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Selenate Enhances STAT3 Transcriptional Activity in Endothelial Cells: Differential Actions of Selenate and Selenite on LIF Cytokine Signaling and Cell Viability

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Abstract

Sodium selenate may have utility in treating Alzheimer's disease and diabetes; however, its impact on the associated proinflammatory cytokine signaling of endothelial cells has not been investigated. We report that treatment of human microvascular endothelial cells with sodium selenate at a pharmacological dose (100 μM) enhanced tyrosine phosphorylation of nuclear STAT3 on Y705 in response to IL-6-type cytokine, leukemia inhibitory factor (LIF), indicative of enhanced STAT3 activity. Accordingly, STAT3 nuclear binding to DNA was increased, as well as LIF-induced gene expression of chemokine (C-C motif) ligand 2 (CCL2). CCL2 plays a key role in inflammatory processes associated with neuronal degenerative and vascular diseases. The enhancing action of selenate on LIF-induced STAT3 Y705 phosphorylation was replicated by vanadate and a specific inhibitor of protein tyrosine phosphatase, non-receptor type 1 (PTP1B). Moreover, we observed that selenite, the cellular reduction bioproduct of selenate but not selenate itself, inhibited enzymatic activity of human recombinant PTP1B. Our findings support the conclusion that in human microvascular endothelial cells selenate has a vanadate-like effect in inhibiting PTP1B and enhancing proinflammatory STAT3 activation. These findings raise the possibility that beneficial actions of supranutritional levels of selenate for treating Alzheimer's and diabetes may be offset by a proinflammatory action on endothelial cells.

Keywords

Inflammation; endothelial cell; protein tyrosine phosphatase; gene expression; chemokine (C-C motif) ligand 2; vanadate

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1. Introduction

Sodium selenate (Na_2SO_4) is a water-soluble molecule that is commonly used to supplement commercial fertilizers in order to introduce the essential micronutrient selenium into the food chain and prevent diseases caused by selenium deficiency [1]. Recently, sodium selenate was demonstrated to reverse memory deficits in Alzheimer's disease models ostensibly by causing dephosphorylation of tau proteins through activation of serine/threonine protein phosphatase 2A (PP2A) [2]. For that reason, sodium selenate is suggested as a new medication in treating Alzheimer's [2]. In addition, sodium selenate has been shown to improve insulin sensitivity in several animal models of type 1 and 2 diabetes mellitus by several possible mechanisms, including inhibition of protein tyrosine phosphatases, increased peroxisome proliferator-activated receptor γ (PPARγ) expression, and improved cellular redox status [3–7]. Although sodium selenate was thought to hold promise as a preventative agent for cancers, recent meta-analysis cast doubts on that possibility [8].

Diabetes and Alzheimer's disease are both associated with chronic inflammation that has an especially negative impact on endothelial cells [9–12]. Moreover, the inflammatory state is associated with elevated levels of interleukin 6 (IL-6) family cytokines, i.e., IL-6 and/or leukemia inhibitory factor (LIF) [13–15], which further sustain inflammation. The IL-6 cytokines signal through the homo- or heterodimerization of the gp130 transmembrane receptor that couples to Just Another Kinase - Signal Transducers and Activators of Transcription (JAK-STAT) signaling, most notably activation of the STAT3 transcription factor [16]. Canonical STAT3 gene induction is absolutely dependent upon Y705 phosphorylation, but is thought to be further enhanced by S727 phosphorylation [16]. However, the actions of sodium selenate on endothelial cells and IL-6 type cytokine signaling have received scant attention. The original rationale for this study was to assess whether sodium selenate would decrease STAT3 activity in endothelial cells by activating PP2A, which is reported to dephosphorylate the S727 residue of STAT3 [17]. Contrary findings were obtained that indicate sodium selenate has pro-inflammatory actions on endothelial cells.

2. Experimental section

2.1. Materials

Sodium selenate (Cat. # S8295), sodium selenite (Cat. # S5261), and hydrocortisone were purchased from Sigma-Aldrich (St. Louis, MO). Calyculin A was from Santa Cruz Biotechnology (Santa Cruz, CA). Epidermal growth factor was from BD Biosciences (Bedford, MA). Sodium orthovanadate that was "activated" (i.e., depolymerised) so as to convert it into a more potent inhibitor of protein tyrosine phosphatases [18] was from Boston Bioproducts (Ashland, MA). Cell culture reagents were from Invitrogen (Carlsbad, CA). Fetal bovine serum was from Thermo Scientific (Waltham, MA). PTP1B inhibitor (3-(3,5-dibromo-4-hydroxy-benzoyl)-2-ethyl-benzofuran-6-sulfonicacid-(4-(thiazol-2-ylsulfamyl)-phenyl)-amide [19] and LIF were from EMD Millipore (Billerica, MA). Antibodies for total and STAT3 phosphorylated on Y705 (pY705) were from Cell Signaling Technology (Danvers, MA). Anti-STAT3 phosphorylated on S727 (pS727) antibody was from Millipore. Nuclear extracts of proteins were prepared using a kit from Active Motif (Carlsbad, CA). The TransAM STAT3 kit for the binding of nuclear extracts to a STAT3 consensus oligonucleotide was from Active Motif. SDS-PAGE reagents and 4–20% Tris-HCl Ready Gels were from Bio-Rad Laboratories (Hercules, CA). Western blot Odyssey blocking buffer, nitrocellulose membranes, molecular weight marker, and IRDye Secondary Antibodies were from LI-COR Biosciences (Lincoln, NE). The T cell protein tyrosine

phosphatase (TC-PTP) inhibitor [20] was a gift from Dr. Zhong-Yin Zhang (Indiana University).

2.2 Cell culture

Human microvascular endothelial cells (HMEC-1) were obtained from the Centers for Disease Control and Prevention (CDC) and grown in MCDB 131 supplemented with 15% FBS, 10 ng/mL epidermal growth factor, 10 mM glutamine, 1 μg/mL hydrocortisone, and antibiotic-antimycotic. For experiments, cells grown on 100 mm dishes to near confluency were incubated in medium with 0.5% FBS beginning 12–15 h beforehand.

