IL-1β directly excites isolated rat supraoptic neurons via upregulation of the osmosensory cation current

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Chakfe, Yassar, Zizhen Zhang and Charles W. Bourque. IL-1β directly excites isolated rat supraoptic neurons via upregulation of the osmosensory cation current. Am J Physiol Regul Integr Comp Physiol 290: R1183–R1190, 2006. First published November 17, 2005; doi:10.1152/ajpregu.00716.2005.—Previous studies have shown that IL-1β can excite the magnocellular neurosecretory cells (MNCs) of the hypothalamus. However, it is not known whether IL-1β can have direct IL-1 receptor type 1 (IL-1R1)-mediated effects on MNCs, and little is known about the cellular mechanisms by which IL-1β influences electrical activity in these cells. Here, we used patch-clamp recordings to examine the effects of IL-1β on acutely isolated rat MNCs. We found that IL-1β directly excites MNCs in a dose-dependent manner and that this response can be blocked by an inhibitor of the IL-1R1. Voltage-clamp analysis of the current evoked by IL-1β revealed a linear current-voltage relationship between −90 and −20 mV, and a reversal potential near −35 mV. This value was not affected by reducing the concentration of chloride ions in the external solution, indicating the involvement of a nonselective cation conductance. The effects of IL-1β were inhibited by Na-salicylate, an inhibitor of cyclooxygenase. Moreover, the effects of IL-1β were mimicked and occluded by PGE2, and were inhibited by AH-23848, an inhibitor of cyclooxygenase-2 (COX-2) activity (15), thereby increasing the synthesis and release of PGs. Moreover, whole cell patch-clamp recordings have shown that both IL-1β and PGs produced either by adjacent cells (i.e., via a paracrine effect), or by the MNCs themselves (i.e., via an autocrine effect) depolarize MNCs through the activation of a nonselective cation conductance that underlies osmoreception.

vasopressin; prostaglandin; EP4 receptor; osmosensitivity; neurohypophysis

RELEASE OF THE INFLAMMATORY cytokine IL-1β into the circulation is believed to recruit central neuronal responses that mediate host defense through a cascade of events that is initially triggered in brain vascular cells. Specifically, IL-1β binding to the IL-1 receptor type 1 (IL-1R1) expressed in vascular cells is believed to induce the expression of cyclooxygenase-2 (COX-2) activity (15), thereby increasing the synthesis and release of PGs. PGs can then diffuse across the blood-brain barrier to induce neuronal responses (e.g., 25). However, previous studies have shown that IL-1β is also released within the brain parenchyma during systemic infection (e.g., 23, 33) and in response to traumatic injury (e.g., 19) or ischemia (e.g., 6). During systemic infection, high levels of IL-1β are observed predominantly in the hypothalamus where, together with vascular IL-1β, they may initiate host defense responses through alterations in behavioral and neuroendocrine function. For example, hypothalamic IL-1β has been implicated in the mediation of sickness behavior (2), loss of appetite (17), fever (9), and activation of the hypothalamo-pituitary-adrenal axis (32) and in the release of oxytocin (29) and vasopressin (16, 31, 36) from the neurohypophysis. The pressor and antidiuretic effects associated with vasopressin release are particularly important during the early phase of septic shock, because they may help maintain tissue perfusion under conditions where powerful vasodilatory substances are being released (13).

Recent studies have provided important insight into the cellular mechanisms by which IL-1β may increase vasopressin secretion from the neurohypophysis. Indeed, recordings from hypothalamic slices have shown that IL-1β can depolarize magnocellular neurosecretory cells (MNCs) in the supraoptic (18) and paraventricular nuclei (10). The depolarizing effects of IL-1β observed in these studies were insensitive to tetrodotoxin and were blocked by inhibitors of COX, suggesting that they were mediated postsynaptically, through a mechanism involving the production of PGs. Moreover, whole cell patch-clamp recordings have shown that both IL-1β (10) and PGs (10, 28, 30) depolarize MNCs through the activation of a nonselective cation conductance. Taken together, these findings support the hypothesis that IL-1β may induce vasopressin release by exciting MNCs through a mechanism that involves the production of PGs (18). However, the identity of the cells responsible for detecting IL-1β and producing PGs remains unknown. Indeed, because PGs diffuse freely across cell membranes (9, 26), their release is regulated primarily by the rate of synthesis rather than by action potential-dependent exocytosis. Thus the effects of bath-applied IL-1β observed on MNCs in the presence of tetrodotoxin in brain slices could have involved PGs produced either by adjacent cells (i.e., via a paracrine effect), or by the MNCs themselves (i.e., via an autocrine effect). Although two immunocytochemical studies have shown that the IL-1R1 receptor responsible for mediating the

