Published in final edited form as:

J Neuroendocrinol. 2008 November 1; 20(11): 1224–1232. doi:10.1111/j.1365-2826.2008.01783.x.

Interleukin-1 β Release in the SON Area during Osmotic Stimulation Requires Neural Function[‡]

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Abstract

Interleukin-1 β (IL-1 β) is present throughout the magnocellular neuroendocrine system and co-depletes with oxytocin and vasopressin from the neural lobe during salt-loading. To examine if IL-1 β is released from the dendrites/soma of magnocellular neurons during osmotic stimulation, microdialysis adjacent to the supraoptic nucleus (SON) in conscious rats was combined with immunocapillary electrophoresis and laser-induced fluorescence detection to quantify cytokine in 5 min dialysates collected before (0-180 min; basal), and after (180-240 min), hypertonic saline injected s.c. (1.5 M NaCl). Osmotic release of IL-1 β was compared after inhibiting local voltage-gated channels for Na⁺ (tetrodotoxin; TTX) and Ca²⁺ (cadmium and nickel) or by reducing intracellular Ca²⁺ stores (thapsigargin). Immunohistochemistry combined with microdialysis was used to localize cytokine sources (IL-1 β ⁺) and microglia (OX-42⁺). Under conditions of microdialysis, basal release of IL-1 β ⁺ in the SON area was measurable and stable (pg/ml; mean $\pm$ SEM) from 0-60 min (2.2 $\pm$ 0.06), 60-120 min (2.32 $\pm$ 0.05) and 120-180 min (2.33 $\pm$ 0.06), likely originating locally from activated microglia (OX42⁺; IL-1 β ⁺; ameboid, hypertrophied) and magnocellular neurons expressing IL-1 β . In response to osmotic stimulation, IL-1 β increased progressively in dialysates of the SON area by a mechanism dependent on intracellular Ca²⁺ stores sensitive to thapsigargin and, similar to dendritic secretion of oxytocin and vasopressin, required local voltage-gated Na⁺ and Ca²⁺ channels for activation by osmoregulatory pathways from the forebrain. During osmotic stimulation, neurally dependent release of IL-1 β in the SON area likely upregulates osmosensitive cation currents on magnocellular neurons (observed *in vitro* by others), to facilitate dendritic release of neurohypophysial hormones.

Keywords

magnocellular system; microdialysis; oxytocin; vasopressin; microglia

INTRODUCTION

The magnocellular neuroendocrine system, with cell somas and dendrites in the supraoptic (SON) and paraventricular (PVN) nuclei, has an essential role in osmoregulation (1). Elevations in Na⁺ and osmolality activate magnocellular neurons directly (2) and indirectly, via innervating osmosensitive neurons in the forebrain (3), to enhance vasopressin and oxytocin release into the hypothalamus and peripheral circulation (4). Resulting renal water re-

[‡]The definitive version is available at www.blackwell-synergy.com.

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absorption and natriuresis restore plasma tonicity, while vasopressin and oxytocin release from the dendrites influence innervating afferent fibers in the SON (5), the firing rates of magnocellular neurons (6) and plasticity of local neuro-glia interactions (7,8).

IL-1 β expressed in oxytocinergic and vasopressinergic neurons (9) may enhance magnocellular responses to osmotic stimulation. An autocrine feedback is possible as cytokine and its biologically active receptor (Type 1; IL-1R1) in magnocellular neurons respond to chronic dehydration (9,10). Coincident with increased expression of IL-1R1 (10), IL-1 β with oxytocin and vasopressin and their corresponding granules co-deplete from the neural lobe during salt loading (9,11), making possible cytokine-stimulated release of neurohypophysial hormones, as occurs *in vitro* (12). Likewise, IL-1 β may accompany dendritic/somatic release of oxytocin and vasopressin during osmotic stimulation (13), since cytokine is present in magnocellular soma (9) and can upregulate osmosensory cation currents in *isolated* supraoptic neurons, *in vitro* (14).

To examine whether IL-1 β is released *in vivo*, microdialysis of the SON area in conscious rats was combined with immunoaffinity capillary electrophoresis with laser-induced fluorescence detection (ICE-LIF; (15), enabling quantification of IL-1 β in 20 μ l samples collected at 5 min intervals under *basal* conditions of microdialysis (1-3 hrs) and after an acute *osmotic stimulus* (4 hr). Changes in IL-1 β concentration during dialysis of drugs defined local requirements of voltage-gated Na⁺ and Ca²⁺ channels responding to osmotic stimulation. Sensitivity to tetrodotoxin (TTX) identified dependence on action potentials (16), leaving unaffected cytokine release from probe injury (17). Reliance on voltage-gated Ca²⁺ channels of N-, L- and T- subtypes was characterized using cadmium with nickel (Cd,Ni;(18) in perfusion fluid without calcium, known to reduce afferent activation (19), magnocellular neuron responses (20) and dendritic release of oxytocin and vasopressin (13). Thapsigargin defined the role of sensitive intracellular Ca²⁺ stores (21) and whether “priming the pump” is involved in cytokine release (22,23), as for dendritic secretion of oxytocin (24) and vasopressin (25) in the SON during osmotic stimulation.

Since cytokine released in the SON area could derive from innervating fibers (26), magnocellular neurons themselves (9) or from glia (17), another microdialysis study was combined with immunohistochemistry to identify potential cellular sources (IL-1 β ⁺) in the SON area, including microglia (OX-42⁺; antibody to rat microglial membrane domain homologous to human complement receptor type 3 antigen; (27).

MATERIALS AND METHODS

The studies reported were performed in accordance with the Guide for the Care and Use of Laboratory Animals as published by the National Research Council (1996 edition) and guidelines from The Penn State College of Medicine.

Surgery for implantation of the microdialysis guide cannula

Adult male Sprague Dawley rats (300 - 400 gms) were anesthetised with sodium pentobarbital (40 mg/kg, i.p.) and supplemented with ketamine (100 mg/kg, i.m.). A guide cannula for the microdialysis probe (CMA-12; CMA Microdialysis, North Chelmsford, MA) was stereotactically implanted with its tip 2 mm above the SON in the left hemisphere (AP – 0.97 mm; L 1.83 mm; D 7.15 mm; flat skull). After surgery, animals were housed individually for 6 or 7 days until the experimental day.

