57 ± 5%) in calcium current became apparent. In these neurons, the two mechanisms modulating calcium channel activity had been activated by the peptide and, being of nearly equal magnitude, had produced only a small activation. Once the facilitation pathway was blocked by nimodipine, however, the underlying inhibitory effect was unmasked. This response would be expected if the facilitation was mediated through DHP-sensitive channels.

Discussion

AII affects activity of a wide variety of cell types including vascular smooth muscle, cardiomyocytes, adrenal glomerulosa, astrocytes, and neurons (I. S. Allen et al., 1988; Iredale et al., 1992; McMillian et al., 1992; Pinzani et al., 1992; Saito et al., 1992; Tremblay et al., 1992). Most of the known systemic physiological responses to AII are mediated through the AT_1 receptor via signal transduction pathways involving activation of phosphoinositide hydrolysis or inhibition of adenylate cyclase activity. The physiological functions of AT_2 receptor activation, on the other hand, are for the most part unknown (Inagami and Harris, 1993; Timmermans et al., 1993).
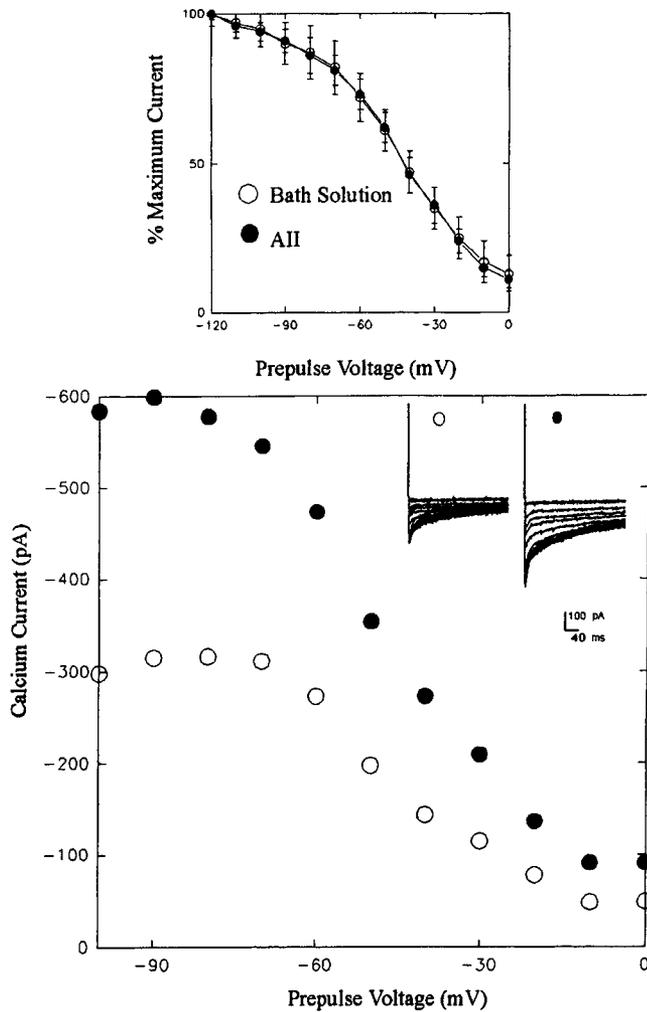
Figure 10. Top, Effect of AII on the voltage dependence of inactivation of whole-cell calcium current in neurons demonstrating AII-induced facilitation. Protocol is same as Figure 4. Currents were normalized to the peak current during the test pulse following a prepulse to −120 mV. Bottom, Current traces and inactivation curve showing effect of AII in an individual neuron.

Figure 11. Current–voltage relationship and representative current traces are compared for control and AII in the absence and presence of the AT_1 receptor antagonist losartan ($n = 4$). The pulse protocol, 300 msec voltage steps from −120 mV to +30 mV, was delivered 1/3 sec.

Direct evidence for AII effects on calcium channels has been obtained in cardiac myocytes, adrenal glomerulosa cells, adrenal medullary chromaffin cells, neurons, and vascular smooth muscle. These effects have been excitatory and are discussed below.

