NEUROENDOCRINE AND SYMPATHETIC CONTROL OF SPLENIC MACROPHAGE IL-1 SECRETION

BY

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(M.D.)

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Dedication

TO MY PARENTS, WIFE AND SISTER

ACROSS THE PACIFIC OCEAN

TO ALL OF MY FRIENDS IN WINNIPEG

Acknowledgements

I would like to thank my supervisor, Dr. Arnold. H. Greenberg, for his excellent guidance and support. I would also like to thank Dr. Dennis Dyck for his support and help.

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List of Abbreviations

ACTH	Adrenocorticotropic Hormone
ADX	Adrenalectomy
AIDS	Acquired Immune Deficiency Syndrome
BBB	Blood-Brain Barrier
BSA	Bovine Serum Albumin
C5a	Complement 5a
cAMP	Cyclic Adenosine Monophosphate
CNS	Central Nervous System
CRF	Corticotropin-Releasing Factor
CSF	Cerebrospinal Fluid
CVOs	Circumventricular organs
EDTA	Ethylenedinitrolotetraacetic Acid
EEG	Electroencephalogram
FSH	Follicle-stimulating hormone
HIV	Human Immunodeficiency Virus
HPA axis	Hypothalamic-Pituitary-Adrenal axis
HPLC	High-pressure Liquid Chromatography
ICV	Intracerebroventricular
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ip.	Intrperitoneal
IS	Immune System
iv.	Intravenous

LH	luteinizing hormone
LPS	Lipopolysaccharide
MHC	Major Histocompability Complex
mRNA	Messenger Ribonucleic Acid
NE	Norepinephrine
NK Cell	Natural Killer Cell
OVLT	Organum vasculosum Lamina Terminalis
PGE_2	Prostaglandin E_2
POMC	Proopiomelanocortin
PVN	Paraventricular Nucleus
RIA	Radioimmunoassay
SNS	Splenic Nerve Section
SP	Substance P
SRBC	Sheep Red Blood Cells
TGF	Transforming Growth Factor
TNF	Tumour Necrosis Factor
TMEV	Theiler's murine encephalomyelitis virus
TSH	Thyrotropin
VIP	Vasoactive Intestinal peptide

Abstract

The interaction between the immune system (IS) and the central nervous system (CNS) has been studied in rats. Following systemic administration of interleukin-1 beta (IL-1ß), dose-related effects on adrenal and splenic macrophage functions have been shown. While systemic administration of 10 and 100 ng of IL-1ß increased blood corticosterone level and decreased IL-1 production from lipopolysaccharide (LPS) activated splenic macrophages, systemic administration of 5 ng of IL-1 did not increase corticosterone level and stimulated IL-1 secretion from LPS activated splenic macrophages. These results indicated that at high doses, IL-1 can affect neuroendocrine function and produce a negative feedback effect on immune function. To examine whether IL-1 acted through the CNS, experiments were designed to examine the effects of administrated centrally IL-1. Intracerebroventricular (ICV) injection of IL-1ß produced a dose-dependent increase in blood corticosterone and adrenocorticotropic hormone (ACTH) levels, indicating an activation of the pituitary-adrenal axis. Following ICV IL-1ß, a suppression of IL-1 but not TGF-ß secretion was observed from LPS stimulated splenic macrophages, indicating a differential regulation for these two cytokines. Both adrenalectomy (ADX) and spleen nerve section (SNS) prevented the suppressive effect of ICV IL-1 on macrophage IL-1 secretion and ADX also resulted in stimulation

of IL-1 secretion. The combination of ADX and SNS produced an even greater stimulatory effect of ICV IL-1ß on splenic macrophage IL-1 secretion relative to that seen with ADX or SNS alone. Further studies showed that SNS alone, without ICV IL-1ß, resulted in stimulation of splenic macrophage IL-1 secretion and that systemic injections of hydrocortisone (40 mg) inhibited splenic macrophage IL-1 production. These data indicate that both the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system are involved in the inhibitory control of splenic macrophage IL-1 production and support a bidirectional communication between the CNS and the immune system.

Review of the Literature

1. Introduction

Among all regulatory systems in the body, the immune system (IS), characterized by its cellular and humoral responses to foreign and self antigens, by its specific and non-specific protection against infections, tumours and self-destructive factors, is a unique system which displays highly autonomous and self-regulating features[1]. Some early evidences, such as Pavlov's classic conditioning experiments[2] and Selye's observation of thymic involution during stress[3], showed the interaction between the central nervous system (CNS) and the IS. But major developments in this field didn't begin until the 1970's. Today, despite the fact that much work remains to be done, there are few who question that the CNS can specifically control the function of the IS[4].