Microdialysis

Rats with the guide cannula implanted were weighed, briefly anesthetized (halothane/oxygen mixture) to insert the microdialysis probe (CMA-12; polyethersulphone; 100,000 Dalton pore) with its 2 mm membrane extended into the SON area and then dialyzed (2 or 4 $\mu\text{L}/\text{min}$) continually with sterile perfusion fluid (PF; CMA #P000151; mmol/L: 147 NaCl, 2.7 KCl, 1.2 CaCl_2 , 0.85 MgCl_2 ; pH 5.78) for 6 hrs. All the probes, tubing and syringes used for microdialysis were gas sterilized as for clinical use (ethylene oxide; 15 hr cycle) by the Sterile Processing Department of the Penn State M.S. Hershey Medical Center. Two hours after inserting the probe, 20 μL samples of dialysate were collected at 5 or 10 minute intervals for 4 hours to monitor basal release of IL-1 β (hours 1-3) and drug effects after osmotic stimulation (hour 4) by sterile hypertonic saline (HS; 1.5 M NaCl; 15 ml/kg) injected s.c (Fig. 1). Vehicle or drugs were dialyzed locally into the SON area for 30 or 60 minutes during hours 2 and 4 to affect release of IL-1 β in the SON area. Animals were decapitated 60 min after HS. The brain was blocked then frozen, sectioned (25 μm) and stained (Nissl) for assessment of the probe position relative to the SON. By light microscopy the shortest distance of the probe tip to the SON and the volume of the nucleus were quantified using image software (Pax-it Enhanced Module). A "hit" required the probe tip to be within a 500 μm diffusion radius of the SON (28).

Immunoaffinity capillary electrophoresis with laser-induced fluorescent detection (ICE-LIF) for quantification of IL-1 β in microdialysates of the SON area

The immunoaffinity capillary was prepared as described previously (15). An antibody to IL-1 β was enzymatically digested using the Pierce ImmunoPure F (Ab) $_2$ preparation kit. The resulting divalent F(Ab) $_2$ fragment was further reduced to two monovalent Fab fragments by incubation with an equal volume of 200 mmol/L Cleland's reagent for 30 min at 37°C (15). Equal amounts of each antibody fragment (Fab) were used to coat an immunoaffinity capillary (Bio-Rad Laboratories; Hercules, CA) cut to a length of 45 cm. The capillary was filled with 500 nL of an aqueous solution of 10% 3-aminopropyl-triethoxysilane and incubated at 100°C for 60 min. This treatment was repeated four times then the capillary was filled with 10 mmol/L of hydrochloric acid and incubated at 100°C for 60 min. After washing the capillary in distilled water, a thiol-activated surface was prepared by allowing 500 nL of sulphosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SSMCC; 1 mg/ml) dissolved in sodium borate (50 mmol/L; pH 7.6) to be taken up by capillary action. It was then sequentially incubated at 30°C for 60 min, flushed with 50 mmol/L borate, filled with the Fab mixture and incubated overnight at 4°C. This procedure produced an antibody coating on approximately 6 cm of the capillary, representing an antibody excess of ca. 90-fold. Approximately a 100:1 ratio of antibody to antigen is required for efficient analyte capture during short incubation times. The capillary was then flushed three times with 100 mmol/L phosphate buffer, pH 7.4, and mounted onto a modified capillary electrophoresis unit (Model 3850 — ISCO, Inc.; Lincoln, NE).

Total protein levels in standard solutions of IL-1 β and in samples of microdialysates were quantified and then derivatized with fluorochrome Cy5 (15). After separation of microaggregates by centrifugation, 30 nL dilutions of the cytokine standard and microdialysates were injected into the capillary using a split-flow injector. Each sample was in direct contact with the immobilized antibody coating for 5 min before the capillary was purged with 200 μL of 100 mmol/L phosphate buffer (pH 7.4) to which 0.01% Brij 35 was added. This removed all unbound materials, including free fluorochrome, before recovery and analysis of the bound analytes. Capillaries were placed into reservoirs containing 100 mMol/L phosphate buffer/0.01% Brij (pH 1.5), and the bound analytes electro-eluted at 100 μA constant current.

A laser-induced fluorescence (LIF) detector was used to enhance sensitivity and to continuously monitor (on-line) electrophoretic separation of the bound analytes (15). Data obtained from amplification (x100) of the spectrometer signal was relayed to a 1000 kHz A/D board and analyzed using an MS Windows-based software package supplied with the spectrometer. The concentration was calculated by comparison to a standard curve constructed from known amounts of pure cytokine. The lower level of detection for ICE-LIF ranged from 1.6 to 1.9 pg/ml for IL-1 β (29), with inter- and intra- assay coefficients of variation averaging 4.0% and $3.0 \pm 0.6\%$, respectively.

Immunohistochemistry

Unoperated rats (control) or those having the probe inserted with, or without, microdialysis of sterile perfusion fluid in the SON area for 6 h (4 μ l/min) were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) before fixing the brain *in situ* by perfusing 0.9% saline (1 min; 50-100 mls) and then 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4; 15 min; 500 mL) through the ascending aorta. The brain was removed from the skull and immersed in the same fixative for 7 hrs at 4°C, with careful attention to treat all the tissues identically. Following post-fixation, the tissue was stored at 4°C in 0.1 phosphate buffer (pH 7.4) containing 20% sucrose that was replaced after 12 hrs of immersion.

Multiple series of cryostat sections (30 μ m) through the SON, beginning near the bregma (AP – 0.4 mm) and ending just after the PVN (AP – 2.0 mm from bregma), were collected in groups of 4 with #1 sections stained with thionin (Nissl), and the remainder processed for immunohistochemistry using primary antibodies for OX-18 (OX-18⁺; antibody to MHC Type I antigen, Accurate; OBT0025; monoclonal; 1:7,500 dilution; #2 sections), OX-42 (CEDARLANE, CL042AP, CL042AP-2; monoclonal antibody 1: 20,000 dilution; #3 sections) and glial fibrillary acidic protein (GFAP; DAKO, rabbit anti-GFAP polyclonal antibody, Z0334; 1:18,000 dilution; #4 sections). From another series of animals treated similarly, tissue sections were thionin-stained (#1 sections) or immuno-reactive product localized for OX-42 (#2 sections) and IL-1 β (R&D Systems; AF-501-NA; goat polyclonal anti E. coli-derived recombinant rat IL-1 β ; 1:1000 dilution; #3 sections). Specificity of reaction product was evaluated by omitting the primary or secondary antibody.

Initially, endogenous peroxidase was inactivated with hydrogen peroxide, then tissue sections were incubated free-floating in 0.01 M phosphate buffered saline (PBS) containing 1% normal horse (OX-18, OX-42, IL-1 β) or donkey (GFAP) serum (Vector Lab., Burlingame, CA), 0.3% Triton X-100 (Sigma, St. Louis, MO) and the specific antibody for 3 days at 4°C. Subsequently, the immuno-reactive product was visualized using the avidin-biotin complex method (Vectastin elite ABC kit; Vector Lab., Burlingame, CA). Sections were incubated at room temperature in PBS containing biotinylated horse anti-mouse or donkey anti-rabbit IgG, Triton-X and normal blocking serum for 1 hour then avidin-biotinylated horseradish peroxidase complex for another hour, followed by incubation for 10 minutes in 0.05 M Tris buffer (pH 7.2) containing 0.03% 3', 3'-diaminobenzidine (Sigma) and 0.0075% H₂O₂. Each step was followed by washes in PBS. After thorough rinses in distilled water, sections were mounted on slides, dehydrated in ethanol, cleared in xylene, and coverslipped in Permount® (Fisher Scientific, Fair Lawn, NJ).