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effects of IL-1β is expressed in hypothalamic nuclei containing MNCs (8, 15), these studies have provided contradictory results concerning the presence of such receptors on the somata of MNCs. In this study, therefore, we examined the effects of IL-1β on acutely isolated MNCs, under experimental conditions that eliminate the involvement of potential paracrine effects.

EXPERIMENTAL PROCEDURES

Isolation of MNC somata. MNC somata were isolated from brains of male Long-Evans rats (100–200 g) killed by decapitation as previously described (5, 35) and in accordance with a protocol approved by the Animal Care Committee of McGill University. Briefly, coronal slices of hypothalamus (about 1-mm thick) were obtained and blocks (~1 mm³) containing the supraoptic nucleus were removed with the use of iridectomy scissors. Tissue blocks were incubated for 90 min at 33°C in 10 ml of an oxygenated (100% O₂; pH 6.9–7.0) PIPES saline comprising (in mM): 120 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 20 PIPES (disodium salt), 25 d-glucose, as well as 0.7 mg/ml trypsin (type XI; Sigma, St. Louis, MO). Blocks were subsequently washed in trypsin-free oxygenated PIPES saline (pH 7.3–7.4) and kept (<8 h) until use. When required, blocks were triturated with fire-polished Pasteur pipettes (0.2–0.5 mm ID), and the suspension was plated onto 35-mm petri dishes (Corning Costar, Cambridge, MA).

Whole cell recording. Dishes were mounted onto the stage of an inverted-phase contrast microscope and perfused (2 ml/min) with a HEPES saline solution (22–24°C; pH 7.35) comprising (in mM): 120 NaCl, 4 NaOH, 3 KCl, 1 MgCl₂, 10 HEPES, 1 CaCl₂, and 1.6 EGTA. Membrane voltage (dc, 5 kHz) and current (dc, 200 Hz) were recorded through an Axopatch 1D amplifier (Axon Instruments, Union City, CA) and captured using a Digidata 1200B interface driven by Clampex 8 software (Axon Instruments).

Production and measurement of changes in cell volume. In some experiments, we stimulated the stretch-inhibited cation (SIC) channels in MNCs by reducing cell volume via the application of negative pressure (~100 mmHg) via the recording pipette. The amplitude of the suction stimulus was controlled by an air-filled syringe connected to the tubing via a T-junction. Volume changes (%V) were monitored by digital imaging and morphometry, the maximal cross-sectional area (CSA; in pixels) was determined by the software. All windows version 4.02 (Scion, Frederick, MD). The maximal cross-sectional area (CSA; in pixels) was determined by the software. All windows version 4.02 (Scion, Frederick, MD). The maximal cross-sectional area (CSA; in pixels) was determined by the software. All windows version 4.02 (Scion, Frederick, MD). The maximal cross-sectional area (CSA; in pixels) was determined by the software. All windows version 4.02 (Scion, Frederick, MD). The maximal cross-sectional area (CSA; in pixels) was determined by the software. All windows version 4.02 (Scion, Frederick, MD).

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Action of IL-1β on isolated MNCs. To define any direct effect of IL-1β on MNCs, we performed whole cell voltage-clamp recordings from neurons acutely isolated from the supraoptic nucleus of adult rats. Moreover, to avoid the possibility that substances released from other types of cells present in the dish might induce indirect paracrine effects, the fluid perfusion device was positioned so that no other cell was located upstream of that being recorded (~300 μm away) and no other cell was present upstream of the cell being tested, thereby excluding the possibility of paracrine effects. During direct cell perfusion experiments, complete exchange of the solution perfusing the cell was achieved in less than 20 ms.

