

Soluble Cytokine Receptor Subunits Modulate Catecholaminergic Cell Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AP and SSZ designed the study and wrote the protocol. Authors AP and SSZ performed the statistical analysis, managed the literature search, and wrote the first draft of the manuscript with assistance from author JB and IN. All authors read and approved the final manuscript.

Short Communication

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ABSTRACT

Soluble cytokine receptors (SCR) modulate cytokine signaling and are increased in states of abnormal immune activation. SCR's are also increased in psychiatric and neurological disorders associated with altered brain catecholaminergic activity; although, there is no evidence that any soluble cytokine receptor alone modulates such activity. One such soluble cytokine, soluble IL-2 receptor (sIL-2R) modulates lymphocyte activity. Here, we discovered that soluble IL-2 receptors (sIL-2R α , sIL-2R β) dose-dependently increase cAMP levels in differentiated PC12 cells. sIL-2R γ and sIL-6R α have produced no effect. Co-treatment of sIL-2Rs with IL-2 alters cAMP levels in a concentration-dependent manner. This is the first evidence that soluble receptors modulate catecholaminergic cell activity. Thus, soluble cytokine receptors, including the α and β subunits, may represent new therapeutic targets for relevant psychiatric and neurological disorders.

Keywords: *Soluble cytokine receptors; interleukin-2; interleukin-6; PC12 cells; cAMP; Catecholaminergic activity.*

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1. INTRODUCTION

Soluble cytokine receptors (SCR) are increased in states of abnormal immune activation. They are also increased in psychiatric and neurological disorders associated with altered brain catecholaminergic activity [1-2]. Although, there is no evidence that any soluble cytokine receptor alone modulates such activity. The finding that SCRs are novel subunit-specific modulators of catecholaminergic activity suggests new therapeutic targets for relevant psychiatric and neurological disorders. Cytokines are important regulators of numerous immunological events, (e.g., cell proliferation and apoptosis) and are potent modulators of brain function [3]. Cytokine activity is tightly regulated to prevent adverse effects secondary to dysregulated signaling. Soluble cytokine receptors (SCRs) are circulating proteins that regulate cytokine activity by binding their target cytokines and regulating signaling in antagonistic or agonistic manners [4]. SCRs are generated in numerous ways, including proteolytic cleavage of cell-surface receptors, alternative splicing and shedding and extracellular release of membrane bound receptors. The first SCR to be characterized was the soluble IL-2 receptor (sIL-2R) [5]. In vitro studies have shown that the alpha (α) subunit of sIL-2R is released from activated cells [6]. In addition, cell line cells that express Human T-Lymphotropic virus-1 (HTLV-I) release large quantities of sIL-2R α into their culture supernatant [7]. sIL-2R β is released in culture supernatant of human T cell leukemia virus I positive T cell lines [8].

sIL-2R was shown to be able to modulate T cell activity and might play an immune-inhibitory role under disease conditions [9]. Indeed, sIL-2R levels are increased in states of abnormal immune activation, including autoimmune conditions. Inasmuch as SCRs may potentiate the effects of their target proteins, it seems reasonable to suggest that increased levels of SCRs may have pathological repercussions. In recent years, evidence has accumulated suggesting that SCR's, are also elevated in the serum or CSF of patients suffering from psychiatric and neurological disorders. For example, sIL-2Rs are increased in schizophrenic patients, notably ones with tardive dyskinesia and muscle force instability [10]. The link between sIL-2Rs and a subgroup of patients suggests that they may act as etiological agents and not simply as biomarkers of immune activation. Soluble cytokine receptors (sIL-2R α , sIL-2R β) induce subunit-specific behavioral responses and accumulate in the cerebral cortex and basal forebrain [11]. Additionally, soluble interleukin-6 receptor induces motor stereotypies and co-localizes with gp130 in regions linked to Cortico-Striato-Thalamo-Cortical circuits [12]. Although, there is no evidence that sIL-2Rs modulate relevant cell activity. Here, we determined whether sIL-2R affects (1) activity of catecholaminergic cells (PC 12 cells) differentiated with neural growth factor (NGF), and (2) whether these effects are (a) subunit-specific and (b) cytokine receptor-specific. We discovered that sIL-2R α and sIL-2R β , but not sIL-2R γ or sIL-6R α modulate cAMP levels of PC 12 cells.

2. MATERIALS AND METHODS

2.1 Soluble Cytokines/Soluble Cytokine Receptors

Recombinant mouse soluble interleukin-6 receptor alpha (sIL-6R α), recombinant carrier free sIL-2R β and sIL-2R γ were purchased from R and D Systems (Minneapolis, MN). Recombinant sIL-2R α and recombinant rat IL-2 were obtained from Peprotech (Rockhill, NJ).