Quantification of microglia in the SON—Microglia containing immunoreactive product for OX-42 were qualitatively identified morphologically as ramified, hypertrophied or ameboid (29) in the SON outlined from cresyl violet stained sections. Ramified microglia were defined by thin radial projecting processes from the soma; hypertrophied microglia had enlarged and darkened soma with shorter, thicker and less branched processes; ameboid microglia had densely stained and enlarged cell bodies with, or without, a few short processes (30). The

combined total of all forms of microglia was determined by the disector method (31) on each tissue section in the left (side of probe) and right hemispheres (contra-lateral to probe) with measurements of the area of the SON and tissue thickness for calculation of density, i.e. density = total OX-42⁺ cells /area x tissue thickness. The antibody for OX-42 also labels monocytes/macrophages from the circulation, as well as some lymphocytes, because of similar ontogenetic derivation (32).

Study 1. Effects of local inhibition of Na⁺ and Ca²⁺ channels on IL-1 β release during microdialysis of the SON area after acute osmotic stimulation in conscious rats.

Sterile perfusion fluid from CMA was continuously dialyzed (4 μ L/min) via the probe (CMA-12) with 5 min samples collected for 4 hrs until decapitation (Fig. 1). In hours 1 and 3 perfusion fluid was dialyzed with, or without, addition in hour 2 of tetrodotoxin (TTX; 5 μ M; (16) or calcium-free perfusion fluid (0^{Ca2+}Cd, Ni) containing Cd (0.3 mM) and Ni (0.3 mM) for 30 or 60 minutes (i.e. from 60 to 90 or 120 min), and again in hour 4 after HS (i.e. from 180 to 210 or 240 min; Fig. 1). These doses of TTX and 0^{Ca2+}Cd, Ni have been shown effective when microdialyzed in the SON (33). Basal levels of IL-1 β were, however, too low to quantify inhibitory effects of the drugs in hour 2.

Study 2. Effects of dialyzed thapsigargin on IL-1 β secretion during microdialysis of the SON area after acute osmotic stimulation in conscious rats

For 3.5 -hour (0-210 minutes), conscious rats were continuously dialyzed in the SON area with sterile perfusion fluid (CMA; 2 μ L/min) and samples collected every 10 min. At 60 min, either vehicle (1% DMSO) or thapsigargin (50 μ M) was dialyzed for 30 minutes from 60-90 min. HS was injected i.p. 30 min later at 120 min, with decapitation after 1.5 hr (210 min). Using this protocol Ludwig and colleagues demonstrated enhanced dendritic release of oxytocin (24) and vasopressin (25) from magnocellular neurons in response to osmotic stimulation, likely resulting from thapsigargin “priming” the membrane transport pump (23) via complex changes in intracellular Ca²⁺ and store-operated ionic fluxes following inhibition of Ca²⁺-ATPases (21). Basal levels of IL-1 β were, however, too low to quantify inhibitory effects of thapsigargin in hour 2.

Study 3. Response of microglia in the SON area to microdialysis

Immunohistochemistry of fixed-frozen brain tissue was used to evaluate cellular changes in the SON from groups of rats (n = 3-5) that were unoperated (controls) or had only the probe inserted into the guide cannula with, or without, microdialysis (4 μ L/min) for 6 hrs or HS injected s.c. 1 hour before killing. Cell types and morphological changes were identified by comparing distributions on adjacent serial tissue sections (30 μ m) using Nissl to localize magnocellular neurons in the SON and antigen markers for microglia preferentially surrounding blood vessels or dispersed (27) and for astrocytes (antibody to GFAP) having their soma in the ventral glia limitans with fine processes projecting vertically to surround magnocellular neurons and their dendrites (34). Animals were treated similarly as in the microdialysis studies except that, instead of decapitation, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and the brain fixed *in situ* before sending to FD Neurotechnologies, Inc. (Ellicott City, MD) for sectioning and immunohistochemistry. The slides were returned for our qualitative and quantitative analyses.

Study 4. Effects of HS on plasma osmolality with time after s.c. injection

Unoperated male SD rats (40 total; 10/treatment) were injected with HS, s.c., then decapitated 15, 30, 60 and 120 min later. Plasma was separated and osmolality determined by freezing point depression (MicroOsmette; Precision Instruments).

Statistics

Osmotic stimulated release of IL-1 β —The concentration of IL-1 β in dialysates of the SON area was transformed to the natural log then compared in the same animal dialyzed with perfusion fluid just before (180 min; control) and after HS injection (185 to 240 min) using 1-way ANOVA for repeated measures and the Newman Keul's t test to define changes with time following osmotic stimulation.

Drug effects on osmotic stimulated release of IL-1 β —Differences in means of log transformed data from groups of animals treated with either perfusion fluid (control) or drug (TTX, 0 $^{Ca^{2+}}$ Cd, Ni) for 30 or 60 min before, and after, HS were analyzed by 2-way ANOVA for repeated measures and the Newman Keul's T test (n=3-7 rats/treatment). Effects of thapsigargin and its vehicle (1% DMSO) after osmotic stimulation (i.e. 100 – 180 min) were compared using each animal as its own control (n=5 or 6).

Quantification of microglia in the SON—Differences in density of OX-42 $^{+}$ cells in the SON related to the guide cannula, microdialysis and osmotic stimulation were determined by 1-way ANOVA and the Tukey t test.

Changes in plasma osmolality after HS—Differences in plasma osmolality at 0, 15, 60 and 120 minutes after HS were determined by 1-way ANOVA and the Tukey t test (n=7-10).

RESULTS

Basal release of IL-1 β in the SON area is stable during microdialysis and increases after osmotic stimulation

IL-1 β was measurable in dialysate samples from hours 1-3 in all the rats. Under conditions of microdialysis, *basal* release of IL-1 β^{+} in the SON area was stable (pg/ml; mean $\pm$ SEM; n=5 rats) from 0-60 min (2.2 ± 0.06), 60-120 min (2.32 ± 0.05) and 120-180 min (2.33 ± 0.06) and similar to cytokine levels released from the PVN (35). Importantly, the closest distance from the probe tip to the SON and the area of the nucleus dialyzed in drug- and vehicle- treated animals did not differ ($P > 0.05$) from those given only perfusion fluid (*distance to SON*: $78 \pm 48 \mu\text{m}$; range: 0 to $194.4 \mu\text{m}$; *SON area*: $106 \pm 18 \times 10^3 \mu\text{m}^2$; range 59.3 to $143.8 \times 10^3 \mu\text{m}^2$).