Statistics. Values are reported as means ± SE. Comparisons between groups were made using the Student’s t-test, the paired t-test, or a Mann-Whitney rank sum test, as appropriate. One-way ANOVA (Sigmastat 2.1; Jandel, San Rafael, CA) was performed for the comparison of multiple groups. Where differences were found, Student-Newman-Keuls test for multiple comparisons was performed post hoc to identify specific distinctions. Differences between means were considered significant when P < 0.05. Fitting of the three-parameter logistic equation through dose-response data points was performed using Sigmaplot 8 (SPSS Science, Chicago, IL).

RESULTS

Actions of IL-1β on isolated MNCs. To define any direct effect of IL-1β on MNCs, we performed whole cell voltage-clamp recordings from neurons acutely isolated from the supraoptic nucleus of adult rats. Moreover, to avoid the possibility that substances released from other types of cells present in the dish might induce indirect paracrine effects, the fluid perfusion device was positioned so that no other cell was located upstream of that being recorded (see EXPERIMENTAL PROCEDURES). As illustrated in Fig. 1A, sudden exposure (delay <20 ms) to a sustained concentration of IL-1β induced the appearance of a slowly developing inward current. During these experiments, membrane conductance was measured from the amplitude of current responses to 500-ms hyperpolarizing steps (~40 mV) applied every 5 s. As shown in Fig. 1A, the inward current induced by IL-1β was accompanied by a progressive and reversible increase in membrane conductance.
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For each cell tested, we quantified the increase in conductance induced by a specific concentration of IL-1β by subtracting the baseline conductance of the cell from that observed 120 s following the onset of the application. As plotted in Fig. 1B, different concentrations of IL-1β induced a dose-dependent increase in membrane conductance, with a half-maximal effect (EC50) at 15 ± 13 nM and an apparent maximum change of +1.3 nS (r2 = 0.80). All cells tested with a concentration of IL-1β > 20 pM (n = 40) showed positive responses to the drug.

We next examined the effects of IL-1β on isolated MNCs tested under current clamp conditions. Because no differences were noted in the time course and amplitude of responses evoked by direct cell perfusion or bath application of IL-1β under voltage-clamp (data not shown), the latter (simpler) approach was used in these experiments. As illustrated in Fig. 2A, depolarizing and excitatory responses evoked by concentrations of 40–200 pM reached a peak within 30–60 s and recovered within 0.5–5 min following washout. The excitatory effects of IL-1β repeated 2–3 times in the same cell following complete washout. Higher concentrations (>40 nM) produced depolarizing effects that generally did not reverse for the remainder of most recordings (10–30 min). In contrast, applications of IL-1β at concentrations of 0.04–4 pM were ineffective (n = 6; Fig. 2A), in agreement with the lack of significant effects of the drug on membrane conductance under voltage clamp (Fig. 1B). Finally, to confirm the specificity of these effects, we examined the actions of IL-1β in the presence of recombinant human IL-1R1 antagonist (IL-1ra). In these experiments, IL-1ra was applied 40–60 s before IL-1β, and the drug remained present while the agonist was being applied. When present at a concentration of 160 pM, IL-1ra blocked the responses in only two of six cells tested with IL-1β (400 pM; data not shown). In contrast, when applied at a concentration of 1.2 nM, IL-1ra blocked the excitatory actions of IL-1β in 7 of 10 cells tested. In the three cells that showed residual effects of IL-1β in the presence of IL-1ra, the depolarizing responses were smaller (1.5 ± 0.4 mV) than those observed in the absence of antagonist (2.3 ± 0.1 mV; n = 11). These results suggest that the excitatory effects of IL-1β on isolated MNCs are mediated specifically by IL-1R1.