2.2 PC12 Cell Cultures, Treatment and Cyclic AMP Assay

PC12 cells (ATCC, Manassas, VA) were propagated in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 2mM L-glutamine, 10% horse serum (Invitrogen), 5% fetal bovine serum (Invitrogen) and appropriate antibiotics in a humidified incubator maintained at 37°C with 5% CO₂. PC12 cells were differentiated on poly-lysine-coated culture slides (Sigma, St. Louis, MO) in RPMI 1640 medium containing 1% horse serum, 2 mM L-glutamine and antibiotics with 50 ng/mL mouse nerve growth factor, (NGF 2.5S) (Chemicon, Temecula, CA) for 4 days[13]. Differentiated PC12 cells were exposed to 10-100ng/ml sIL-2Rs (α , β and γ), sIL-6R α , IL-2 alone, or IL-2 combined with different concentration of sIL-2Rs for 24hr and the intracellular cAMP level was measured by using the cAMPBiotrak Enzyme immune assay (EIA) kit (GE Healthcare, Piscataway, NJ, USA). The cAMP levels were measured by following the recommendations of the kit manufacturer (GE Healthcare). The final absorbance values were measured at 450 nm on a microplate reader (Bio-Rad, Hercules, CA, USA). The amount of cAMP in each well was determined by comparing the absorbance values of the treated variables with a series of standards provided with the kit. For each experiment, the standards were assayed in parallel and used to generate a standard curve with a linear detection range that spanned 13–3200 fmol/well. Samples were also verified with western blotting to make sure that Adenylyl cyclase levels were not different among PC12 cells treated with soluble cytokines and soluble cytokines receptors [14].

3. EXPERIMENTS AND RESULTS

We differentiated PC12 cells with 50ng/ml NGF and treated the cells with different concentration of sIL-2R subunits (α , β or γ ; 0.5-100ng/ml) (Fig 1A). The results showed that SCR treatment significantly increased cAMP levels ($F_{12,93}=3.017$, $P<0.05$). Newman-Keuls ($\alpha=0.05$) multiple comparisons confirmed that both sIL-2R α (0.5ng/ml and 10ng/ml groups) and sIL-2R β (0.5ng/ml, 10ng/ml, 50ng/ml and 100ng/ml groups) treated PC12 cells showed elevated cAMP activity compared with controls. In contrast, sIL-2R γ ($F_{4,37}= 0.8088$, $P>0.05$; Fig. 1A) and sIL-6R α ($F_{3,25} = 1.806$, $P>0.05$; Fig. 1B) had no effect. This is the first evidence that SCRs, in the absence of the target cytokine, modulate catecholaminergic cell activity, and this occurs in a cytokine receptor and subunit-specific manner. Because cAMP is degraded into inactive metabolites via hydrolysis by phosphodiesterase (PDE), it is possible that PDE affects the response to SCRs by altering the levels of this nucleotide, herein, in studies that measured changes in cAMP levels. We co-treated sIL-2R with the PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) (0.5 mmol/L). IBMX dramatically increased cAMP levels up to 2 fold in both control and SCR groups, but the percentage increase induced by sIL-2Rs are not significantly altered in the absence or presence of IBMX (data not shown), which suggests the increase of cAMP level induced by sIL-2R is not regulated by PDE. We also determined whether IL-2, which is the target cytokine for sIL-2R, modulates intracellular cAMP levels in PC12 cells. As illustrated in Fig. 1C, multiple comparisons confirmed that compared to controls, significantly increased cAMP level was induced by 50ng IL-2 treatment ($F_{3,25}= 4.973$, $**P<0.01$; Fig. 1C). Inasmuch as biological effect of cytokines may be antagonized or potentiated by their soluble receptors, we also determined cAMP levels in PC12 cells co-treated with sIL-2R and IL-2, and whether effects vary with the relative concentrations of these substances [12]. The results showed that increased cAMP levels induced by co-treatment of sIL-2Rs and IL-2 significantly exceeded levels in groups treated with IL-2 or sIL-2R alone ($F_{3,25}= 58.81$, $**P<0.01$; Fig. 1D). In contrast with these effects, significant reductions in cAMP levels were induced by 50ng IL-2+100ng sIL-2R α treatment ($**P<0.01$, Fig. 1D). We also treated PC12 cells with different combination of IL-2 and sIL-

2R α /or sIL-2R β (100ng/ml IL-2+50ng/ml sIL-2R α group; 50ng/ml IL-2+100ng/ml sIL-2R α group; 100ng IL-2+100ng sIL-2R β group; 50ng IL-2+50ng sIL-2R β group; 50ng IL-2+100ng sIL-2R β group), and these combinations do not cause significant changes of cAMP level in PC12 cells (data not shown).

