Original Article

Vasopressin inhibits mitogen-activated protein kinases and activated protein-1 in macrophages

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ABSTRACT

Objectives: We have previously shown that vasopressin could inhibit the upregulation of inflammatory mediators. Expression of inflammatory mediators is tightly regulated by the upstream transcriptional pathway mitogen-activated protein kinases (MAPKs) and activated protein-1 (AP-1). In this study, we elucidated whether vasopressin could inhibit the upregulation of MAPKs/AP-1.

Methods: Murine macrophages (RAW264.7 cells) randomly received lipopolysaccharide (LPS; 100 ng/mL) or LPS plus vasopressin (1000 pg/mL) (designated as the LPS and the LPS + V groups, respectively).

Control groups were run simultaneously. For MAPKs, cells were harvested at 0 minutes, 15 minutes, 30 minutes, 45 minutes, and 60 minutes after reaction. For AP-1, cells were harvested at 60 minutes after reaction. Between-group differences in MAPKs (i.e., extracellular regulated kinase, c-Jun N-terminal kinase, and p38 MAPK) and AP-1 expressions were compared.

Results: Immunoblotting assay data revealed that extracellular regulated kinase concentrations of the LPS + V group that harvested at 45 minutes and 60 minutes, but not at 15 minutes and 30 minutes, were significantly lower than those of the LPS group (all p < 0.001). Concentrations of p38 MAPK of the LPS + V group that harvested at 15 minutes, 30 minutes, 45 minutes, and 60 minutes were also significantly lower than those of the LPS group (all p < 0.001). In addition, immunohistochemistry assay revealed that the AP-1 fluorescence signals of the LPS + V group were weaker than those of the LPS group.

Conclusion: Vasopressin inhibits MAPKs and AP-1 in endotoxin-activated macrophages.

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1. Introduction

Vasopressin is a nanopeptide that is synthesized in paraventricular and supraoptic nuclei in the hypothalamus. The established physiological characteristics of vasopressin include vasoconstriction, antidiuresis, and behavioral regulation. Moreover, clinical data revealed that septic shock patients tend to have low circulating levels of endogenous vasopressin. Therefore, clinical guidelines have suggested exogenous vasopressin supplement as part of the therapies against severe sepsis.

The concept of vasopressin supplement against sepsis is supported by previous data that exogenous vasopressin replacement could decrease sepsis-induced pulmonary inflammation and preserve renal and mesenteric blood flow. We have further confirmed that vasopressin could significantly inhibit the upregulation of inflammatory mediators. Together, these data conform the potent anti-inflammatory effects of vasopressin.

Expression of inflammatory molecules is tightly regulated by the upstream regulatory pathway mitogen-activated protein kinases (MAPKs) and activated protein-1 (AP-1). To date, the question of whether vasopressin can exert significant effects on inhibiting the upregulation of MAPKs/AP-1 remains unstudied. To
elucidate further, we thus conducted this cellular study with the hypothesis that vasopressin can inhibit the upregulation of MAPKs [i.e., extracellular regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK] and AP-1 in endotoxin-activated murine macrophages.

2. Materials and methods

2.1. Cell cultures and cell activation protocols

This study employed RAW264.7 cells, an immortalized murine macrophage-like cell line, to facilitate the investigation. Cultured with mixtures of Dulbecco’s modified Eagle’s medium (Life Technologies, Grand Island, NY, USA), 10% fetal bovine serum, and 1% penicillin/streptomycin (Life Technologies), RAW264.7 cells were incubated in a humidified chamber at 37°C and maintained with a gas mixture of 95% air and 5% CO2. To activate cells, confluent RAW264.7 cells were stimulated with lipopolysaccharide (LPS, 100 ng/mL), a gram (-) endotoxin from Escherichia coli (serotype 0127:B8; Sigma-Aldrich, St. Louis, MO, USA), according to our previous report.9

2.2. Experimental protocols

Confluent RAW264.7 cells randomly received phosphate buffered saline (PBS, Life Technologies), vasopressin (1000 pg/mL, Life Technologies), LPS (100 ng/mL, Sigma-Aldrich), or LPS plus vasopressin and designated as the PBS, the V, the LPS, and the LPS+V groups, respectively. Vasopressin was administered immediately after LPS. The dosage and timing of administration of vasopressin were also determined according to our previous report.9