In response to peripheral osmotic stimulation IL-1 β levels in dialysates of the SON area increased immediately to plateau at 30-45 min (Fig. 2), following linear kinetics with time after HS injection (0-60 min: $r^2 = 0.85$; $y = 2.342x - 413.81$; Fig. 2). The dose volume of hypertonic saline administered (15 ml/kg b.w.; s.c.), likely elevated circulating osmolality rapidly with a correspondingly short onset of central cytokine release detected at lower levels with the enhanced sensitivity of ICE-LIF (Fig. 2). Although stressed initially after the injection, animals typically slept during the dialysis period. These changes correspond with increases in: 1. plasma osmolality of unoperated animals (mOsmol/kg H $_2$ O; mean $\pm$ SEM; Control 294.6 ± 0.5 , n=10; HS-15 min 309.4 ± 1.7 , n=7; HS-60 min 319.0 ± 2.9 , n=6; HS-120 min 315.0 ± 1.8 , n=7; control < all others, $p < 0.05$; HS-15 < HS-60; $p < 0.05$) and 2. reported increases of dendritic/somatic release of oxytocin and vasopressin (4,13), presumably stimulated by osmotically-activated forebrain pathways (1) involving glutamate-mediated (3) exocytosis (36).

Osmotic stimulated release of IL-1 β in the SON area is dependent on neural function sensitive to TTX requiring voltage-gated calcium channels

Like as reported for dendritic secretion of oxytocin and vasopressin (37,38), blocking Na $^{+}$ channels required for axonic conduction with TTX (39), or dialyzing 0 $^{Ca^{2+}}$ Cd, Ni to inhibit exocytosis of neurotransmitters from afferent terminals requiring voltage-gated Ca $^{2+}$ channels (38,40), markedly reduced, or prevented, IL-1 β release from the SON area during osmotic

stimulation (Fig. 3). The osmotic response was attenuated markedly by both drug treatments, reducing IL-1 β concentration in the dialysates immediately (0-5 min) and for longer than 1 hr (Fig. 3).

Thapsigargin-sensitive intracellular stores of Ca²⁺ are required for osmotic stimulated release of IL-1 β in the SON area

Dialysis of 1% DMSO, the vehicle for thapsigargin, did not alter the cytokine response to osmotic stimulation since, like perfusion fluid alone, IL-1 β released in the SON area increased progressively with time after HS injection (Fig. 4). Osmotically-stimulated cytokine release, however, was prevented or attenuated after thapsigargin, an inhibitory response lasting longer than 1 hr (Fig. 4).

Microdialysis of the SON area activates microglia

Throughout the SON, magnocellular neurons are bordered and separated by protoplasmic astroglia and processes of glia having their soma in the ventral glia limitans, with quiescent ramified microglia interspersed (30,41). In our study, patterns of immunoreactivity demonstrated astroglia (GFAP⁺) in the ventral glia limitans and microglia preferentially near blood vessels (OX-18⁺; 41) or ramified throughout the SON (OX-42⁺; 27; 30; Fig. 5). IL-1 β was expressed in magnocellular neurons, as described previously (9), and in cells of the ventral glial region (and meninges) having fibrous projections dorsally through the SON similar to astrocytes known to express GFAP (Fig. 5). To our knowledge, astroglia of the ventral glia limitans in the SON have not been reported previously to express IL- β (9,26). Although the primary antibody for IL-1 β from R&D Systems does not cross-react with recombinant rat IL-1 α or numerous other cytokines, it is possible that a larger precursor of the cytokine that also binds this antiserum (42) contributes to the immunoreactivity observed (Fig. 5). Although a probe inserted for 6 hrs *without* dialysis did not alter consistently the form of microglia or expression of IL-1 β in the SON, dialysis of sterile perfusion fluid for 6 hrs caused many OX-42⁺ cells to become hypertrophied and amoeboid, with fewer ramified near the probe (Fig. 5). This activation response corresponded with induction of IL-1 β in the microglia (Fig. 5), but not proliferation, as the density of OX-42⁺ cells (#cells/ μ m³; mean $\pm$ SEM; n=3 rats/treatment; 6 tissue sections/rat) in the SON ipsi- (Control: $5.61 \pm 0.51 \times 10^{-5}$) and contra- (Control: $5.87 \pm 0.32 \times 10^{-5}$) lateral to the probe remained unchanged by any treatment ($P > 0.05$).

DISCUSSION

The enhanced sensitivity and nanotechnology afforded by ICE-LIF, coupled with the capability of quantifying analytes in low sample volumes made possible, for the first time, measurement of IL-1 β in dialysates collected over minutes from the SON area of conscious rats. Our initial studies documented progressive increases of IL-1 β released in the SON area during peripheral osmotic stimulation attenuated, in part, by anesthesia (43). Subsequent studies distinguished characteristics of osmotically-stimulated release of cytokine differing in requirements for voltage-gated Na⁺ and Ca²⁺ channels, but with a common dependence on intracellular stores of Ca²⁺ sensitive to thapsigargin. Comparing IL-1 β release with dendritic secretion of oxytocin and vasopressin from magnocellular neurons documents a similar dependence on afferent neural activation during osmotic stimulation (13), whereas for *basal* levels, activated microglia and magnocellular neurons are likely sources of the cytokine.

Basal release of IL-1 β in the SON area

Under basal conditions of microdialysis, quantifiable levels of IL-1 β were released in the SON area with a consistency similar to that reported for cytokine sampled repeatedly by push-pull perfusion of the hypothalamus lateral to the PVN (35). Ranging from 2.20 ± 0.06 to $2.33 \pm$

0.06 pg/ml, the *basal* levels of IL-1 β likely derive from activated microglia expressing the cytokine. Local microglia near the probe became hypertrophied and amoeboid in form, with induction of IL-1 β in response to dialysis of the SON area (17,35,44). Importantly, this microglial activation was not caused by mechanical injury from the probe or guide cannula, nor was it intensified by osmotic stimulation. The acidic pH of the commercially available perfusion fluid from CMA that was dialyzed, or possibly a bio-incompatibility of the probe membrane or perfusion fluid interaction with the tubing, may have activated microglia locally in the SON area to release cytokine (45). Since amoeboid microglia function like macrophages (46), with expression and secretion of IL-1 β and its precursor (47), those activated near the probe are likely sources of cytokine in dialysates, as well as are magnocellular neurons (9) and local cytokinergic neural pathways (26).