2.3 Experimental Design

For maintenance and experimental purposes, cells were kept at 37°C in a humidified incubator with 95% air and 5% CO_2. Cells in 10 mL of medium were pretreated for 2 h with 100 μM sodium selenate or sodium selenite by adding 40 μL of a 25 mM stock solution prepared in distilled water and filter sterilized. Control cells were treated with an equal volume of H_2O. Cells were then incubated (without changing the medium) for an additional hour with or without the addition of 2 ng/mL LIF. For assessing the role of PTP1B, cells were pretreated 1 h with 50 μM PTP1B inhibitor or 0.02% v/v DMSO (vehicle) before dosing with LIF. Dishes were placed on ice, the medium removed, and washed with ice cold Hanks' balanced salt solution (RNA extraction) or PBS with phosphatase inhibitors (nuclear extractions).

2.4 Western blot analysis

Protein in nuclear extracts was measured using the Bio-Rad DC Protein Assay. Equal amounts of protein were resolved by SDS-PAGE and transferred to nitrocellulose membranes and probed simultaneously either for pY705 STAT3 and STAT3 or pS727 STAT3 and STAT3. Immunoreactive bands were quantified using the LI-COR Odyssey detection system.

2.5 Cell counts and viability

Cell viability was assessed by measuring reduction of alamarBlue (Invitrogen), which is a measure of innate metabolic activity and status of the intracellular reducing environment. HMEC-1 were treated for 2 h with vehicle, 100 μM sodium selenate, or 100 μM sodium selenite. AlamarBlue (10% v/v) was then added and the incubation continued for 1 h. Reduced and oxidized forms of alamarBlue were determined by measuring absorbance of the medium at 570 and 600 nm, respectively. Percent of alamarBlue reduced by cells was calculated according to the manufacturer's instructions. For determining cell number, HMEC-1 were incubated for 24 h with vehicle, 100 μM sodium selenate, 100 μM sodium selenite, or 100 μM calyculin A (positive control). The medium was removed and the growth surface of the dishes coated with 1 mL TrypLE Express (Invitrogen) and incubated at 37°C for 5 min. Plates were gently smacked on the working surface of the hood to help detach the cells and 3 mL of growth medium with 15% FBS was added. HMEC-1 were completely dislodged using a cell lifter and further dispersed by gently pipetting up and down several times. Numbers of total and dead cells were determined with a NucleoCounter (ChemoMetec). Cell lift off did not occur with either selenate or selenite over 24 h; however, it was observed with calyculin A. For that reason, the medium of cells treated with calyculin A was collected and spun at 150×g for 5 min. The pelleted cells were resuspended in 2 mL of growth medium. The number of total and dead cells in the resuspension was added to the number of cells left attached to the dish.

2.6 STAT3 binding

Aliquots of nuclear extracts containing equal amounts of protein (4 μg) were assayed for STAT3 binding using an Elisa-based assay from Active Motif as described by the manufacturer. Plates were read using a BioTek Synergy 2 multi-mode microplate reader.

2.7 PTP1B activity

A colorimetric assay kit from EMD Chemicals USA was used to assess the effects of sodium selenate and sodium selenite on PTP1B activity. Vehicle (water) or test compound at two different concentrations was added to the reaction mixture containing 25 ng/mL PTP1B and 75 μM substrate. Reactions were carried out in duplicate. Wells containing enzyme, but no substrate, served as the blank. The reaction was carried out for 20 min at 30°C. Developed plates were read using the BioTek Synergy 2 plate reader.

2.8 Real Time PCR

RNA was extracted using the RNAqueous kit from Applied Biosystems/Ambion (Austin, TX). cDNA was prepared using the SuperScript VILO cDNA synthesis Kit (Invitrogen). Amplification was carried out with the TaqMan Gene Expression Master Mix and TaqMan Gene expression assays (Applied Biosystems). The TaqMan Gene Expression primers used were CCL2 (Hs00234140_m1), suppressor of cytokine signaling 3 (SOCS3; Hs02330328_s1), and GAPDH (Hs99999905_m1). Real time PCR was carried out with the BioRad iQ5 and expression normalized using the housekeeping gene GAPDH. Data were analysed with iQTM 5 optical system software (Bio-Rad).

2.9 Statistical Analysis

Results are expressed as mean ± SEM for n independent experiments. Statistical significance was assessed by one way ANOVA followed by an appropriate post hoc test. A P value 0.05 was considered significant.

3. Results

3.1 Selenate enhances LIF-induced nuclear STAT3 phosphorylation

Canonically STAT3 is activated by phosphorylation on Y705, which leads to STAT3 homo- or heterodimer formation, nuclear translocation, and enhanced DNA binding. To assess what effect sodium selenate has on canonical STAT3 signaling, human microvascular endothelial cells were pretreated for 2 h with 100 μM sodium selenate, and then treated for 1 h with the IL-6 type cytokine LIF. Levels of STAT3 phosphorylated on Y705 were enhanced with LIF as expected (Figs. 1A and 1B). Pretreatment with sodium selenate further increased LIF-induced STAT3 Y705 phosphorylation by 72%. By itself, sodium selenate showed a tendency to increase nuclear STAT3 Y705 phosphorylation levels, but this did not reach statistical significance. Sodium selenate was shown to enhance enzymatic activity of purified PP2A [2] and PP2A was linked to STAT3 S727 dephosphorylation in HeLa cells [21], T-cells [17], and rat vascular smooth muscle cells [22]. However, there was no significant difference in nuclear STAT3 S727 phosphorylation between LIF and LIF with sodium selenate (Figs. 1C and 1D). Rather, unexpectedly, pretreatment of HMEC-1 with sodium selenate made the increase in STAT3 S727 phosphorylation seen with LIF treatment at 1 h more readily observable (Figs. 1C and 1D). As seen from Figs 2A and 2B, pretreatment of HMEC-1 with the same concentration of sodium selenite (and under the same conditions as sodium selenate) did not affect LIF-induced nuclear STAT3 Y705 phosphorylation.