**Ionic basis of IL-1β actions in MNCs.** The ionic basis of the IL-1β-induced excitation was next examined under voltage clamp. Steady-state current-voltage analysis (Fig. 3A) revealed that the ionic current evoked by IL-1β had a linear slope between −90 and −20 mV and an average reversal potential (Erev) of −36 ± 2 mV (n = 9). Reducing the concentration of chloride ions in the external solution from 127 to 7 mM did not significantly affect the Erev of the current (−33 ± 1 mV; n = 4; P > 0.05; see Fig. 3B), indicating that the channels modulated by IL-1β are not permeable to anions. Because no other anions are available to mediate an outward current, and because the value of Erev does not correspond to the equilibrium potential for any of the particular cations in solution, we conclude that the IL-1β-induced excitation is mediated through the activation of voltage-independent nonselective cation channels.

**Effects of IL-1β on isolated MNCs require intrinsic COX activity.** Previous studies of MNCs in hypothalamic slices (10, 18) have indicated that the excitatory actions of IL-1β in that preparation could be inhibited by Na-salicylate, a general inhibitor of COX. We therefore examined whether Na-salicyl-
late could block IL-1β-mediated responses in isolated MNCs. In these experiments, 100 μM salicylate was included in all extracellular solutions (including a 15- to 30-min pretreatment) and in solution that filled the recording pipette. Moreover, to exclude the possibility of paracrine effects, drugs were applied using direct perfusion with no other cell lying upstream of the cell being tested. As illustrated in Fig. 4, the progressive increase in membrane conductance evoked by application of 20 nM IL-1β was dramatically reduced in the presence of salicylate (Fig. 4A). Indeed, the conductance increase measured 120 s following the onset of the application was significantly lower in the presence of salicylate (0.11 ± 0.05 nS, n = 7) compared with that evoked under control conditions (0.56 ± 0.07 nS; n = 16; P = 0.005, Fig. 4B).

**Effects of IL-1β involve autocrine production of PGs and EP4 receptors.** Previous studies have shown that PGE2 can excite MNCs via the activation of a nonselective cation conductance (10, 30) and that this effect is mediated specifically by the activation of postsynaptic EP4 receptors (28). We therefore examined whether the effects of IL-1β are due to an autocrine production of PGs by MNCs. Application of 1 μM PGE2 for 120 s under conditions preventing paracrine actions resulted in the activation of a nonselective cation current reversing near −35 mV (n = 3; data not shown). As illustrated in Fig. 5, the increase in membrane conductance induced by PGE2 (1 μM) was significantly inhibited when tested in the presence of 10 μM of the EP4 receptor antagonist AH-23848 (0.11 ± 0.08 nS, n = 8 vs. 0.47 ± 0.09 nS, n = 13 in PGE2 control; P = 0.014). Moreover, AH-23848 significantly inhibited responses induced by direct perfusion of 20 nM IL-1β (0.04 ± 0.05 nS, n = 6 vs. 0.56 ± 0.07 nS, n = 16 in IL-1β control; P = 0.003). Finally, when both IL-1β (20 nM) and PGE2 (1 μM) were applied to MNCs, the effect of IL-1β was occluded by PGE2 (P = 0.227, one-way ANOVA among groups: IL-1β, PGE2, and IL-1β+PGE2; Fig. 5).

**IL-1β stimulates the osmosensory conductance in MNCs.** The cation permeability and voltage independence of the current stimulated by IL-1β and PGE2 are similar to those of the

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**Fig. 3.** Current-voltage (I-V) analysis of the effects of IL-1β in MNCs. A, left: steady-state I-V relations obtained by applying a slow (15 mV/s) voltage ramp in the absence (control) and presence of 400 nM IL-1β; right: I-V relation of the IL-1β-evoked ionic current obtained by subtracting the steady-state I-V relations shown on left. Note that the current is voltage insensitive and that the reversal potential (E_{reversal}) lies near −35 mV. B: I-V analysis of the IL-1β-evoked current recorded in the presence of a low Cl− extracellular solution. Note the similarity of the steady-state (left) and subtracted (right) I-V relations recorded in low Cl− concentration ([Cl−]) solution (7 mM) compared with those recorded in normal (127 mM) [Cl−] solution (A).