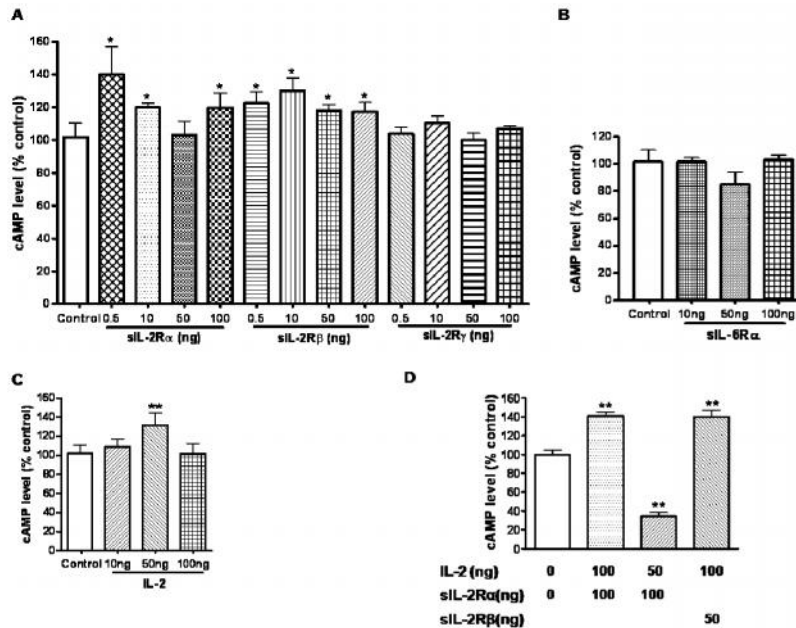


Fig. 1. Sil-2R increases intracellular cAMP level in catecholaminergic cells. (A) Differentiated PC12 cells were treated with different concentration of sIL-2R (α , β and γ subunits), and intracellular cAMP level was measured as an indicator of dopamine function of dopaminergic cells. **(B)** cAMP level of sIL-6R α treated differentiated PC12 cells was used as a comparison with that of sIL-2R treated cells. **(C)** IL-2, as a corresponding cytokine of sIL-2R also can promote cAMP producing in dopaminergic cells. **(D)** Different concentration combination of IL-2 and sIL-2R α /sIL-2R β had varied effects on cAMP level of differentiated PC12 cells

4. DISCUSSION

Based on these findings, the following conclusions can be made: (1) Soluble cytokine receptors (sIL-2Rs) alone induce marked increase in catecholaminergic cells activity; (2) these effects are (a) cytokine receptor and (b) subunit-specific and, (3) co-treatment with sIL-2R and IL-2 results in potentiating or inhibitory effects.

These suggestions are supported by the evidence that certain SCRs alone may later induce immune cell signaling via interactions with a trans-membrane protein [15]; thus, it is possible that sIL-2Rs modulate catecholaminergic cell signaling via a similar mechanism. sIL-2R's effects on catecholaminergic cell activity enhanced by the addition of IL-2 suggests that sIL-2R might serve as an agonist of IL-2, indicating an enhanced sensitivity of catecholaminergic cells to IL-2 or a prolonged half-life of the cytokine. Additionally, depending upon concentration, soluble cytokine receptor may serve as competitive antagonist to cytokine

leading to reduced sensitivity of catecholaminergic cells to IL-2 or very limited/less effective activation Fig. 1D.

The binding of IL-2 to the IL-2 receptor on human T-cells is a key regulatory event, which is absolutely required for T-cell-mediated immune responses. Hakimi et al reported the binding of IL-2 to sIL-2R in a dose-dependent manner in an immunosorbent receptor assay [16]. Our results in PC12 cells showed that different combinations of IL-2+sIL-2Rs had different effects on cAMP level, which suggests that the effects of IL-2+sIL-2Rs is related to the proportion of IL-2 to soluble receptor concentration.

Although one group found that the complex of IL-6 and soluble IL-6 receptor (sIL-6R α) results in neuron-specific differentiation and formation of a neuronal network in PC12 cells [15], IL-6 or sIL-6R α alone had no effects. Indeed, there is no evidence that any SCR alone modulates such activity.

In summary, we discovered that sIL-2Rs modulate catecholaminergic cell activity in highly specific manner. These findings suggest that the function of IL-2 and its soluble receptor are not restricted to stimulation of growth and differentiation of immune cells but also to modulating the activity of catecholaminergic cells. Of further importance, the present findings suggest a mechanism by which sIL-2R, which is implicated in psychiatric and neurological disorders associated with aberrations in brain catecholaminergic cell activity, may act as an etio-pathological agent in these disorders. As such, the present findings suggest novel therapeutic targets for the treatment of such disorders.

5. CONCLUSION

This is the first evidence that soluble cytokine receptors, in the absence of the target cytokine, modulate catecholaminergic cell activity; and this occurs in a cytokine receptor and subunit-specific manners. These present finding suggest novel therapeutic targets for the treatment of such disorders.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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