3.2 Effects of selenate and selenite on cell viability

In our experiments, HMEC-1 were treated with sodium selenate at a concentration (100 μM) that is pharmacological and was used by others to assess the utility of sodium selenate to enhance PP2A activity in both *in vitro* and *in vivo* models of Alzheimer's disease [2,23]. To address the possibility that this concentration of sodium selenate or selenite had adverse effects on cell metabolism or viability over the 3 h of exposure that was used, the impact of these salts on reduction of alamarBlue by HMEC-1 was assessed. No difference was found in the % reduction of alamarBlue among cells treated with vehicle (51.7 ± 5.2), 100 μM sodium selenate (55.1 ± 5.2), or 100 μM sodium selenite (59.0 ± 8.0), n = 3. Exposure of HMEC-1 to 100 μM selenate for 24 h did not affect cell number; however, 100 μM selenite modestly reduced the number of cells consistent with either inhibition of proliferation or induction of cell death (Fig. 3). The PP1/PP2A inhibitor calyculin A clearly had an adverse effect on cells, causing a marked decrease in both total and live cells, as well as the ratio of live to total cells (Fig. 3). Observations made under a light microscopy were consistent with these findings: no membrane blebbing or cell lift-off was seen with either selenate or selinite treatments, although the number of cells appeared to be less with the latter; with calyculin A cells adopted a rounded appearance and floating cells were seen.

3.3 Selenate enhances nuclear STAT3 binding

We next addressed whether enhanced nuclear STAT3 Y705 phosphorylation was associated with increased binding of nuclear extracts to a STAT3 consensus binding motif. As Figure 4 shows, LIF increased STAT3 binding in nuclear extracts by nearly 10-fold. Pretreatment with sodium selenate further increased nuclear STAT3 binding by 68%. By itself, selenate did not affect STAT3 binding.

3.4 Effect of selenate on LIF-induced gene expression

To assess the functional significance of enhanced STAT3 Y705 phosphorylation and DNA binding, we examined whether LIF-induced gene expression was also enhanced by sodium selenate. We chose to look at expression of 2 genes that are induced by LIF in HMEC-1 and which are reported to contain STAT3 binding sites in their promoters based on experimental evidence [24,25] and computational (P-Match) analysis [unpublished observation]. At 1 h, 2 ng/mL LIF produced an 8.7 ± 2.3 (n = 5) and 22.3 ± 3.4 (n = 5) fold increase in CCL2 and SOCS3 mRNA levels, respectively. As Figure 5 shows, selenate pretreatment produced a significant increase in LIF-induced CCL2 mRNA levels (P = 0.0036). Although there was a tendency for sodium selenate to enhance LIF-induced SOCS3 expression, this did not reach statistical significance (P = 0.0594). However, it should be noted that the fold-increase induced by LIF (without sodium selenate) in SOCS3 expression was greater than that for CCL2 expression, consistent with the possibility that induction of SOCS3 expression by LIF was already near maximal.

3.5 Vanadate and a PTP1B inhibitor mimic the actions of selenate

Sodium orthovanadate is a well characterized inhibitor of protein tyrosine phosphatases [26]. We thus sought to compare the actions of sodium selenate in enhancing nuclear STAT3 Y705 phosphorylation to those of vanadate. As Figure 6A shows, vanadate produced a comparable increase (71%) in LIF-induced nuclear STAT3 tyrosine phosphorylation as selenate, suggesting that sodium selenate was acting to inhibit a protein tyrosine phosphatase. We next sought to determine which protein tyrosine phosphatase was being targeted by sodium selenate. TC-PTP is a protein tyrosine phosphatase found in the nucleus and reported to target STAT3 [27,28]. However, pretreatment of HMEC-1 with a 100 nM concentration of a highly specific TC-PTP inhibitor [20] that has cellular activity in the range of 5–20 nM failed to enhance nuclear STAT3 Y705 phosphorylation (data not

shown). In contrast, pretreatment with a specific PTP1B inhibitor [19] produced a 98% increase in LIF-induced STAT3 Y705 phosphorylation and also alone enhanced STAT3 tyrosine phosphorylation 3-fold (Figs. 6B & 6C). STAT3 pY705 was previously shown to be a direct target for dephosphorylation by PTP1B [29].

3.6 Selenite but not selenate inhibits PTP1B activity

The selenate ion contains 4 oxygen atoms and based on its structure is not expected to inhibit protein tyrosine phosphatases. On the other hand, the reduced bioproduct of selenate, the selenite anion has 3 oxygen atoms and structurally resembles vanadate. To establish the possibility that PTP1B was being targeted by selenite and not selenate in our cells, we assessed the effect of these ions on PTP1B activity using a colorimetric assay. As Figure 7 shows, under optimal conditions for PTP1B activity and relatively high level of enzyme compared to the cellular situation, neither sodium selenate nor sodium selenite affected PTP1B enzymatic activity at 500 μM. However, at a 10-fold higher concentration, selenite, but not selenate, produced a marked inhibition in PTP1B activity.

4. Discussion

Sodium selenate is a water soluble salt that is readily absorbed in the gastrointestinal tract with excellent bioavailability [1]. Previously, sodium selenate was proven to activate PP2A (a serine-threonine phosphatase) in neuroblastoma cells over a concentration range of 0.1 nM to 10 μM [2] or 10 – 100 μM [23]. However, we did not observe a reduction in STAT3 S727 phosphorylation with sodium selenate in human microvascular endothelial cells. Rather, we report for the first time that sodium selenate enhances the levels of phosphorylated tyrosine 705 (pY705) of STAT3 when co-administered with the cytokine LIF (Figs. 1A & 1B). It is worth mentioning that sodium selenate did not affect basal levels of tyrosine phosphorylation in HMEC-1 when administered alone. This indicates that the effect of sodium selenate on STAT3 pY705 levels in HMEC-1 is only observed when co-administered with an agonist that activates this transcription factor. Enhanced STAT3 tyrosine phosphorylation had functional significance, as STAT3 binding and gene expression were enhanced (Figs. 4 & 5). We observed increased gene expression of CCL2 in response to LIF in endothelial cells pretreated with sodium selenate. CCL2 is a cytokine/chemokine that plays a role in chemotaxis for monocytes and basophils and is a factor in the pathogenesis of inflammatory diseases with monocytic infiltration, e.g. rheumatoid arthritis, and atherosclerosis [30].