AII-induced inhibition

The concentration–response curve for AII-induced inhibition of calcium current is consistent with binding studies for the AT_1 receptor by Healy et al. (1989). Block of the AII-induced inhibition by losartan confirms the role of the AT_1 receptor. This receptor is widely accepted to be a member of the G-protein receptor family, a notion that is supported by the present findings using GTPγs and pertussis toxin. Of the calcium currents thus far identified in nodose neurons, the inhibition appeared to be restricted to the CTX-sensitive channel.

Many other neuromodulators also inhibit neuronal calcium currents through PTX-sensitive G-proteins (Hescheler et al., 1987; Dolphin and Scott, 1989; Crain and Shen, 1990; Wiley et al., 1990) and several inhibitors have been shown to act specifically on CTX-sensitive channels (Cox and Dunlap, 1992;
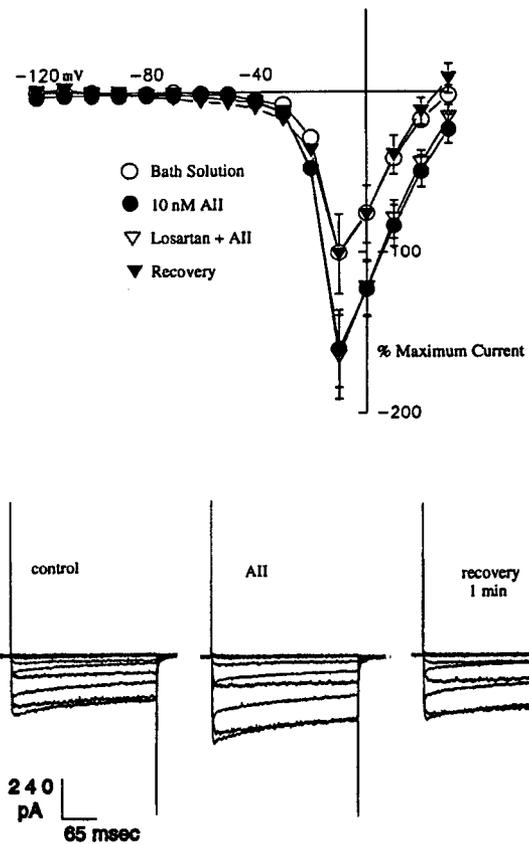
Mogul et al., 1993; Yawo and Chuhma, 1993), as in the present study. Thus, the AII-induced inhibition of nodose cell calcium current appears to be a member of a large family of similar systems.

AII-induced facilitation

AII-induced facilitation was seen with less frequency than inhibition. It is clear that the losartan-sensitive AT_1 receptor is not involved in the facilitation, but further identification could not be confirmed due to the partial agonist effects of the two AT_2-specific antagonists. Chaki and Inagami (1992) have recently identified an AII receptor in a neuroblastoma cell line that is not blocked by AT_1 or AT_2 antagonists. Thus, the possible alternatives to the AT_1 receptor are not limited to AT_2 receptors.

The reported effects of AII on calcium channels have been excitatory and not uniformly on the same channel type. In frog atrial myocytes the low-threshold calcium current is increased while a DHP-sensitive high-threshold current is unaffected (Bonvallet and Rougier, 1989). Cohen et al. (1988) also report an increase in low-threshold calcium current in adrenal glomerulosa cells. In contrast, in neonatal rat cardiac myocytes and in portal vein smooth muscle the high-threshold DHP-sensitive current is stimulated by AII (I. S. Allen et al., 1988). In the present study the DHP-sensitive calcium channel was shown to underlie an AII-induced facilitation and an effect on low-threshold channels was not evident. However, this latter current is absent in approximately 50% of the cells, is very small in the
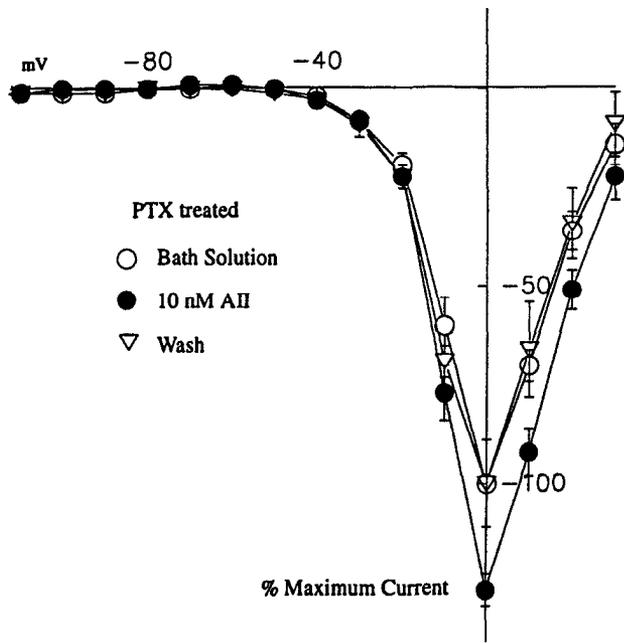
Figure 12. The effect of overnight incubation with pertussis toxin (200 ng/ml) on AII-induced facilitation. The control cells responded to AII with a 21% increase in calcium current (not shown). PTX did not alter the facilitation (27%; *n* = 6).