The interaction between the CNS and the IS is supported by accumulating evidence in recent years, such as behavioral conditioning of alterations of immunological reactivity[5] [6], the effects of brain lesions[7] and stress[8] on immune responses, and physiological and chemical changes in the brain during immune responses[9]. These links probably include glucocorticosteroids secreted from the adrenal gland, catecholamines and neuropeptides secreted by sympathetic terminals and the adrenal medulla, certain pituitary hormones, and polypeptides produced by cells of immune

system[10].

The first part of this review will briefly discuss bi-directional communication between the CNS and the IS and the second part will be focused on one of the best-characterized mediators of CNS-IS communication, Interleukin-1.

2. Bi-directional Communication Between the Brain and the Immune System : An Overview

One of the more important developments from studies of CNS-IS interaction is the idea that the immune system may function as a sensory organ[9]. It has been proposed that the immune system may sense stimuli that are not recognized by the central and peripheral nervous systems[11]. These stimuli include bacteria, tumours, viruses, self and modified-self antigens, etc. The recognition of such stimuli by immunocytes is then converted into information in the form of peptide hormones, lymphokines, and monokines that is conveyed to the neuroendocrine system which in turn produces a physiological change. Contrariwise, the central and peripheral nervous system recognition of stimuli results in similar hormonal information being conveyed to receptors on immunocytes resulting in an immunologic change[12]. Via these feedback loops the immune system and the central nervous system are believed to communicate with each other[13].

2.1. The Signals from IS to CNS

That the immune system can signal the brain has been suggested by increasing amounts of experimental evidence.

Besedovsky and co-workers showed that after specific antigenic challenges with sheep red blood cells (SRBC) there is an increase in the firing rate of hypothalamic neurones[14]. They also observed an increase in plasma corticosterone concentrations in mice and rats after antigenic challenge. Since plasma glucocorticoid concentrations are usually considered to be regulated via hypothalamic control of the pituitary gland, these data clearly implicate the brain[15]. Immunization with SRBC also changed the rate of synthesis of NE in the hypothalamus 4 days after administration[16] and the decrease in hypothalamic NE was confined to the paraventricular nucleus (PVN)[17] of the hypothalamus. This is significant in that this nucleus is the location of the cells that release CRF into the portal circulation inducing the anterior pituitary to release ACTH[18]. These findings suggest that some mediators produced by immunological cells (i.e. cytokines and monokines) are able to affect the biological activity of neurones in the brain.

There is more direct evidence supporting the hypothesis that lymphokines released during an immune response can alter CNS activity and by this way the immune system can transmit its signals into the brain[12]. Many immune lymphokines, such as interferon (IFN) α and ß, IL-1, IL-2, thymosin etc. have been found to modify brain function (See Table 1). When interleukin-1 and interleukin-2 are injected systemically,

they can directly effect brain areas where the blood-brain barrier (BBB) is either absent or possibly more permeable due to some pathological conditions[19] [20]. Among all immune peptides which have been studied for their function of effects on the CNS, IL-1 has attracted the most attention. one of the most prominent effect of IL-1 on the CNS is its ability to elevate glucocorticoid[21].

Wekerle et al.[22] reported that activated T-lymphocytes can readily cross the endothelial blood-brain barrier, which suggests that some immune cells can directly enter the CNS. However, the vertebrate central nervous system (CNS) has been traditionally thought to be inaccessible by cells of the immune system.

Another observation by Pouplard et al. found that Fc portion of immunoglobulins can bind to anterior pituitary ACTH producing cells. This finding may indicate another signal transduction pathway from the IS to the brain mediated by Fc receptor activation[23].

Table 1. Neuroendocrine effects of lymphokines and monokines. (From Blalock, J. E., Physiological Reviews. Vol.69, No.1, 1989).

TABLE 3. *Neuroendocrine effects of lymphokines and monokines*

Lymphokine or Monokine	Neuroendocrine Effect
IFN-α and/or β	Adrenal steroidogenesis Induction of melanin synthesis Enhancement of iodine uptake by thyroid cells Excitation of neurons Suppression of morphine withdrawal reactions Catalepsy and analgesia
IL 1	Fever Promotion of slow-wave sleep Hypothalamic release of CRF Pituitary release of ACTH and endorphins Elevation of glucocorticoid levels
Thymosin α_1	Elevation of ACTH and glucocorticoid levels
Thymosin β_4	Hypothalamic release of LHRH
IL 2	Pituitary release of ACTH and endorphins Elevation of glucocorticoid levels

2.2. The Signals From CNS to IS

There is increasing evidence that the CNS can modulate the immune system. This immunoregulatory function of the CNS can be triggered as a feedback response to the signals from the IS as mentioned above or it can be initiated by other factors such as psychosocial factors and possibly psychopathological conditions[10]. Behavioral conditioning studies suggest that the CNS can detect alterations in immune reactivity and can subsequently initiate a change in immune responses with exposure to the conditioned stimulus[5] [24]. The involvement of CNS circuitry in modulation of immune responses has also been tested directly in lesioning studies[25]. It is hypothesized that stress responses lead to altered release of neurohormones and/or neuropeptides which affect immune cell functions[26].