To our knowledge, our study is the first to show that the addition of sodium selenate to any cell type results in enhanced tyrosine phosphorylation of a protein. Others previously reported that the chronic feeding of type 2 diabetic dbdb mice with supranutritional doses of sodium selenate ameliorated insulin sensitivity and that this was associated with reduced cytosolic protein tyrosine phasphatase activity in liver and skeletal muscle in comparison with selenium deficiency or supranutritional doses of selenite [4,31]. These findings would suggest that the beneficial action of selenate *in vivo* was to enhance the tyrosine phosphorylation events linked to insulin signaling. Moreover, this group did not see any effect of sodium selenate *in vitro* on the PTP enzymatic activity of crude liver homogenates. However, they did report that sodium selenite was able to inhibit hepatic PTP and concluded that sodium selenate was most likely metabolized *in vivo* to sodium selenite. Our findings support this possibility and suggest that endothelial cells have the capacity to reduce selenate to selenite. The exact mechanism by which metabolism of selenate occurs in human microvascular endothelial cells is not known and is an area for future investigation.

For the first time, we report that sodium selenite inhibits purified recombinant PTP1B, which is unlikely to be an action specific for this particular protein tyrosine phosphatase.

Our study does not provide definitive evidence that the actions of sodium selenate in enhancing LIF-induced STAT3 tyrosine phosphorylation are due solely to the targeting of PTP1B. Other protein tyrosine phosphatases could be involved. However, we do provide evidence using a specific inhibitor that PTP1B is involved in opposing LIF-induced STAT3 Y705 phosphorylation in HMEC-1 (Figs. 6B & 6C). Moreover, the actions of sodium selenate in enhancing nuclear STAT3 tyrosine phosphorylation could be mimicked by sodium vanadate, supposing the conclusion that a protein tyrosine phosphatase was involved (Fig. 6A).

Although selenite but not selenate inhibited PTP1B *in vitro*, selenate but not selenite enhanced LIF-induced STAT3 Y705 phosphorylation in our cultured microvascular endothelial cells. This point is not inconsistent with our conclusion that selenite, the bioproduct of selenate, was responsible for enhanced STAT3 Y705 phosphorylation in HMEC-1 if (a) the cellular uptake of selenate by HMEC-1 is greater than that of selenite and/or (b) selenite is further metabolized by HMEC-1. Consistent with either possibility is our finding that HMEC-1 cells were fairly tolerant of exposure to 100 μM sodium selenite. Exposure of neuronal cells, such as rat cultured hippocampal slices [2] and human SH-5Y5Y neuroblastoma cells [23], to 100 μM sodium selenite for 12 or 24 h was shown to be toxic, presumably due to the pro-oxidant nature of selenite to cause reactive oxygen species generation [32]. In agreement with our findings with human microvascular endothelial cells (Fig. 3), neuronal cells were found to show no adverse effects from exposure to 100 μM sodium selenate for upwards of 24 h [2,23].

Recently, an anorexigenic effect of selenate was observed. Müller and others noticed a decrease in feed consumption and average body weight of mice kept on a selenium deficient diet if they were also given weekly increasing supranutritional doses of sodium selenate, but not if they were given no supplement or sodium selenite [31]. Since leptin activates STAT3 signaling in the central nervous system and this activation is thought to be the key player in reducing appetite [33], we hypothesize that the anorexigenic effect of selenate is mediated centrally by enhanced STAT3 activation.

In our study, we used a dose of sodium selenate that is unattainable through normal dietary intake, but we think that our results have pharmacological relevance. Currently, sodium selenate is proposed as a new medication for Alzheimer's disease [2]. However, it is evident from this study that sodium selenate has an enhancing effect on the JAK-STAT3 signaling pathway. This may, subsequently, promote the end-products of this pathway, with some being inflammatory in nature [34]. We suspect that long-term treatment with sodium selenate may work hand-in-hand with LIF and IL-6 to promote general inflammatory status in the body. Furthermore, chronic selenate might accelerate atherosclerosis and other inflammatory diseases, e.g. rheumatoid arthritis, associated with increased IL-6 cytokine signaling [35]. STAT3 activation is also implicated in tumorigenesis [36]. Therefore, it is credible, from the given data above, that long-term treatment for Alzheimer's patients with sodium selenate might precipitate tumorigenesis. While our study was done acutely, the findings do underscore the question of how selenate is metabolized by different cells. In brief, the safety of sodium selenate as a medication for Alzheimer's disease should be evaluated further by *in vivo* experiments focusing on STAT3 inflammatory signaling.

5. Conclusions

Here we show that administration of sodium selenate at a pharmacological dose to human microvascular cells (HMEC-1), increased STAT3 Y705 phosphorylation in response to LIF. In addition, this treatment increased STAT3 DNA binding and transcriptional activity. Our findings suggest that increased STAT3 Y705 phosphorylation and DNA binding may

enhance the physiological and pathological outcomes of pro-inflammatory cytokine signaling. Thus, the safety of sodium selenate at supranutritional levels as a medication for Alzheimer's disease and diabetes will need to be carefully assessed.