IL-1 β released in the SON area during acute osmotic stimulation corresponds with enhanced dendritic secretion of oxytocin and vasopressin from magnocellular neurons reported by others.

During peripheral osmotic stimulation, the dendrites and soma of magnocellular neurons release neurohypophysial hormones by *exocytosis* (36), requiring local voltage-gated Ca²⁺ channels activated by neural reflexes from the lamina terminalis dependent upon Na⁺-conductance and action potentials (13,37,38). Innervating glutamatergic fibers from the osmo-sensitive forebrain having TTX-sensitivity and Ca²⁺ dependent neurotransmitter release are required to stimulate dendritic/somatic secretion of oxytocin and vasopressin in response to HS administered peripherally (3,13). Identical neural requirements governed IL-1 β secretion in the SON area during osmotic stimulation. Dialysate levels of cytokine increased progressively with time after HS, as plasma osmolality and [Na⁺] rise and neural reflexes from the osmoregulatory forebrain are activated with requirements of voltage-gated Na⁺ and Ca²⁺ channels locally in the SON. Albeit microglia activated by microdialysis and expressing IL-1 β in the SON could be a prominent source of enhanced cytokine released during osmotic stimulation, this appears less likely since proliferation did not occur and many were in the ramified, or resting state, in the SON after HS (manuscript in preparation). Although IL-1 β ergic fibers (26) could also be activated by osmotic stimulation, it is proposed that cytokine in small vesicle-like granules, responsive to chronic salt-loading (9,11), is released from the dendrites and soma of magnocellular neurons coincident with Ca²⁺-dependent exocytosis of oxytocin and vasopressin from larger dense core vesicles (22,36,38). Measuring co-release was attempted in our experiments, but even with ICE-LIF, oxytocin and vasopressin concentrations remained, for the most part, below detectable limits in the dialysate samples. Presumably, using a probe optimized for quantifying IL-1 β in dialysates (i.e. pore size 100,000 Kd) markedly compromised recovery of the neurohypophysial peptides, *in vivo*.

Although voltage-gated Na⁺ and Ca²⁺ channels are necessary, intracellular Ca²⁺ requirements remain distinct for IL-1 β during osmotic stimulation. While presumably driven by the same osmo-excitatory glutamatergic pathway from the forebrain (1), it should be emphasized that the mechanism for IL-1 β release in the SON area differs from dendritic release of neurohypophysial peptides in being dependent on thapsigargin-sensitive Ca²⁺ stores, or associated compensatory responses. Dialysis of the drug with its intracellular Ca²⁺ changes in magnocellular neurons (48) *inhibited* osmotically-induced secretion of cytokine, whereas *priming* of dendritic pumps (23) with *potentiated* oxytocin and vasopressin release after hypertonic saline, s.c., is well-documented using an identical protocol for microdialysis (24, 25). Recent molecular and biochemical evidence also supports a secretory mechanism for IL-1 β different from “classical” exocytosis, with similarity to other proteins lacking appropriate signal sequences (49).

Further studies comparing intra-nuclear release patterns after peripheral HS and its activation of afferent osmoregulatory pathways (3,13,37,38) with those following direct hyperosmotic activation of magnocellular neurons locally in the SON (4) are needed to more completely compare the mechanism(s) and cellular sources of cytokine secreted into dialysates with that of dendritic/somatic release of the neurohypophysial peptides (13). It will also be important to distinguish what, if any, role the stress and cutaneous inflammation associated with HS contributes to IL-1 β secreted in the SON area during osmotic stimulation.

Proposed functions for IL-1 β released in the SON area

Intrinsically, magnocellular neurons are activated by local hypertonicity that causes cellular shrinkage, opening of stretch-inactivated non-selective cationic channels (2) with significant Ca²⁺ entry and release from internal stores combined with enhanced flux of monovalent cations, K⁺ and Na⁺ (50). Since these non-selective cationic channels can be upregulated by IL-1 β applied to isolated magnocellular neurons, *in vitro* (14), a similar response is proposed for cytokine released in the SON area during osmotic stimulation, *in vivo*. As shown by Chakfe, Zhang and Bouque (14), IL-1 β interaction with its type 1 receptors up-regulates intrinsic stretch-inactivated non-selective cationic channels on magnocellular neurons necessary for osmoreception by an autocrine mechanism dependent on cyclooxygenase and PGE₂ synthesis activating EP₄ receptors to increase Ca²⁺ influx for membrane depolarization (50). Our studies provide support that this cytokine response may occur *in vivo*, mediated by enhanced secretion of IL-1 β in the SON area during peripheral osmotic stimulation. After release, a similar cascade of cytokine binding to IL-1R1 (51) affecting cyclooxygenase is proposed, that ultimately facilitates oxytocin and vasopressin release both from dendrites and magnocellular terminals in the neurohypophysis during peripheral osmotic stimulation (4,50). Similarly, cytokine released from microglia transitioning from stellate to an amoeboid-activated morphology in the SON area may contribute to magnocellular activation with chronic salt loading (30), as well as during microdialysis. Astrocytes could also be responders, being shown to express metalloproteinases (52) as well as IL-1 β (53;Fig. 5) when activated by the cytokine. Functionally, modulation of postsynaptic glutamatergic neurotransmission could result (54), a pathway enhanced by osmotic stimulation (3), or possibly morphological plasticity dependent on local release of oxytocin in the SON concurrent with neuronal activation by hyperosmotic stimulation (4,55). Collectively, in these ways, autocrine and paracrine feedback from IL-1 β released locally in the SON, potentially from neurons and glia (56), could modulate the magnocellular neuroendocrine response to effectively compensate for fluid/electrolyte imbalance during osmotic-dehydration.

Dependence of IL-1 β release in the SON area on intracellular Ca²⁺ stores sensitive to thapsigargin, suggests another role for the cytokine. Dynamics of intracellular Ca²⁺ changes within magnocellular neurons after thapsigargin, influences not only dendritic release of oxytocin and vasopressin to the osmotic challenge (24), but also compensatory cellular responses to stress on the endoplasmic reticulum exerted by the drug (57). Since neuronal autophagy (58) and apoptosis (59) pathways are activated by thapsigargin, further cellular stress would be minimized by reducing cytokine release with its potentiated Ca²⁺ response via an autocrine loop (14) and neuronal death via NMDA receptors (60). Simultaneously, potentiated dendritic release of oxytocin would afford protection from cellular damage via autoreceptors increasing IP₃ levels, a cellular response known to diminish autophagy (58). Alternately, an impact of thapsigargin on other modulators of magnocellular neurons could attenuate indirectly IL-1 β secretion in the SON, such as feed-back from oxytocin/vasopressin autoregulation (6) and norepinephrine release inhibited by glutamate (33). Clearance of Ca²⁺ from the soma of magnocellular neurons in the SON is also dependent on thapsigargin-sensitive Ca²⁺-ATPase pumps (48), making possible a more general influence on magnocellular neurons with consequent inhibition of IL-1 β release.