2.2.1. Neuroendocrine Regulation

Until recently, most interactions between the immune and neuroendocrine system were attributed to steroid hormones[27] [28]. Now, however, we are beginning to recognize that many peptide hormones can directly modulate immune responses[12].

Neuroendocrine outflow from the brain is achieved through

the posterior and anterior pituitary[29]. The pituitary is regulated mainly through the hypothalamus, limbic forebrain, and brain stem circuitry. This pathway is also called hypothalamus-pituitary-endocrine target-organ axis. The immunoregulatory effect of the hypothalamic-pituitary-adrenal (HPA) axis is at present the best-studied response. Focus on this axis started with the observation of an elevation of circulating glucocorticoid levels that coincided with peak antibody titres during an immune response[15].

CRF can be synthesized and released from the hypothalamus during the stress-related response and plays an important role in the immunoregulatory function by the HPA axis[30]. Its endocrine effects include actions at the pituitary level to stimulate the synthesis and release of POMC-derived peptides such as ACTH, as well as ß-endorphin. These peptide hormones may have an indirect or direct effect on immune functions[31] [32]. ACTH not only stimulates the adrenals to release glucosteroids hormones, which can have a suppressive effect on functions of many immune cells, but also can have direct effects on some immune responses[30].

2.2.2. Autonomic Innervation

There is extensive autonomic innervation of all lymphoid organs, including the thymus, spleen, lymph nodes and bone marrow. Most of the data indicate sympathetic innervation[33].

Felten et al[34] first proposed that organs of the immune system be included as target organs of direct autonomic innervation. They found autonomic nerve fibers in specific compartments of both primary and secondary lymphoid organs. Most interestingly, these nerve fibers were associated with lymphocytes and macrophages in these lymphoid organs. Along with these nerve endings, several neurotransmitters have been found to be co-localized together, including NE, SP, VIP, NPY etc. According to the demonstration of close relationship with immune cells in lymphoid organs, they proposed that NE plays a role in the modulation of immune responses.

Some investigators have observed alterations in immune function following sympathectomy produced by treatment with the catecholamine neurotoxine, 6-hydroxydopamine[35] [36]. Studies have shown that an antigenic challenge with SRBC decreased splenic content of NE 3-4 days after immunization and at the time of the peak immune response[37]. These results suggest that the sympathetic innervation of organs involved may be functionally related to the immune response.

2.3. Common mediators, shared receptors and cross-reactions

The interaction between the CNS and the IS is suggested not only by the signal transduction between these two systems

but also by the common mediators and shared receptors.

2.3.1. Neuroendocrine peptides produced by the immune system

Cells of the immune system are able to produce peptide hormones that were previously thought to be restricted to the neuroendocrine system. Blalock et al.[38] have suggested that when stimulated by antigens lymphocytes produce small quantities of ACTH, beta-endorphin, and certain other hormones (such as TSH). Other neuropeptides such as opiates, substance P (SP) etc have also been found to be produced by immune cells[12].

2.3.2. Immunopeptides produced by the CNS

While the immune system can produce peptides that were previously thought to be restricted to the neuroendocrine system, the neuroendocrine system is also able to produce peptides and proteins that are classically associated with the immune system, i.e., lymphokines and monokines.

One of the first lymphokines and /or monokines shown to be produced by the CNS was IL 1. The synthesis of this molecule has been reported to occur in astrocytes, glial cells, and in the brain of endotoxin-treated mice[39 40]. There is a high density of IL-1 immunoreactive fibres in human

hypothalamus. By means of in situ hybridization, IL-1, IL-2 and IL-3 mRNA have been localized in discrete areas of mouse brain where they are found in both neuronal cell bodies and astrocytes. Other experiments have confirmed the existence of IL-2 immunoreactivity and IL-2-binding sites in several areas of rat brain[41]. Interferon-gamma-like (IFN-gamma) immunoreactivity has also been found in certain neurons of the central and peripheral nervous system[42]. Transforming growth factor beta (TGF-beta), was also identified by immunocytochemistry in the brain tissues of patients with acquired immune deficiency syndrome (AIDS). The TGF-beta staining was localized to cells of monocytic lineage as well as astrocytes, especially in areas of brain pathology. The identification of TGF-beta within the CNS implicates this cytokine in the immunopathologic processes responsible for AIDS-related CNS dysfunction[43].

Some cytokines have been found in the CNS during pathological conditions. The interleukins 1 and 2 (IL-1, IL-2) and interferon-gamma have been measured in homogenate from the CNS and sera from Theiler's murine encephalomyelitis virus (TMEV) infected animals[44]. Elevated levels of interleukin-6 in cerebrospinal fluid was observed from patients with systemic lupus erythematosus and central nervous system involvement[45].