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Table of Abbreviations

CCL2	chemokine (C-C motif) ligand 2
HMEC-1	Human microvascular endothelial cells 1
IL-6	interleukin 6
JAK	Just Another Kinase
LIF	leukemia inhibitory factor
PBS	phosphate buffered saline
PP1	protein phosphatase 1
PP2A	protein phosphatase 2A
PPARγ	peroxisome proliferator-activated receptor γ
PTP1B	protein tyrosine phosphatase, non-receptor type 1
SOCS3	suppressor of cytokine signaling 3
STAT	Signal Transducers and Activators of Transcription
TC-PTP	T cell protein tyrosine phosphatase

References

1. Hartikainen H. J Trace Elem Med Biol. 2005; 18:309–318. [PubMed: 16028492]
2. Corcoran NM, Martin D, Hutter-Paier B, Windisch M, Nguyen T, Nheu L, Sundstrom LE, Costello AJ, Hovens CM. J Clin Neurosci. 2010; 17:1025–1033. [PubMed: 20537899]
3. Battell ML, Delgatty HL, McNeill JH. Mol Cell Biochem. 1998; 179:27–34. [PubMed: 9543346]
4. Mueller AS, Pallauf J. J Nutr Biochem. 2006; 17:548–560. [PubMed: 16443359]
5. Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Diabetologia. 1996; 39:3–11. [PubMed: 8720597]
6. Iizuka Y, Ueda Y, Yagi Y, Sakurai E. Biol Trace Elem Res. 2010; 138:265–271. [PubMed: 20177813]
7. Atalay M, Bilginoglu A, Kokkola T, Oksala N, Turan B. Mol Cell Biochem. 2011; 351:125–131. [PubMed: 21246260]
8. Dennert G, Zwahlen M, Brinkman M, Vinceti M, Zeegers MP, Horneber M. Cochrane Database Syst Rev. 2011; 5:CD005195. [PubMed: 21563143]
9. Booz GW. Free Radic Biol Med. 2011; 51:1054–1061. [PubMed: 21238581]
10. Grammas P, Martinez J, Miller B. Expert Rev Mol Med. 2011; 13:e19. [PubMed: 21676288]
11. Garcia C, Feve B, Ferré P, Halimi S, Baizri H, Bordier L, Guiu G, Dupuy O, Bauduceau B, Mayaudon H. Diabetes Metab. 2010; 36:327–338. [PubMed: 20851652]
12. van den Oever IA, Raterman HG, Nurmohamed MT, Simsek S. Mediators Inflamm. 2010; 2010:792393. [PubMed: 20634940]

13. Soilu-Hänninen M, Broberg E, Röyttä M, Mattila P, Rinne J, Hukkanen V. Acta Neurol Scand. 2010; 121:44–50. [PubMed: 20074285]
14. Rensink AA, Gellekink H, Otte-Höller I, ten Donkelaar HJ, de Waal RM, Verbeek MM, Kremer B. Acta Neuropathol. 2002; 104:525–533. [PubMed: 12410400]
15. Goldberg RB. J Clin Endocrinol Metab. 2009; 94:3171–3182. [PubMed: 19509100]
16. Kurdi M, Booz GW. J Cardiovasc Pharmacol. 2007; 50:126–141. [PubMed: 17703129]
17. Woetmann A, Nielsen M, Christensen ST, Brockdorff J, Kaltoft K, Engel AM, Skov S, Brender C, Geisler C, Svejgaard A, Rygaard J, Leick V, Odum N. Proc Natl Acad Sci USA. 1999; 96:10620–10625. [PubMed: 10485875]
18. Gordon JA. Methods Enzymol. 1991; 201:477–482. [PubMed: 1943774]
19. Sun T, Ye F, Ding H, Chen K, Jiang H, Shen X. Cytokine. 2006; 35:88–94. [PubMed: 16949833]
20. Zhang S, Chen L, Luo Y, Gunawan A, Lawrence DS, Zhang ZY. J Am Chem Soc. 2009; 131:13072–13079. [PubMed: 19737019]
21. Togi S, Kamitani S, Kawakami S, Ikeda O, Muromoto R, Nanbo A, Matsuda T. Biochem Biophys Res Commun. 2009; 379:616–620. [PubMed: 19121623]
22. Liang H, Venema VJ, Wang X, Ju H, Venema RC, Marrero MB. J Biol Chem. 1999; 274:19846–19851. [PubMed: 10391929]
23. van Eersel J, Ke YD, Liu X, Delerue F, Kril JJ, Götz J, Ittner LM. Proc Natl Acad Sci USA. 2010; 107:13888–13893. [PubMed: 20643941]
24. Auernhammer CJ, Kopp FB, Vlotides G, Dorn F, Isele NB, Spöttl G, Cengic N, Weber MM, Senaldi G, Engelhardt D. Neuroimmunomodulation. 2004; 11:224–232. [PubMed: 15249728]
25. Ikeda T, Sato K, Kuwada N, Matsumura T, Yamashita T, Kimura F, Hatake K, Ikeda K, Motoyoshi K. J Leukoc Biol. 2002; 72:1198–1205. [PubMed: 12488502]
26. Heneberg P. Curr Med Chem. 2009; 16:706–733. [PubMed: 19199933]
27. Tiganis T, Flint AJ, Adam SA, Tonks NK. J Biol Chem. 1997; 272:21548–21557. [PubMed: 9261175]
28. Yamamoto T, Sekine Y, Kashima K, Kubota A, Sato N, Aoki N, Matsuda T. Biochem Biophys Res Commun. 2002; 297:811–817. [PubMed: 12359225]
29. Lund IK, Hansen JA, Andersen HS, Møller NP, Billestrup N. J Mol Endocrinol. 2005; 34:339–351. [PubMed: 15821101]
30. Rollins BJ. Mol Med Today. 1996; 2:198–204. [PubMed: 8796888]
31. Müller AS, Most E, Pallauf J. J Anim Physiol Anim Nutr (Berl). 2005; 89:94–104. [PubMed: 15787978]
32. Brozmanová J, Mániková D, Vlčková V, Chovanec M. Arch Toxicol. 2010; 84:919–938. [PubMed: 20871980]
33. Hall JE, da Silva AA, do Carmo JM, Dubinion J, Hamza S, Munusamy S, Smith G, Stec DE. J Biol Chem. 2010; 285:17271–17276. [PubMed: 20348094]
34. Kurdi M, Booz GW. Congest Heart Fail. 2010; 16:234–238. [PubMed: 20887622]
35. Libby P. Am J Med. 2008; 121(Suppl 1):S21–S31. [PubMed: 18926166]
36. Aggarwal BB, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, Koca C, Dey S, Sung B. Ann N Y Acad Sci. 2009; 1171:59–76. [PubMed: 19723038]