Figure 14. Current–voltage relationships of a representative neuron (*n* = 2) showing the lack of effect of AII (10 nM) in the presence of 1 μM Bay K 8644. The pulse protocol described in Figure 11 was used to activate calcium current in the following sequence: in the presence of AII (●), after removal of AII (O), upon subsequent addition of Bay K (♦), followed by Bay K and AII (▲).

remainder, and therefore is difficult to evaluate. The activation of calcium current by AII in the nodose neurons resembles that in cardiac myocytes where both the early and late components of DHP current are stimulated (I. S. Allen et al., 1988). In further comparisons, the myocyte study showed only a very small shift (+2.6 mV) in the inactivation curve with AII; our study was similar, with no change in voltage dependence of inactivation.

The DHP-sensitive channel is well characterized in other neuronal systems and has been reported to exist in a "reserve pool"
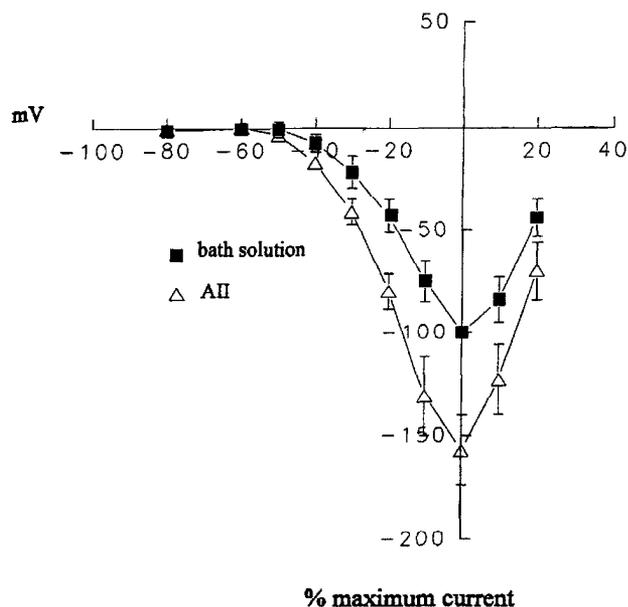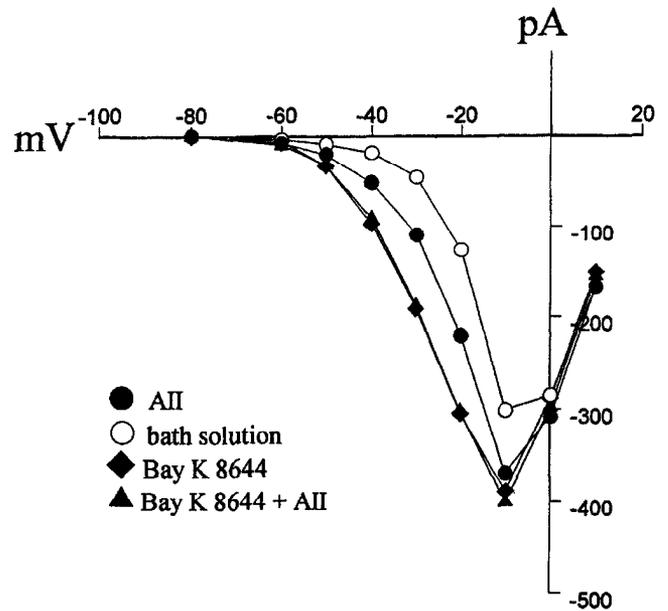
(Artalejo et al., 1990), as is proposed in this study. When activated by Bay K 8644 or AII (or perhaps other factors as well), these reserve channels can then contribute markedly to the whole-cell calcium current.