**Fig. 4.** IL-1β effects are blocked by salicylate. A: graph plots the average time course of mean ± SE changes in membrane conductance observed on adding 20 nM IL-1β (bar) to the bath in the presence (open circles) and absence (control, solid circles) of 100 μM Na-salicylate. In these experiments, 100 μM salicylate was included in both the external and internal (i.e., recording pipette) solutions. Membrane conductance was monitored from the current response to a −40 mV voltage step applied every 5 s (V_{hold} = −66 mV). Conductance changes in each cell were calculated by subtracting the average of all control values for that cell from conductance values measured at each time point. B: bar histograms show means ± SE conductance changes observed 2 min following the onset of exposure to IL-1β for the two groups of cells. *P < 0.05.
osmosensory conductance that contributes to osmoreception (20–22, 34) and Na⁺ detection in MNCs (35). Moreover, previous studies have shown that the neuropeptides ANG II, cholecystokinin, and neurotensin can excite these cells through an upregulation of the osmosensory cation conductance and that the depolarizing effects of these peptides are magnified when elicited under conditions that increase the basal probability of opening of the osmosensing SIC channels (5). We therefore examined the effects of enhancing the background osmosensory current on the amplitude of the response evoked by IL-1β. As illustrated in Fig. 6, A and B, the current induced by IL-1β was significantly enhanced when the volume of the cell was reduced (~15%) by application of negative pressure (-100 mmHg) to the recording pipette (-143 ± 21 pA in the presence of suction vs. −37 ± 5 pA in control; P < 0.05, paired t-test; n = 3). Previous studies have also shown that the osmosensory conductance of MNCs is blocked by Gd³⁺ with an IC₅₀ near 35 μM (22). We therefore examined the effects of Gd³⁺ on responses evoked by IL-1β in 4 MNCs. As shown in Fig. 6, C and D, bath application of 100 μM Gd³⁺ quickly eliminated the inward current evoked by IL-1β, as well as the basal inward current that was present at rest. Thus, following the addition of IL-1β, membrane conductance increased from 1.08 ± 0.08 nS to 1.96 ± 0.26 nS (P < 0.05), reflecting the upregulation of osmosensory conductance. On addition of Gd³⁺, membrane conductance decreased to 0.50 ± 0.17 nS, (n = 4; P < 0.05; one-way ANOVA), consistent with a suppression of both evoked and basal SIC conductance (5, 20–22, 35).

IL-1β does not affect the volume or mechanosensitivity of MNCs. Because the SIC conductance of MNCs is mechanically activated during decreases in cell volume, the upregulation of this conductance in the presence of IL-1β might be due to a drug-evoked decrease in cell volume. However, application of 20 nM IL-1β alone did not provoke significant changes in cell volume (volume at 120 s was 99.98 ± 0.04% of control; P = 0.97; n = 7). A previous study has indicated that the osmoreponsiveness of the neurohypophyseal axis is increased during endotoxemia (12). Thus it is possible that the intrinsic osmosensitivity of MNCs is increased in the presence of IL-1β, even if the cytokine does not itself provoke a decrease in cell volume. Because the osmosensitivity of MNCs is due to the mechanical regulation of the SIC conductance during osmotically evoked changes in cell volume, we examined whether IL-1β could increase the mechanosensitivity of MNCs. Cells were maintained at a holding potential of −66 mV, and hyperpolarizing steps to −106 mV were applied every 5 s to monitor membrane conductance. Images were captured simultaneously to monitor changes in cell volume. As illustrated in Fig. 7A, reducing pipette pressure for 60 s caused a progressive decrease in cell volume accompanied by the emergence of an inward current transient (Vhold = −66 mV) that reflects the activation of SIC channels. Large vertical transients are current responses caused by the voltage ramps used for I-V analysis (not shown). B: means ± SE amplitude of the IL-1β evoked current recorded at −66 mV in the absence (before suction) and presence (after suction) of IL-1β (100 nM) (n = 4; *P < 0.05; paired Student’s t-test). C: current trace showing that the inward current evoked by IL-1β (bar) can be blocked by bath application of Gd³⁺ (open bar). The net outward current observed in Gd³⁺ compared with control baseline (dashed line) is due to the blockade of all SIC channels, including those already open at rest. D: means ± SE membrane conductance recorded in the presence of IL-1β before (IL-1β; n = 4) and after (IL-1β + Gd) addition of 100 μM Gd³⁺ to the bath. *P < 0.05 (one-way ANOVA).
inward current and an increase in membrane conductance. This experiment was repeated in eight MNCs under control conditions, and in eight MNCs exposed to 20 nM IL-1β. As shown in Fig. 7B, no significant differences were noted in the mean amplitudes of the suction-evoked volume decrease (−16.3 ± 0.9% control vs. −14.3 ± 0.9% IL-1β; P = 0.141) or in the conductance increase (+0.42 ± 0.17 nS control vs. +0.32 ± 0.20 nS IL-1β; P = 0.72), observed in the two groups of cells. Moreover, the mean MI (conductance change/normalized volume change) was not different in control (2.6 ± 1.1 pS/nΔV) and IL-1β-treated cells (2.3 ± 1.5 pS/nΔV; P = 0.61).