ACKNOWLEDGEMENTS

This research was supported by the NIMH (R01-MH65271 to J.S.-L.). The authors appreciate technical support from Mindy Lull, Xiaofen Chen, Ann Pruss, David Widitz and X. Wang. A portion of this research was published previously as an abstract: Long, A., T.M. Phillips, X. Wang, X. Chen, A. Pruss, K. Seaton and J.Y. Summy-Long. Basal and osmotic-stimulated release of interleukin-1 β from the supraoptic nucleus: Dependence on Na⁺ and Ca⁺⁺ channels. Program No. 422.14. 2004 Abstract Viewer/Itinerary Planner; Washington, DC: Society for Neuroscience.

References

1. Bourque CW, Ciura S, Trudel E, Stachniak TJE, Sharif-Naeini. Hydromineral Neuroendocrinology: Neurophysiological characterization of mammalian osmosensitive neurones. *Exp Physiol* 2007;92:499–505. [PubMed: 17350993]
2. Oliet SH, Bourque CW. Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. *Nature* 1993;364:341–343. [PubMed: 7687327]
3. Bourque CW, Richard D. Axonal projections from the organum vasculosum lamina terminalis to the supraoptic nucleus: functional analysis and presynaptic modulation. *Clin Exp Pharmacol Physiol* 2001;28:570–574. [PubMed: 11458884]
4. Neumann I, Ludwig M, Engelmann M, Pittman QJ, Landgraf R. Simultaneous Microdialysis in Blood and Brain: Oxytocin and Vasopressin Release in Response to Central and Peripheral Osmotic Stimulation and Suckling in the Rat. *Neuroendocrin* 1993;58:637–645.
5. Kombian SB, Mougnot D, Pittman QJ. Dendritically Released Peptides Act as Retrograde Modulators of Afferent Excitation in the Supraoptic Nucleus In Vitro. *Neuron* 2003;19:903–912. [PubMed: 9354336]
6. Moos F, Gouzènes L, Brown D, Dayanithi G, Sabatier N, Boissin L, Rabié A, Richard P. New aspects of firing pattern autocontrol in oxytocin and vasopressin neurones. *Adv Exp Med Biol* 1998;449:153–162. [PubMed: 10026798]
7. Hatton GI. Dynamic neuronal-glial interactions: an overview 20 years later. *Peptides* 2004;25:403–411. [PubMed: 15134863]
8. Theodosis DT. Oxytocin-secreting neurons: A physiological model of morphological neuronal and glial plasticity in the adult hypothalamus. *Frontiers in Neuroendocrinology* 2002;23:101–135. [PubMed: 11906204]
9. Watt JA, Hobbs NK. Interleukin-1 β immunoreactivity in identified neurons of the rat magnocellular neurosecretory system: evidence for activity-dependent release. *J Neurosci Res* 2000;60:478–489. [PubMed: 10797550]
10. Watt JACranston HJPaden CMInterleukin-1 receptor type 1 expression in the magnocellular neurosecretory system of the rat: Evidence for activity-dependent regulation. *Soc Neurosci Abstr* 2004; Program No 73.2. 2004 Abstract Viewer/Itinerary Planner, Society for Neuroscience Washington, DC
11. Summy-Long JY, Hu S, Pruss A, Chen X, Phillips TM. Response of Interleukin-1 β in the Magnocellular System to Salt-Loading. *J Neuroendocrin* 2006;18:926–937.
12. Christensen JD, Hansen EW, Fjalland B. Influence of interleukin-1 β on the secretion of oxytocin and vasopressin from the isolated rat neurohypophysis. *Pharmacology & Toxicology* 1990;67:81–83. [PubMed: 2395821]
13. Ludwig M. Dendritic Release of Vasopressin and Oxytocin. *J Neuroendocrin* 1998;10:881–895.
14. Chakfe Y, Zhang Z, Bourque CW. IL-1 β directly excites isolated rat supraoptic neurons via upregulation of the osmosensory cation current. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2006;290:R1183–R1190.
15. Phillips TM. Analysis of single-cell cultures by immunoaffinity capillary electrophoresis with laser-induced fluorescence detection. *Luminescence* 2001;16:145–152. [PubMed: 11312540]
16. Westerink BH, Tuntler J, Damsma G, Rollema H, Vries JB. The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis. *Naunyn-Schmiedeberg's Arch Pharmacol* 1987;336:502–507.