Dual nature of AII-induced effects

What determines the relative expression of the two types of calcium current responses? The nodose ganglion is itself a het-

Figure 13. Current–voltage relationship in the presence and absence of 10 nM AII for cells that had been incubated in 1 μM CTX for 15–30 min (*n* = 4).
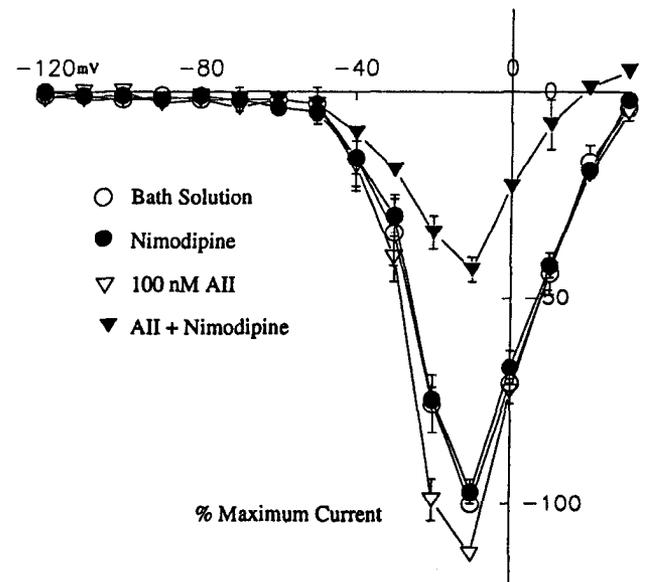

Figure 15. Dual effects of AII. Perfusion with 2 μM nimodipine alone had no effect on whole-cell calcium current, while a small change in calcium current was seen upon perfusion with AII alone. Subsequent perfusion of AII and nimodipine together, however, eliminated a facilitation and caused a reduction in calcium current (*n* = 4).

erogeneous structure representing many different sensory modalities. Cell-to-cell variation was therefore anticipated, but, surprisingly, the variation was between cells prepared from different litters and not among cells of the same litter. One possibility to account for the presence of the facilitory versus inhibitory response was a difference in the relative expression of calcium channel types. For instance, cells without a reserve pool of DHP-sensitive channels would not show a facilitation of calcium current. Experiments using Bay K 8644 to activate DHP-sensitive channels indicated that these channels were present even in cells which exhibited no facilitation. Furthermore, CTX-sensitive channels, substrate for the inhibitory effect, were always present. The variation is therefore more likely due to changes in expression of types of AII receptors or in the systems that couple the receptors to ion channels. Changes in the expression of AII receptors with development have been reported (Tsutsumi and Saavedra, 1991). Since the data presented in this report were obtained from neonatal animals, a comparison with the responses of fetal and adult nodose neurons may provide an answer to the question of differential expression.

Functional role of AII receptors in the NTS

Casto and Phillips (1986) and Kubo and Kihara (1990) reported that microinjection into the NTS of high concentrations of AII (>100 ng) causes an increase in blood pressure, while application of lower concentrations leads to depressor effects. The resultant U-shaped dose–response curve may be due to the dual effects of AII reported in the present study. In a preliminary report a single concentration of AII was shown to increase the amplitude of the excitatory postsynaptic potential of NTS neurons responding to stimulation of the afferent tract containing the nodose axons. The postsynaptic neuron was unaffected (Yang and Andresen, 1991). This suggests that AII receptors are located at the presynaptic terminal although it does not indicate which of the AII receptor subtype(s) is present.

In summary, the data presented here indicate AII-induced inhibition and facilitation are mediated through different receptors, different cellular pathways, and different ion channels. The inhibitory effect involves AT_1 receptors, PTX-sensitive G-proteins, and CTX-sensitive calcium channels, while the facilitation utilizes non-AT_1 receptors, a PTX-insensitive coupling system, and a reserve pool of DHP-sensitive channels.

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