Thus it appears that the CNS, like the immune system, can

produce hormones (lymphokines/monokines) that have both neuroendocrine and immune functions. The sharing of ligands between the two systems provides a biochemical basis by which both systems can communicate.

2.3.3. Shared receptors

Besides sharing the common mediators, the CNS and Immune systems also share receptors for those mediators. Neuroendocrine peptide receptors have been found in immune cells, such as receptor for ACTH[31], ß-endorphin[46], enkephalin[31], VIP[47], GH[48], substance P[49] and insulin[50]. Cytokine/monokine receptors have also been found in the CNS, such as receptor for IL-1[51], IL-2[52], IFN[53], and PAF[54]. Studies also show that some of those receptors may have functional influences on each other[55].

2.3.4. Cross reactions

Other evidence shows that brain cells such as astrocytes, oligodendrocytes, microglia, motor and sensory neurones, and pituitary cells can respond to cytokines derived from T cells and macrophages, such as IL-1 , IL-2, IL-3, IL-6, neuroleukin, tumour necrosis factor, alpha-, beta- or gamma-interferon[56]. On the other hand, immune cells can also respond to neurohormones, neuropeptides or neurotransmitters. Studies

have demonstrated immunoregulational effects of growth hormone (GH)[57] [58] [59], ACTH[31] [60] [61], endorphins[62], glucocorticocoids[63], NE[64] [65], VIP[66], SP[67], somatostatin[66].

Taken together, these two systems contain and use the same set of signal molecules in the form of hormones, lymphokines, and monokines for inter- and intrasystem communication and regulation. They also harbour the same array of receptors for the shared ligands. Thus, it is very reasonable to infer that there is a "cross talk" between the immune and neuroendocrine systems.

2.4. Summary

It is now apparent that the immune and nervous systems communicate bidirectionally through short, intermediate, and long communication loops. The immunoregulatory roles of the CNS are believed to be mediated via the endocrine and the autonomic nervous systems. The concept that the CNS modulates immune functions implies that the immune system feeds back information to the CNS. These loops are achieved through mediators, such as hormones, neurotransmitters, and cytokines that interact with receptors found on cells of both systems. These mediators utilize common second messager systems,

probably interacting directly or indirectly to alter the responsiveness or expression of receptors for these signal molecules, providing for integrated responses when acting on neurons or lymphocytes (Figure 1).

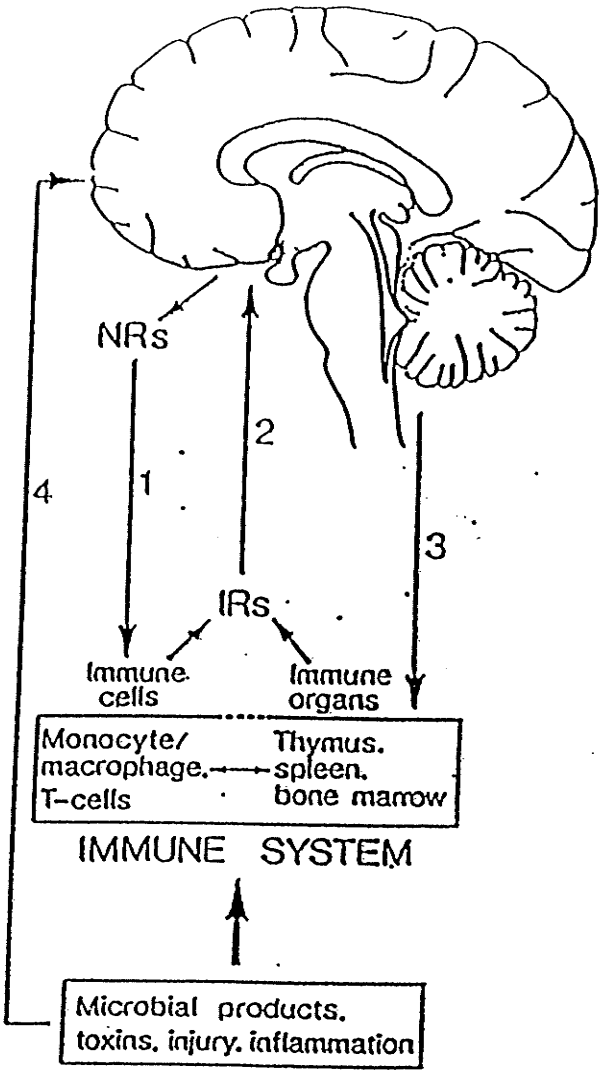

Figure 1. Bidirectional Communication between the CNS and the IS. (a). Signals from the CNS to the IS: neuroregulators (NRs) (1) and autonomic innervation (3). (b). Signals from the IS to the CNS: immunoregulators (IRs)(2). (c). Both systems receive signals from environment, such as infectious agents. (From Plata-Salaman, C., Neuroscience & Biobehavioral Reviews, Vol. 15, p.186, 1991)

3. IL-1: The Best-known Mediator in the Communication between the Brain and the Immune system

3.1. General Features

Interleukin 1 (IL-1), named more than a decade ago as the first interleukin[68], was described initially as a product of activated macrophages. Since then, IL-1 has been shown to be synthesized by and act upon a wide variety of cells. It is now believed that IL-1 is a basic mediator of intercellular communication both within the immune system and between the immune system and virtually all other organ systems.