2.3. Immunoblotting assay for MAPKs

Cell cultures were harvested at 0 minutes, 15 minutes, 30 minutes, 45 minutes, and 60 minutes after reaction with LPS or at comparable time points in groups without LPS. Cell harvesting and cell culture processes were performed as we have previously reported.11 In brief, RAW264.7 cells were washed, scraped, and centrifuged at 1500g for 5 minutes. The cell pellet was resuspended in 5 mL cell lysis buffer [10mM HEPES (pH 7.9), 1.5mM MgCl2, 10mM KCl, 0.5mM dithiothreitol, and 0.2mM phenylmethylsulfonyl fluoride] and centrifuged again at 1500g for 5 minutes. Cells were resuspended again in cell lysis buffer and allowed to swell on ice for 10 minutes followed by homogenization. Homogenates were centrifuged at 3300g for 15 minutes at 4°C. The supernatants were saved and the pellets were discarded. The protein concentration of each sample was measured using a bicinchoninic acid protein assay (Pierce Biotechnology Inc., Rockford, IL, USA), according to our previous report.9

Moreover, the concentrations of ERK of the LPS group were significantly higher than those of the PBS group (all p < 0.005). By contrast, the concentrations of ERK, JNK, and p38 MAPK of the PBS group that harvested at 15 minutes, 30 minutes, 45 minutes, and 60 minutes after reaction were also significantly lower than those of the LPS group (all p < 0.005; Figure 1). Similarly, the concentrations of JNK of the LPS+V group that harvested at 15 minutes, 30 minutes, 45 minutes, and 60 minutes after reaction were significantly higher than those of the PBS group (all p < 0.0001).

Moreover, the concentrations of ERK of the LPS+V group that harvested at 45 minutes and 60 minutes after reaction, but not at 15 minutes and 30 minutes, were significantly lower than those of the LPS group (p = 0.005 and p = 0.013, respectively; Figure 1). Similarly, the concentration of JNK of the LPS+V group that harvested at 15 minutes, 30 minutes, 45 minutes, and 60 minutes after reaction were also significantly lower than those of the LPS group (all p < 0.001; Figure 1). The concentrations of p38 MAPK of the LPS+V group that harvested at 15 minutes, 30 minutes, and 45 minutes, but not at 60 minutes, were also significantly lower than those of the LPS group (all p < 0.001; Figure 1).

3.2. Vasopressin inhibits AP-1

Immunohistochemistry assay data revealed that the fluorescence signals of the PBS and V groups were low (data not shown). By contrast, the fluorescence signals of the LPS+V group were weaker than those of the LPS group (Figure 2).
4. Discussion

Data from this study confirm our hypothesis that vasopressin can inhibit endotoxin-induced upregulation of MAPKs/AP-1 in activated murine macrophages. Using the same model, we have previously demonstrated that vasopressin could inhibit endotoxin-induced upregulation of inflammatory mediators and hence confirmed the potent anti-inflammatory effects of vasopressin. As expression of inflammatory mediators is tightly regulated by the upstream transcriptional pathway MAPKs/AP-1, and hence confirmed the potent anti-inflammatory effects of vasopressin. The mechanisms underlying the anti-inflammatory effects of vasopressin may involve its effects on inhibiting MAPKs/AP-1 and the subsequent inhibition of inflammatory mediators.

Though data from this study confirmed the effects of vasopressin on inhibiting MAPKs/AP-1 upregulation, the underlying mechanisms remain unstudied. As our data revealed that vasopressin could inhibit the upregulation of all three members of MAPKs (i.e., ERK, JNK, and p38 MAPK) as well as AP-1, these data seem to suggest that the mechanisms underlying the effects of vasopressin on inhibiting MAPKs/AP-1 should involve the regulatory pathways of MAPKs/AP-1. It is well-established that endotoxin-induced upregulation of MAPKs/AP-1 is regulated by the complex of toll-like receptor 4 (TLR4)/myeloid differentiation 2 (MD2).12 For activation of the TLR4/MD2 complex, endotoxin needs to be firstly transported by the soluble lipopolysaccharide-binding protein and then be presented to the cell surface pattern recognition receptor cluster of differentiation 14 (CD14).13 CD14 then transfers LPS to the complex of TLR4/MD2, which then leads to the activation of MAPKs/AP-1 and the subsequent upregulation of inflammatory mediators.12 Judging from these data, we thus speculate that vasopressin might act through inhibiting lipopolysaccharide-binding protein, CD14, and/or the TLR4/MD2 complex to exert its effects on inhibiting MAPKs/AP-1 activation.

The mechanisms underlying the effects of vasopressin on inhibiting MAPKs/AP-1 remain to be elucidated; we thus acknowledge this as part of the study limitations. Moreover, in addition to MAPKs/AP-1, expression of inflammatory mediators is
also regulated by the transcriptional pathway nuclear factor-κB. The question of whether vasopressin can exert similar effects on inhibiting nuclear factor-κB expression remains unanswered. We therefore also acknowledge this as part of the study limitations. Finally, this is a cellular study and therefore further data interpretation should be performed with caution.

In conclusion, vasopressin inhibits MAPKs/AP-1 activation in endotoxin-activated macrophages.

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