17. Fassbender K, Schneider S, Bertsch T, Schlueter D, Fatar M, Ragoschke A, Kischka U, Hennerici M. Temporal profile of release of interleukin-1 β in neurotrauma. *Neurosci Letts* 2000;284:135–138. [PubMed: 10773417]
18. Sharp T, Bramwell SR, Grahame-Smith DG. Release of endogenous 5-hydroxytryptamine in rat ventral hippocampus evoked by electrical stimulation of the dorsal raphe nucleus as detected by microdialysis: sensitivity to tetrodotoxin, calcium and calcium antagonists. *Neuroscience* 1990;39:629–637. [PubMed: 2097517]
19. Turner TJ, Adams ME, Dunlap K. Calcium Channels Coupled to Glutamate Release Identified by ω -Aga-IVA. *Science* 1992;258:310–313. [PubMed: 1357749]
20. Fisher TE, Bourque CW. The function of Ca(2+) channel subtypes in exocytotic secretion: new perspectives from synaptic and non-synaptic release. *Progress in Biophysics & Molecular Biology* 2001;77:269–303. [PubMed: 11796142]
21. Thastrup O, Cullen PJ, Drobak BK, Hanley MR, Dawson AP. Thapsigargin, a tumor promoter, discharges intracellular Ca $^{2+}$ stores by specific inhibition of the endoplasmic reticulum Ca $^{2+}$ -ATPase. *Proc Natl Acad Sci USA* 1990;87:2466–2470. [PubMed: 2138778]
22. Morris JF, Ludwig M. Magnocellular Dendrites: Prototypic Receiver/Transmitters. *J Neuroendocrin* 2004;16:403–408.
23. Tobin VA, Hurst G, Norrie L, Dal Rio FP, Bull PM, Ludwig M. Thapsigargin-induced mobilization of dendritic dense-cored vesicles in rat supraoptic nucleus. *Eur J Neurosci* 2004;19:2909–2912. [PubMed: 15147325]
24. Ludwig M, Sabatier N, Bull PM, Landgraf R, Govindan D, Leng G. Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 2002;418:85–89. [PubMed: 12097911]
25. Ludwig M, Bull PM, Tobin VA, Sabatier N, Landgraf R, Dayanithi G, Leng G. Regulation of activity-dependent dendritic vasopressin release from rat supraoptic neurones. *J Physiol* 2005;564:515–522. [PubMed: 15731188]
26. Lechan RM, Toni R, Clark BD, Cannon JG, Shaw AR, Dinarello CA, Reichlin S. Immunoreactive interleukin-1 β localization in the rat forebrain. *Brain Res* 1990;514:135–140. [PubMed: 2357520]
27. Robinson AP, White TM, Mason DW. Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology* 1986;57:239–247. [PubMed: 3512425]
28. Westerink BH, De Vries JB. A method to evaluate the diffusion rate of drugs from a microdialysis probe through brain tissue. *J Neurosci Methods* 2001;109(Aug 15)(1):53–8. [PubMed: 11489300] 2001. p. 53–58.
29. Phillips TM. Multi-analyte analysis of biological fluids with a recycling immunoaffinity column array. *J Biochem Biophys Methods* 2001;49:253–262. [PubMed: 11694283]
30. Ayoub AE, Salm AK. Increased Morphological Diversity of Microglia in the Activated Hypothalamic Supraoptic Nucleus. *J Neurosci* 2003;23:7759–7766. [PubMed: 12944504]
31. Bjugn R, Gunderson HJG. Estimate of the total number of neurons and glial and endothelial cells in the rat spinal cord by means of the optical disector. *J Comp Neurol* 1993;328:406–414. [PubMed: 8440788]
32. Barron KD. The microglial cell. A historical review. *Journal of the Neurological Sciences* 1995;134:57–68. [PubMed: 8847546]
33. Liu X, Onaka T, Yagi K. Facilitation of Ca $^{2+}$ store-dependent noradrenaline release after an N-methyl-D-aspartate receptor antagonist in the rat supraoptic nucleus. *J Neuroendocrin* 2001;13:894–904.
34. Salm AK, Smithson KG, Hatton GI. Lactation-associated redistribution of the glial fibrillary acidic protein within the supraoptic nucleus. An immunocytochemical study. *Cell Tiss Res* 1985;242:9–15.
35. Phelps CP, Sahu A, Oliver J, Ma XL, Kalra PS, Kalra SP, Menzies RA, Chen L, Denker M, Horvath E, Poole LL. In vivo release of interleukin-1 β into hypothalamic extracellular fluids in rats: effects of repeated sampling. *Brain Res* 1995;688:193–197. [PubMed: 8542307]
36. Pow DV, Morris JF. Dendrites of hypothalamic magnocellular neurons release neurohypophysial peptides by exocytosis. *Neuroscience* 1989;32:435–439. [PubMed: 2586758]

37. Ludwig M, Pittman QJ. Talking back: dendritic neurotransmitter release. *Trends in Neuroscience* 2003;26:255–261.
38. Soldo BL, Giovannucci DR, Stuenkel EL, Moises HC. Ca^{2+} and frequency dependence of exocytosis in isolated somata of magnocellular supraoptic neurones of the rat hypothalamus. *J Physiol* 2004;555:699–711. [PubMed: 14645448]
39. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 2000;26:13–25. [PubMed: 10798388]
40. Dunlap K, Luebke JI, Turner TJ. Exocytotic Ca^{2+} channels in mammalian central neurons. *TINS* 1995;18:89–98. [PubMed: 7537420]
41. Mander TH, Morris JF. Immunophenotypic evidence for distinct populations of microglia in the rat hypothalamo-neurohypophyseal system. *Cell Tissue Res* 1995;280:665–673. [PubMed: 7606773]
42. Dinarello CA. ELISA kits based on monoclonal antibodies do not measure total IL-1 beta synthesis. *J Immunol Methods* 1992;148:255–259. [PubMed: 1564330]
43. WiditzDPPhillipsTMBuiVSummy-LongJOsmotic stimulation increases interleukin-1 β release in the supraoptic nucleus of conscious and anesthetized rats2003 Abstract Viewer/Itinerary Planner 2003; Program No. 613.9.
44. Woodroffe MN, Sarna GS, Wadhwa M, Hayes GM, Loughlin AJ, Tinker A, Cuzner ML. Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by in vivo microdialysis: evidence of a role for microglia in cytokine production. *Journal of Neuroimmunology* 1991;33:227–236. [PubMed: 1874973]
45. Eder C, DeCoursey TE. Voltage-gated proton channels in microglia. *Prog Neurobiol* 2001;64:277–305. [PubMed: 11240310]
46. Streit WJ. Microglial response to brain injury: a brief synopsis. *Toxicol Pathol* 2000;28:28–30. [PubMed: 10668987]
47. Chauvet N, Palin K, Verrier D, Poole S, Dantzer R, Lestage J. Rat microglial cells secrete predominantly the precursor of interleukin-1beta in response to lipopolysaccharide. *Eur J Neurosci* 2001;14:609–617. [PubMed: 11556886]
48. Kim M-H, Lee S-H, Park KH, Ho W-K, Lee S-H. Distribution of K^{+} -dependent $\text{Na}^{+}/\text{Ca}^{2+}$ exchangers in the rat supraoptic magnocellular neuron is polarized to axon terminals. *The Journal of Neuroscience* 2003;23:11673–11680. [PubMed: 14684869]
49. Rubartelli A, Bajetto A, Allavena G, Wollman E, Sitia R. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J Biol Chem* 1992;267:24161–24164. [PubMed: 1332947]
50. Zhang Z, Bourque CW. Calcium permeability and flux through osmosensory transduction channels of isolated rat supraoptic nucleus neurons. *Eur J Neurosci* 2006;23:1491–1500. [PubMed: 16553612]
51. Diana A, van Dam AM, Winblad B, Schultzberg M. Co-localization of interleukin-1 receptor type I and interleukin-1 receptor antagonist with vasopressin in magnocellular neurons of the paraventricular and supraoptic nuclei of the rat hypothalamus. *Neuroscience* 1999;89:137–147. [PubMed: 10051223]
52. Wu CY, Hsieh HL, Sun CC, Tseng CP, Yang CM. IL-1 beta induces proMMP-9 expression via c-Src-dependent PDGFR/PI3K/Akt/p300 cascade in rat brain astrocytes. *J Neurochem* 2008;105:1499–1512. [PubMed: 18315570]
53. Wieseler-Frank J, Jekich BM, Mahoney JH, Bland ST, Maier SF, Watkins LR. A novel immune-to-CNS communication pathway: cells of the meninges surrounding the spinal cord CSF space produce proinflammatory cytokines in response to an inflammatory stimulus. *Brain Behav Immun* 2007;21:711–718. [PubMed: 16989980]
54. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, Oliet SH. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 2006;125:775–784. [PubMed: 16713567]
55. Langle SL, Poulain DA, Theodosis DT. Induction of rapid, activity-dependent neuronal-glia remodelling in the adult rat hypothalamus in vitro. *Eur J Neurosci* 2003;18:206–214. [PubMed: 12859353]
56. Halassa MM, Felin T, Haydon PG. The tripartite synapse: roles for gliotransmission in health and disease. *Trends in Molecular medicine* 2007;13:54–63. [PubMed: 17207662]