3.1.1. Gene

IL-1 is coded by two distinct genes located on chromosome 2, which result in two different molecular forms of IL-1, IL-1α and IL-1β[69] [70]. The genomic organizations of both alpha and beta consist of seven exons. The homology exists between these two genes and among different species[71]. Studies of the regulation of IL-1 genes using lipopolysaccharide (LPS) stimulated human monocytes indicated that the beta gene promoter is more efficient than the promoter of the alpha gene[72]. Besides the transcriptional regulation of the proIL-1 molecule, the post-transcriptional and the post-translational

processing also play a fundamental role in the final regulation of IL-1 production[73].

3.1.2. Protein

IL-1α and IL-1ß are initially translated into propeptides from the different sized mRNAs (2.2 kb for IL-1α and 1.8 kb for IL-1ß)[74]. The propeptides with a molecular weight of 31.5 kd have relatively limited biological activity. IL-1 is found in the extracellular compartment but its propeptides are the predominant intracellular forms. The generation of the 17.5 kd mature IL-1 occurs extracelluarly through the action of proteases. Neither IL-1α nor IL-1ß has a signal peptide and this suggests that IL-1 release from the cell is not through the normal route for protein export[75]. IL-1 in its precursor forms is detected in cytosol of the producer cells and some cells produce high intracellular levels of IL-1 but never release significant amounts of it. IL-1α is reported to preferentially occur as the membrane-associated form in macrophage whereas IL-1ß is likely the one which primarily occurs in the extracelluar environment and is responsible for the hormone-like action of this cytokine. Besides the fully mature IL-1 peptide (17.5 kd), a minimal active peptide with about 14 kd and several active fragments with 4.2 and 2 kd have also been detected[76].

The molecular cloning of IL-1 has revealed that the

beta sequences have 78% conserved amino acid homology, compared to 60-70% in the alpha sequences. The homology between human IL-1α and IL-1ß is 26% at the peptide level, thus, they are quite dissimilar. But these two forms of IL-1 are related, especially at the structural level: both are composed of beta pleated sheets, share multiple biological properties and are recognized by the same receptor[77] [78].

3.1.3. Production

A wide variety of cells are capable of producing IL-1α and IL-1ß, including monocyte/macrophages, T lymphocytes, large granular lymphocytes, fibroblasts, keratinocytes, kidney mesangial cells, corneal epithelium cells, endothelial cells, astrocytes, Langerhans' cells, adrenal chromaffin cells, neutrophils and smooth muscle cells. Among these cells, the macrophage and the keratinocyte are probably the major producers of IL-1 in vivo. The monocyte/macrophages synthesize large amounts of IL-1, and IL-1ß mRNA predominates over that of IL-1α in rodent and human cells. The lymphoid organs are the major place where IL-1 mRNA are located[79] [80].

The transcription of IL-1 genes requires a stimulus. Many exogenous and endogenous agents can provide such a stimulus. The products of microorganisms such as endotoxins, and viral hemagglutinins induce IL-1 production by monocytes. C5a, CSF-1, TNF, TGF-ß , IL-1 itself, and other yet uncloned

T cell lymphokines also stimulate monocyte IL-1 production. By MHC-restricted antigen-dependent contact, T cells can also induce macrophage to produce IL-1. Some physical and chemical factors, such as ultraviolet irradiation and various crystals like silica and urate are also stimuli for IL-1 production[81] [82] [83].

IL-1 production can be specifically inhibited by several agents. Corticosteroids, an anti-inflammatory agent, inhibit IL-1 production by monocytes[84]. Prostaglandins inhibit the release of IL-1 from macrophages, while not affecting transcription[85].

3.1.4. Receptor

The action of IL-1 is mediated by the binding of IL-1 to its receptors on the plasma membrane of different cells[86]. The numbers of IL-1 receptors per cell vary from a few hundred on T lymphocytes to a few thousands on fibroblasts. Both high and low affinity binding receptors exist for IL-1. But no significant difference for the binding affinity between IL-1α and IL-1ß to their receptors has been found in peripheral systems. This may be explained by the similar tertiary structure shared by both forms of IL-1. IL-1 binding is not species-specific and human or murine IL-1s react with receptors of the other species.

The variation in size of the IL-1 binding components

in the different cells has been found and N-glycosylation is a possible reason for this difference. Most findings confirmed the existence of a plasma membrane receptor with 70 to 80 kd but the IL-1 receptors with 60 kd and 97 kd have also been reported[87].