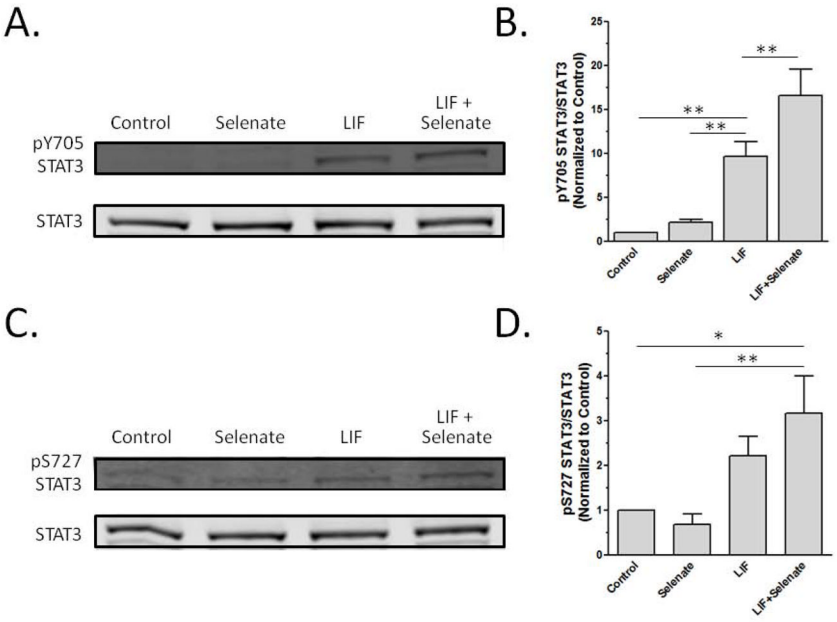

Figure 1.

Selenate enhances nuclear STAT3 phosphorylation. HMEC-1 were pretreated for 2 h with 100 μM sodium selenate or vehicle and then 2 ng/mL LIF or vehicle was added for 1 h. Nuclear extracts were prepared and levels of (A) pY705 STAT3 and STAT3 or (C) pS727 STAT3 and STAT3 evaluated by Western blotting. (B & D) Results were quantified using the LI-COR Odyssey system and the ratio of phosphorylated STAT3 to total STAT3 determined. Results of individual experiments were normalized to the control. Values are mean ± SEM of 7 independent observations. *$P < 0.05$; **$P < 0.01$ (column below line origin vs. column below line end) by ANOVA and Newman-Keuls Multiple Comparison Test.

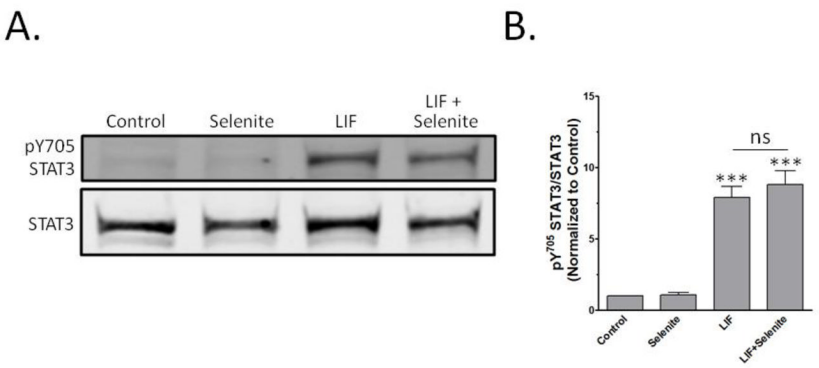

Figure 2.

Selenite does not enhance nuclear STAT3 phosphorylation. HMEC-1 were pretreated for 2 h with 100 μM sodium selenite or vehicle and then 2 ng/mL LIF or vehicle was added for 1 h. (A) Nuclear extracts were prepared and levels of pY705 STAT3 evaluated by Western blotting. (B) Results were quantified using the LI-COR Odyssey system and the ratio of phosphorylated STAT3 to total STAT3 determined. Results of individual experiments were normalized to the control. Values are mean ± SEM of 3 independent observations. ***P < 0.001 vs. control and selenate by ANOVA and Newman-Keuls Multiple Comparison Test. ns = not significantly different.

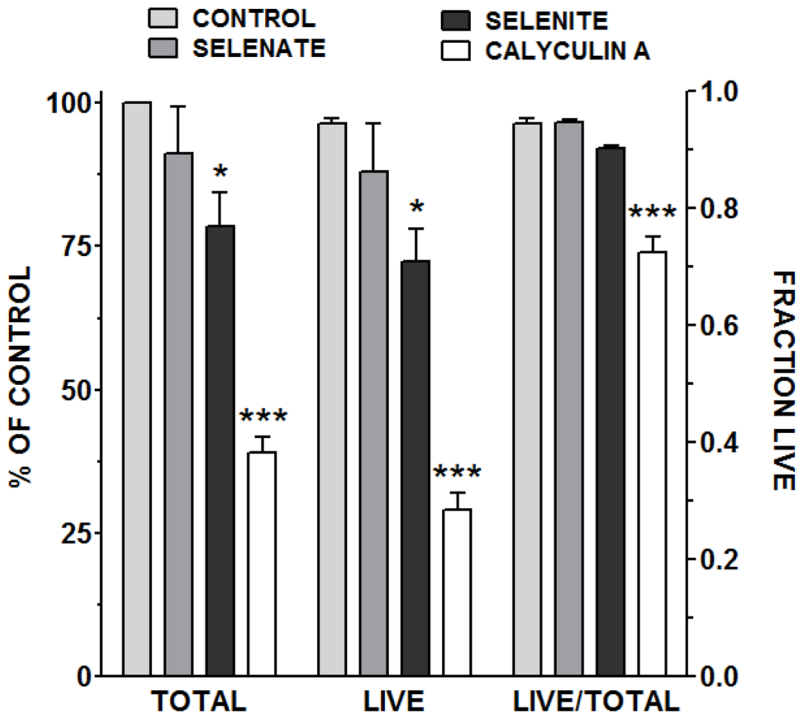

Figure 3.