57. Breckenridge DG, Germain M, Mathai JP, Nguyen M, Shore GC. Regulation of apoptosis by endoplasmic reticulum pathways. *Oncogene* 2003;22:8608–8618. [PubMed: 14634622]
58. Criollo A, Maiuri MC, Tasdemir E, Vitale I, Fiebig AA, Andrews D, Molgo J, Diaz J, Lavandro S, Harper F, Pierron G, di Stefano D, Rizzuto R, Szabadkai G, Kroemer G. Regulation of autophagy by the inositol trisphosphate receptor. *Cell Death and Differentiation* 2007;14:1029–1039. [PubMed: 17256008]
59. Smith MI, Deshmukh M. Endoplasmic reticulum stress-induced apoptosis requires bax for commitment and Apaf-1 for execution in primary neurons. *Cell Death and Differentiation* 2007;14:1011–1019. [PubMed: 17218955]
60. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci* 2003;23:8692–8700. [PubMed: 14507968]

Figure 1.

Seven days after implanting the guide cannula, animals were lightly anesthetized for several minutes with a halothane/O₂ mixture, the probe (CMA-12) was inserted and the SON area continuously dialyzed (2 or 4 μ l) for 6 hrs with commercially available sterile perfusion fluid (CMA). Following 2 hrs of recovery, dialysate samples were collected every 5 min (4 μ l/min) or 10 min (2 μ l/min) from 0 to 240 min for quantification of IL-1 β by ICE-LIF. Dependence on neural function in the SON area was determined as peptide changes in dialysate levels during, and after, dialysis of perfusion fluid with tetrodotoxin (TTX; Na⁺ channel antagonist) or combined cadmium and nickel in perfusion fluid without Ca²⁺ (0 Ca²⁺ Cd, Ni; non-selective inhibitors of voltage-gated Ca²⁺ channels) for 30 or 60 min under *basal* conditions, i.e. from 60 to 90 or 120 min, and after osmotic stimulation (HS; 1.5 M NaCl; 15 ml/kg; s.c. at 180 min), i.e. from 180 min to 210 min or 240 min.

Figure 2.

IL-1 β released in the SON area increases linearly in response to acute osmotic stimulation. Conscious rats, dialyzed continuously in the SON area with sterile perfusion fluid (4 μ l/min), were injected with sterile hypertonic saline (HS; 1.5 M NaCl; 15 ml/kg; s.c.) at 180 min and decapitated 1 hr later as described in Fig. 1. Cytokine was quantified by ICE-LIF in dialysate samples collected at 5 min intervals (i.e., 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 40-45, 55-60 min) in each hour. Effects of osmotic stimulation were identified using log-transformed data analyzed by 1-way analysis of variance for repeated measures and Neuman Keul's t test to identify differences ($P < 0.05$; $n=5$) in IL-1 β before (180 min) and after HS (185 to 240 min). Changes in dialysates levels of IL-1 β relative to time ($r^2=0.85$) after HS were determined by Least Squares Linear Regression Analysis (insert; $n=5$ rats; 45 values from 185 to 240 min). **Perfusion fluid ($P < 0.05$ or < 0.01 ; $n=5$):** 180 min < 185 min < 190 min < 195 min < 200 min to 240 min; 200 min < 210 min to 240 min; 205 min < 225 min to 240 min; 210 min < 240.

Figure 3.

IL-1 β released in the SON area during osmotic stimulation is prevented, or markedly reduced, by dialysis of voltage-gated Na⁺ and Ca²⁺ channel blockers. Animals described in Fig. 1 were continually dialyzed with sterile perfusion fluid in the SON area with, and without, addition of tetrodotoxin (TTX, 5 μ M: 30 min, n=4; 60 min, n=3) or cadmium and nickel (0 Ca²⁺Cd, Ni; 0.3mM: 60 min, n=5) for 30 or 60 min before, and after, s.c. injection of hypertonic saline (HS; 1.5 M NaCl; 15 ml/Kg) at 180 min. Statistical evaluation of log-transformed data (2-way analysis of variance for repeated measures and Newman Keul's t test) identified differences in dialysate levels of IL-1 β with time after osmotic stimulation among treatment groups. ^a P < 0.05 vs. perfusion fluid; ^b P < 0.05 TTX-30' vs TTX-60'

Figure 4.

IL-1 β released in the SON area during osmotic stimulation is prevented by dialysis of thapsigargin. For 3 hours, sterile perfusion fluid was dialyzed (2 μ l/min) continuously in the SON area with addition of vehicle (1% DMSO; n=5) or thapsigargin (50 μ M; n=6) between 30 min and 60 min with injection of hypertonic saline (HS; 1.5 M NaCl; 15 ml/kg; i.p.) at 90 min and decapitation 1.5 hr later (i.e. at 180 min). IL-1 β was quantified by ICE-LIF in dialysate samples collected every 10 min throughout the experiment. One-way analysis of variance for repeated measures and Newman Keul's t test identified differences in the log-transformed data with time and drug treatment (rats/group: n=5, DMSO; n=6, thapsigargin). **HS stimulated release of IL-1 β in vehicle (1% DMSO) treated rats:** $P < 0.05$: 0 to 80 min < 100 min to 180 min; 90 min < 110 min < 120 min to 180 min; 100 min < 120 min to 180 min; 120 min < 140 min to 180 min; 130 min < 150 min to 180 min; 140 min, 150 min < 170 min, 180 min; 160 min < 180 min.

Figure 5.

Immunohistochemical localization in the SON of magnocellular neurons (Nissl stain), astroglia in the ventral glial limitans (glial fibrillary acidic protein; GFAP⁺), microglia predominately around blood vessels (MHC Class I antigen; OX-18⁺) or dispersed throughout the SON (complement receptor 3; OX-42⁺) and IL-1 β . Tissue sections are from rats that were unoperated controls or had the probe (CMA-12) inserted with, and without, microdialysis (4 μ l/min) of the SON area with sterile perfusion fluid (CMA) for 6 hrs. Microdialysis activated microglia (OX-42⁺) in the SON to transition from ramified to hypertrophied and amoeboid forms with enhanced expression of IL-1 β . Magnification: 100 μ m indicated by the scale bar.