Because of the Ig-like structure, the IL-1 receptor has been classified as a member of Ig superfamily[88]. Molecular cloning studies have revealed that the murine IL-1 receptor consists of 557 amino acids (after removing a typical signal peptide). The extracelluar part of the receptor contains the N terminal 319 amino acids and is organized into three Ig-like domains. The transmembrane region has 21 amino acids wherease the cytoplasmic portion of IL-1 receptor has been found to contain 217 amino acids and has been suggested to be a substrate for protein kinase C[89].

Both up- and down-regulation of the IL-1 receptor has been reported. IL-1 downregulates the expression of its own receptor following internalization. Prostaglandins and glucocorticoids upregulate the expression of functional IL-1 receptors on fibroblasts and B cells, but not on macrophages, T cells, or neutrophils[90 91].

Little is known about the post-receptor events that follow the binding of IL-1 to its receptor. It has been shown that IL-1 enhances receptor-induced activation of phospholipase A_2 in 3T3 fibroblasts and increase prostaglandin E_2 (PGE_2) production in astrocytes without affecting

phospholipase C activity[92]. IL-1 is able to increase cyclic AMP (cAMP) production in human fibroblasts and in a human natural killer-like cell line[93] [94]. IL-1 also seems to be involved in the transcriptional regulation of interleukin 2 (IL-2) gene expression. In LBRM-331A5 cells, a mouse T-lymphoma derived cell line, IL-1 enhances expression of c-jun mRNA, whereas the antigenic signal enhances mRNA expression of c-fos. The products of these protooncogenes may contribute to the multiple effects of IL-1[95].

3.1.5. Biological functions

IL-1 is critically involved in the development and maintenance of the inflammatory response. IL-1 is an endogenous pyrogen responsible for the increase in basal temperature after infectious events[96]; it induces acute-phase proteins; it elevates corticosterone levels and is involved in glucose homeostasis and catabolic processes associated with infection; it induces neutrophilia and the release of prostaglandins and proteases in different cells and it alters hepatic drug metabolism[97].

IL-1 also plays a key role in the regulation of immune responses. IL-1 participates in antigen-induced T cell activation and clonal expansion by triggering IL-2 and IL-4 production and IL-2 receptor expression[98] [99]; IL-1 is also one of the factors involved in B cell differentiation and

proliferation and it has haematopoietic activity for the earliest precursors in the bone marrow; As a consequence, the in vivo administration of IL-1 can enhance the immune response to different antigens and it can protect the host from lethal challenges with microorganisms or tumours, and from lethal radiation damage[100 101 102].

Human IL-1 is a cytocidal factor for several tumour cell lines. Some IL-1 biological activities seem to be involved with mechanisms of host tumour killing. IL-1 increases the binding and lysis of natural killer cells to a variety of tumour targets and is chemotactic for monocytes and lymphocytes[103 104].

3.2. Appearance of IL-1 in the CNS

IL-1 has been detected immunohistochemically in human brain[105]. IL-1ß immunoreactive fibers were found in the hypothalamus at the level of the periventricular regions and in the infundibulum, including the region of the median eminence. The high density of IL-1 immunoreactivity reported in the hypothalamic regions that regulate anterior pituitary functions suggests a key role for IL-1 in the modulation of endocrine events. In addition, the CRF-containing cell bodies, located in the paraventricular nucleus of the hypothalamus, correspond to the area of heavily innervated by IL-1ß

immunoreactive fibers. Thus, both a direct and a hypothalamus mediated control of pituitary functions could be exerted by IL-1. Furthermore, the occurrence of IL-1 in cat cerebrospinal fluid has been detected[106].

3.2.1. Local Synthesis:

IL-1 has been identified as a product of mouse brain macrophages[107].Astrocytes and astrocytes-derived cell lines also synthesize IL-1 in response to stimulation in vitro[108].

Various cell types have the ability to synthesize and release IL-1 in the CNS. Intrinsic brain macrophages[109], cerebrovascular endothelial cells[110], microglia[109], astrocytes[111], and neurons[105] have the ability to synthesize and release IL-1 in response to the appropriate stimuli. IL-1ß mRNA has been detected in rat hipocampus and cerebral cortex, strongly suggesting biosynthesis of IL-1ß in the CNS[112].

Various agents induce the synthesis and release of IL-1 by CNS. These agents include bacterial endotoxin (Escherichia coli lipopolysaccharide), neurotropic viruses[113], and activators of protein kinase C[114]. ICV administration of endotoxin induces the appearance of IL-1 in the CSF but was not observed after iv administration of endotoxin. A variety of acute and chronic pathological processes of CNS may result in the appearance of IL-1 in the CNS. IL-1 like activity in

the CSF has been detected in patients with degenerative diseases and in chronic encephalomyelitis in guinea pig.IL-1ß was identified in CSF of patients infected HIV virus[115].