Effect of selenate or selenite on cell viability. HMEC-1 were treated with vehicle (control), 100 μM sodium selenate, 100 μM sodium selenite, or 100 μM calyculin A for 24 h. The numbers of total and live cells under each condition were determined using a NucleoCounter as described in *2.5 Cell counts and viability.* Numbers of total and live cells are expressed as a percent of the respective control (vehicle-treated) number (left y-axis). The fraction of live cells is the ratio LIVE/TOTAL (right y-axis). Values are mean ± SEM of 3 independent observations. *P < 0.05 and **P < 0.001 vs. respective control by ANOVA and Dunnett's Multiple Comparison Test.

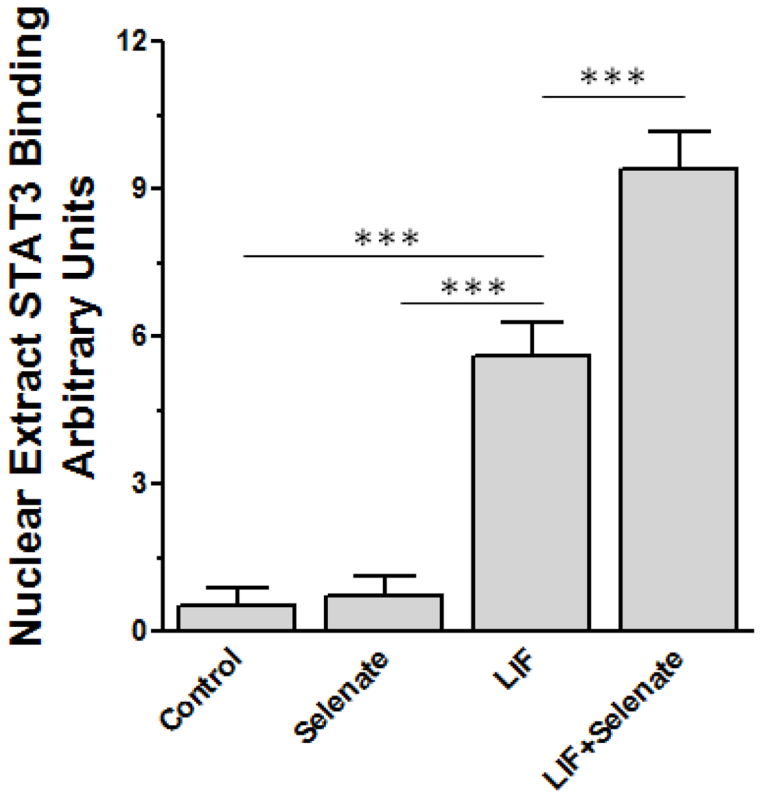

Figure 4.

Selenate enhances nuclear STAT3 binding activity. HMEC-1 were pretreated for 2 h with 100 μM sodium selenate or vehicle and then 2 ng/mL LIF or vehicle was added for 1 h. Nuclear extracts were prepared and equal protein amounts assessed for binding to a STAT3 consensus binding motif using a fluorescent ELISA-based assay. Values are mean ± SEM of 7 independent observations. Statistical significance was determined using ANOVA and the Newman-Keuls Multiple Comparison Test. ***P < 0.001 (column below line origin vs. column below line end).

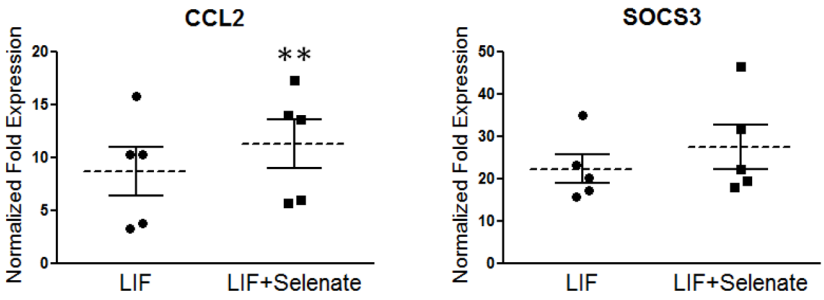

Figure 5.

Effect of selenate on LIF-induced gene expression. HMEC-1 were pretreated for 2 h with 100 μM sodium selenate or vehicle and then 2 ng/mL LIF or vehicle was added for 1 h. RNA was extracted, reverse transcribed, and analyzed for SOCS3 and CCL2 expression by real time PCR. Results were normalized to levels of GAPDH and expressed as the fold increase over control levels (vehicle only). Values from 5 independent experiments are shown along with the mean (dotted line) ± SEM. ** $P < 0.01$ by paired t-test.