**DISCUSSION**

Hypothalamic expression of IL-1β increases rapidly following systemic infection (23, 26, 33), and the central and peripheral release of IL-1β that occurs under these conditions is believed to affect neuronal activity in a manner that orchestrates the behavioral and neuroendocrine responses that contribute to host defense (e.g., 1, 2, 9, 13, 16, 17, 25, 32). The release of vasopressin from the neurohypophysis, in particular, is an important adaptive response that contributes to the maintenance of tissue perfusion during the early stage of septic shock (13), and much evidence supports the hypothesis that IL-1β may contribute to the increase in vasopressin release that occurs under such conditions. Indeed, intracerebroventricular infusions of IL-1β provoke the release of vasopressin in vivo (16, 31, 36), and bath application of IL-1β can stimulate vasopressin release from hypothalamic explants in vitro (37). Moreover, bath application of exogenous IL-1β has been shown to excite MNCs in hypothalamic slices (10), a response that is not blocked by tetrodotoxin (10, 18) and that appears to be mediated through the activation of a nonselective cation conductance (10). Thus IL-1β may provoke vasopressin release by increasing the electrical activity of MNCs through effects that take place on or near the somata of these hypothalamic neurons. Interestingly, the excitatory effects of IL-1β on MNCs in hypothalamic slices are antagonized by COX inhibitors (10, 18), indicating that synthesis and release of PGs is required to mediate the effects of IL-1R1 activation. Further support for this hypothesis has come from the observation that EP4 receptors are expressed in the supraoptic nucleus (38) and that PGE2 can excite acutely isolated MNCs by increasing a nonselective cation conductance (30) upon activation of postsynaptic EP4 receptors (28). Although these observations suggest that IL-1R1-mediated PG release may excite MNCs through an effect involving postsynaptic EP4 receptors, they do not indicate whether MNCs respond directly to IL-1β or whether the effects of the cytokine are mediated by PGs released from neighboring IL-1R1 expressing cells. Indeed, the observation that tetrodotoxin does not prevent the depolarizing effects of IL-1β on MNCs in slices (10, 18) indicates that the effects of the cytokine are not mediated by activity-dependent exocytotic release of another transmitter. However, it does not exclude the possibility that a diffusible agent, such as PGE2, might be released from other cells and excite MNCs through a paracrine action.

**MNCs are directly excited by IL-1β.** Application of IL-1β caused a potent excitation of MNCs, an effect that could be blocked by IL-1ra, an antagonist of the IL-1R1 (1, 26). This response was mediated via the intrinsic activation of a nonselective cation conductance under conditions excluding the possibility of paracrine effects, indicating that individual MNCs contain all of the cellular machinery required to transduce the effects of IL-1β. Although we did not identify the neurons recorded as vasopressin- or oxytocin-containing in our experiments, these cells respectively account for 60 and 40% of the MNCs isolated by our procedure (11). Because all cells tested with suprathereshold (>20 pM) concentrations of IL-1β were responsive to the drug, it is likely that both oxytocin- and vasopressin-releasing MNCs express IL-1R1, as well as other signaling elements required for cellular activation. Indeed, previous studies have indicated that IL-1β can modulate the release of both oxytocin and vasopressin (29).