3.2.2. Uptake from the peripheral circulation:

Plasma IL-1 is thought to enter the hypothalamus at the organus vasculosum of the lamina terminals (OVLT), a circumventricular organ (CVOs) with no blood-brain barrier that is located at the anteroventral tip of the third ventricle[105].

A bidirectional transport of IL-1α across the BBB has been demonstrated[116],[117]. Recombinant human IL-1α entered multiple brain regions with 40% entering the cerebral cortex (which lacks CVOs) by a specific transport system. The hypothalamus, has the highest entry rate. In addition, activated T cells may cross the BBB and release IL-1 in particular brain target sites[118].

3.3. IL-1 Receptors in the CNS

The IL-1 receptor in the CNS has a similar mol.wt. to that of receptors on T cells and fibroblasts in peripheral system. However, receptors in the CNS bind IL-1ß more potently than IL-1α. IL-1 receptors are densely localized in the olfactory, hippocampus, cerebellum, cerebral cortex, choroid

plexus, and hypothalamus[51] [119].

Using autoradiographic techniques, Farrar et al[51]. visualized the distribution of [^{125}I]IL-1 receptors on sections of fresh frozen rat brain. The binding of [^{125}I]IL-1 was widespread throughout the brain with a distinctive pattern of distribution. Typical neuron-rich brain areas were found to have high-density binding. Also, IL-1 receptors were found in rat, mouse, and human pituitary membrane preparations.

3.4. Role of IL-1 in the Brain

IL-1 may play a role in the regulation of CNS growth during embryogenesis and in the determination of neuronal survival during development[120]. IL-1 regulates glial cell growth and proliferation and participates in brain gliosis[121].

IL-1 has a variety of neuromodulatory effects, including the stimulation of central noradrenergic system and the metabolism of NE in the hypothalamus. This effect is specific since no comparable changes in the content of other neurotransmitters were detected[122]; IL-1α also increase levels of epinephrine and VIP in cultured adrenal chromaffin cells[123]; An enhancement of CNS opiod receptor binding[124] and a potent hyperalgesia has been reported when rhIL-1α or rhIL-1ß is administrated peripherally or centrally. This effect is not affected by the opiate antagonist naloxone[125]; IL-1 also

regulate NGF synthesis by increasing NGF mRNA[126] as well as beta-amyloid precursor mRNA levels which is involved in amyloid formation in brains of Alzheimer's disease (AD)[112]; Stimulation of eicosanoid production by astrocytes, in particular PGE, thromboxane B_2, and other arachidonic acid metabolites has also been reported. Protein kinase C is involved in this biological action of IL-1[127]; IL-1 increases pro-opiomelanocortin mRNA expression in pituitary cells.[128]; Also, IL-1 induces cognitive changes by decreasing glucose metabolism[129] and decreases EEG synchronization after ICV administration of IL-1[130]; Delay in conduction within myelinated afferent pathways[131] has also been observed.

Peripheral administration of IL-1 induces synthesis of somatostatin and CRF in rat hypothalamus[132]. IL-1α and IL-1ß also induce the synthesis and release of various pituitary hormones including ACTH , thyrotropin, growth hormone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin[133]. However, other studies have shown inhibition of LH[134] and prolactin secretions[135]. It is proposed that prostaglandins are mediators of IL-1ß-induced CRF/ACTH release[136]. Moreover, IL-1, via protein kinases, also enhances the release of beta-endorphin induced by CRF, VIP, forskolin. NE, phorbol ester, and isoproterenol[137]. Systemic IL-1 may influence pituitary hormone release by passage across the median eminence. Studies in culture show that the effects on

pituitary hormone release occur at concentrations within the range of IL-1 in serum[133]. IL-1 also acts directly on the adrenal gland to increase glucocorticoid synthesis[138].

The administration of recombinant IL 1 to rats also results in a dromatic increase in blood ACTH and corticosterone levels in these animals[139]. This effect is independent of the fever response and , of course, could result from either an effect of IL 1 on the hypothalamus or on the pituitary gland. It appears likely to be due to effects on both, as indicated by the ACTH-releasing potential of IL 1 on pituitary cells and corticotropin-releasing factor (CRF)-releasing activity on hypothalami[140].

IL-1 induces fever through a prostaglandin-dependent mechanism activated in hypothalamic structures[141]. Fever induced by ICV administration of IL-1 is also dependent of the central catecholaminergic system, since pretreatment with 6-hydroxydopamine (a catecholamine-depleting agent) blocks the IL-1-induced fever in rodents[142].

One of the early described effects of monocyte-derived IL-1 was the generation of fever. Introduction of IL-1 by intracerebroventricular administration produces a more profound rise in temperature than dose peripheral venous injection. The effect of IL 1 on temperature appears to be localized to the anterior hypothalamus. In addition to its

fever-inducing characteristics, IL 1 also promotes slow-wave sleep.