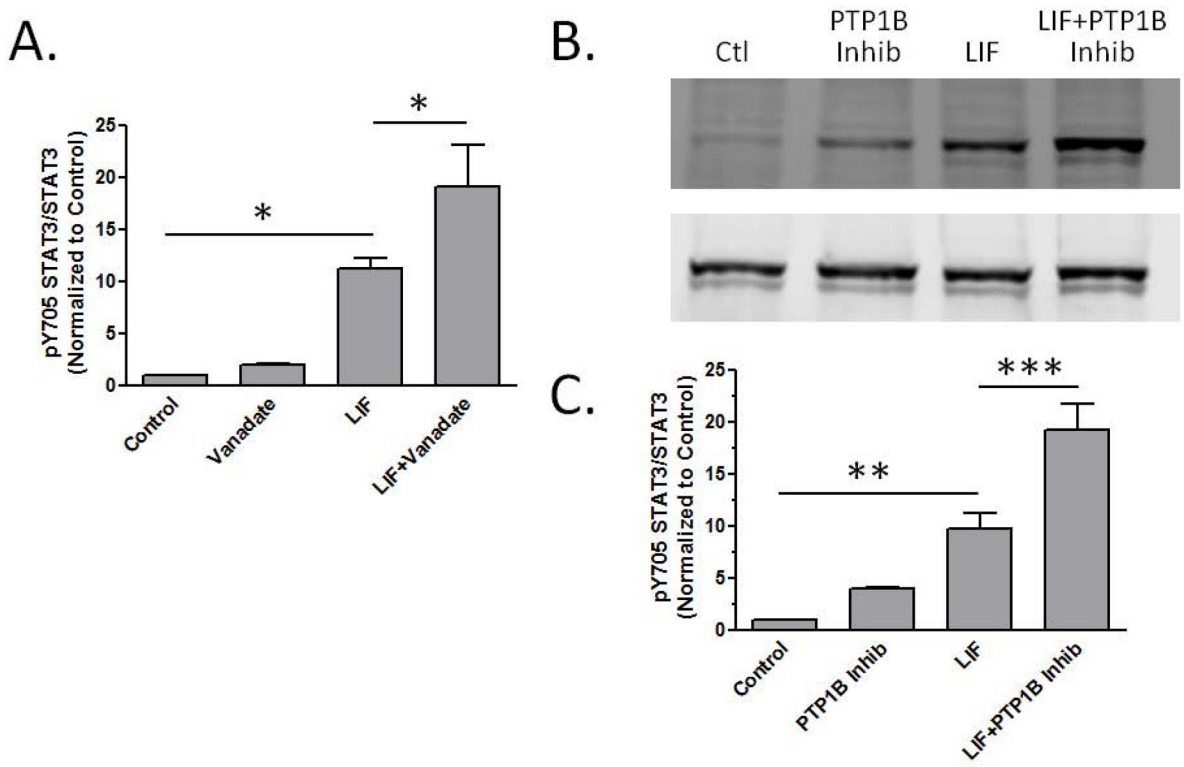

Figure 6.

Vanadate and PTP1B inhibitor enhance nuclear STAT3 Y705 phosphorylation. (A) Cells were pretreated 2h with 100 μM sodium vanadate or vehicle, and then treated for 1 h with 2 ng/mL LIF or vehicle. Nuclear extracts were prepared and analyzed for levels of STAT3 Y705 phosphorylation and STAT3. Results were quantified by the LI-COR Odyssey Detection System. Values are mean ± SEM of 3 independent observations. (B) Cells were pretreated 1h with 50 μM sodium PTP1B inhibitor or vehicle (0.02% v/v DMSO), and then treated for 1 h with 2 ng/mL LIF or vehicle. Nuclear extracts were prepared and analyzed by Western analysis. Shown is a representative blot. (C) Levels of STAT3 Y705 phosphorylation and STAT3 from (B) were quantified by the LI-COR Odyssey Detection System. Values are mean ± SEM of 4 independent observations. *P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Newman-Keuls Multiple Comparison Test.

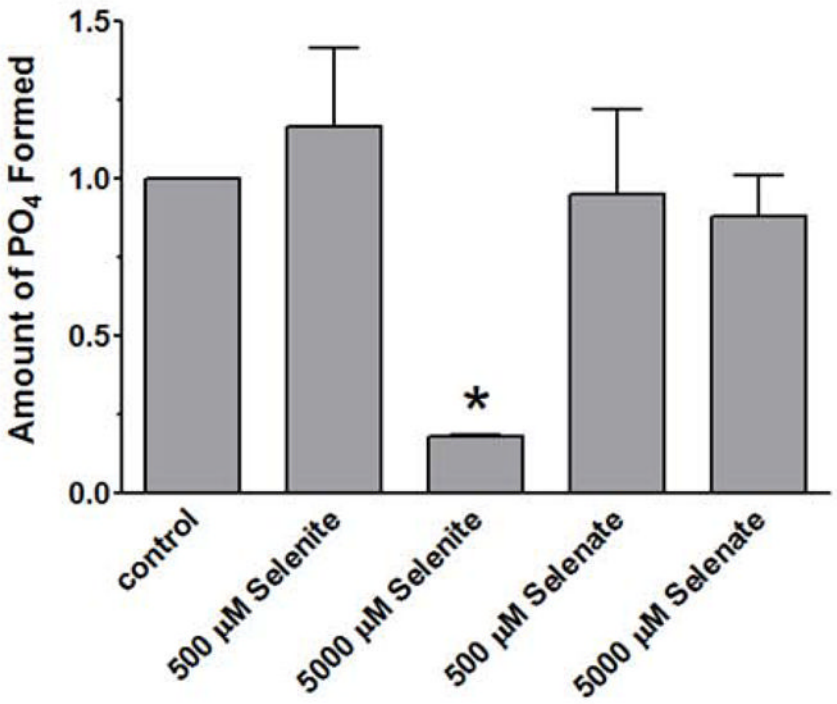

Figure 7.

Effect of selenite or selenate on PTP1B activity. A colorimetric assay was used to assess the direct effect of sodium selenite or sodium selenate on PTP1B enzymatic activity. The assay measures free phosphate formed from a phosphopeptide sequence based on the insulin receptor β subunit domain. The reaction contained human recombinant 25 ng/mL PTP1B and 75 μM substrate. Sodium selenate and sodium selenite were added at a final concentration of 500 or 5000 μM. Absorbance readings were corrected by subtracting the blank value obtained by incubating PTP1B without substrate. Values are mean ± SEM of 3 independent observations. *$P < 0.05$ vs. control by ANOVA and Dunnett's Multiple Comparison Test.