**Effects of IL-1β involve an EP4 receptor-mediated autocrine effect.** The effects of IL-1β observed during direct perfusion experiments were blocked in the presence of sodium salicylate, confirming a requirement for COX activity and PG synthesis in the genesis of the IL-1β response. Because the possibility of a
Effects of IL-1 can be blocked by prior activation of such receptors. Indeed, the studies have shown that PGE2 can also excite MNCs through a direct IL-1R1 activation in MNCs, but do not rule out the if PG synthesis and EP4 receptors act as intermediates in the IL-1R1-mediated PGE2 release by MNC somata can retrogradely modulate synaptic transmission remains to be determined.

**IL-1B upregulates the osmosensory conductance of MNCs.** In a recent report, Grinevich et al. (12) showed that intraperitoneal injection of the bacterial endotoxin lipopolysaccharide causes an increase in vasopressin release that is associated with an increase in the osmoreponsiveness of the hypothalamo-neurohypophyseal system. Because the effects of lipopolysaccharide are mediated, in part, by central actions of IL-1B (e.g., 9), it is reasonable to hypothesize that the effects of IL-1B might be mediated via actions on one of the mechanisms involved in the osmotic regulation of MNCs (14, 34). Voltage-clamp analysis showed that the excitatory effects of IL-1B on isolated MNCs were due to the activation of a Gd3+-sensitive, voltage-insensitive, nonselective cation current that reverses near −35 mV. These properties are similar to those of the SIC channels that contribute to osmoreception (20–22) and sodium detection (35) in MNCs. Moreover, the amplitude of the current evoked by IL-1B was enhanced under conditions that increase the opening probability of SIC channels, as previously shown for ANG II, cholecystokinin, and neurotensin (5). Because these channels are mechanically regulated, we also explored the possibility that SIC conductance activation might have been due to an IL-1B-mediated decrease in cell volume or to a change in mechanosensitivity. Our data showed that IL-1B affects neither the volume nor mechanosensitivity of MNCs. Although our assessment of mechanosensitivity was based on a stimulus that yielded a maximal change in volume of about −15%, it is important to note that the MI computed by our method also applied to the smallest volume changes detectable (see Experimental Procedures). Thus IL-1B is unlikely to affect the activation of SIC channels by physiologically relevant hypertonic stimuli. Although additional studies will be required to define the mechanism by which IL-1B and PGE2 activate the nonselective cation current in MNCs, our observations suggest that this response is mediated specifically by the activation of SIC channels.

**Possible functional role of intrinsic IL-1R1 activation in MNCs.** Although the mechanical coupling between changes in cell volume and SIC channel activity is not altered by IL-1β, the activation of the SIC conductance during IL-1R1 activation will mediate an inward current and, consequently, a depolarization of the membrane potential (10, 18). This effect will promote an increase in the rate of action potential discharge from MNCs and thus enhance the release of vasopressin from axon terminals in the neurohypophysis (24). Moreover, by increasing the overall fraction of excitatory postsynaptic potentials that cross spike threshold, the IL-1β-mediated depolarization might increase the excitatory impact of the glutamatergic projection from osmosensitive organum vasculosum lamina terminalis neurons to MNCs in the supraoptic nucleus and thereby the osmoreponsiveness of the neurohypophysial system (3, 34). Further work will be required to confirm this hypothesis.

In conclusion, our experiments reveal that neurons in the supraoptic nucleus, like those in the subfornical organ (7), appear to express all of the molecular machinery required for IL-1R1-mediated transduction of depolarizing responses to IL-1β. In particular, our results show that the excitatory effects of this cytokine require the COX-dependent synthesis of a PG, and an upregulation of nonselctive cation conductance that depends on the autocrine activation of EP4 receptors. The mechanism by which PG upregulates the cation conductance of MNCs remains to be determined.

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