Microgram doses of IL-1 suppress food intake after intraperitoneal (ip) administration into fasted rats as well as freely fed rats and mice[143]. Also, ICV administration of low doses of rhIL-1ß suppresses food intake. Thus, IL-1 appears to act directly in the CNS to suppress food intake[144].

IL-1 increases NGF expression in rat hippocampal cultures[145]. IL-1 also is able to induce the expression of interleukin 6 (IL-6) mRNA in glioblastoma or astrocytoma cells[146].

Introduction

As mentioned in the literature review of this thesis, Besedovsky and his colleagues first proposed the involvement of IL-1 in the activation of the hypothalamic-pituitary-adrenal axis and the major suppressive effect of ACTH/corticosterone on the immune responses was well-known. There is evidence showing that the sympathetic nervous system innervates lymphoid organs. Felten and his co-workers first hypothesized regulatory effect of sympathetic nerves on the immune system. Although the possibility of the bi-directional communication and control of the immune system by the CNS has been suggested, it is still not clear what were the effects of the autonomic system on a specific immune response. The contributions of both the neuroendocrine and sympathetic innervation on an immune response was in need of study.

Accumulated evidence has proved that IL-1 is one of the most important mediators in both the immune system and the central nervous system, including its effect in bi-directional communication between these two systems. Macrophages are the most important IL-1 producing cells and are also a target for the action of glucocorticoids. It was demonstrated that macrophages in lymphoid organs, including the spleen, are closely associated with autonomic fibers and therefore a likely target of neurotransmitters from the sympathetic nervous system. However, the role of the sympathetic nervous system in splenic macrophage IL-1 regulation is not clear and further study is needed.

In order to characterize further the specific relationship between the IS and the CNS, especially the contribution of both the HPA axis and the sympathetic nervous system to splenic macrophage function, we have conducted a series of experiments to determine whether IL-1 administrated peripherally and centrally can affect immune function through neuroendocrine and/or sympathetic mediated mechanisms.

Materials and methods

Animals

Adult (250-300g) male Sprague-Dawley rats (charles River, Quebec) were used in all experiments. Animals were housed individually in cages with food and water *ad lib*. Lighting was maintained on a 12 hr on and 12 hr off regimen.

Reagents

Recombinant human interleukin-1ß was obtained from Drs. D. Urdal and C. Henney (Immunex Corporation). Highly purified porcine transforming growth factor (TGF-$ß_1$) and anti-TGF-ß antibody were purchased from R&D System (Minneapolis, MN). IL-2 was from Pharmacia Diagnostic Inc., MA, USA).

Surgery and IL-1 Administration

All animals were anaesthetized with Nembutal (20-25 mg/rat) and stainless steel cannulae were implanted into the left lateral ventricle using the stereotoxic coordinates; A-P = 0.8 mm, L = +1.3, D-V = -3.0 mm [147]. Adrenalectomized (ADX) rats were obtained from Charles River and cannulated 10 days following ADX. Splenic nerve section was performed at the same time as cannulation. The spleen was exposed by an abdominal

incision lateral to the midline. Using a microscope, the splenic nerve was located before its bifurcation with the blood supply into the hilar region of the spleen. The nerve was then dissected away from the vasculature and at least 1 cm of nerve was removed from the animal. In some cases the nerve was cut in several locations. Verification of splenic nerve section was achieved by HPLC determination of splenic norepinephrine (NE) content. In all cases, there was an 80-95% reduction in NE content of the spleen at 4 days postsurgery.

Four days after cannulation, rats were given an ICV injection of either recombinant human IL-1ß at doses of 5, 10, or 100 ng in 5 μl saline vehicle. Two hours following injection rats were decapitated and trunk blood and spleens were collected and processed. Half of each spleen was taken for the IL-1 assay and the other half rapidly frozen on dry ice and then stored at -80°C until NE analysis.

Measurement

1. IL-1 Assay

Spleens were desegregated through stainless steel wire mesh and washed in ∝MEM. Red cells were removed by NH_4Cl lysis and remaining cells were washed , resuspended in ∝MEM, 0.1% BSA, 20 mM Hepes, and plated into 24-well plates (Nunclon) at

a concentration of $2x10^6$ splenocytes/ml, 1 ml per well. Following 2 hr incubation at 37°C in 5% CO_2 wells were washed to remove nonadherent cells and 1 ml of medium was added containing either 0, 1, 10, or 100 ug LPS (Salmonella typhimurium, Cat. # L-7261, Sigma) and the remaining adherent cells (>95% nonspecific esterase positive) were incubated for 48 hr at 37°C in 5%CO_2. Supernatants were removed from these cultures, centrifuged, and frozen at -80°C pending bioassay.

For IL-1 bioassay, the NOB1-CTLL assay system was employed[148]. NOB1 cells maintained in RPMI 1640 (GIBCO), 5% fetal calf serum (GIBCO), were adjusted to $5x10^5$ cells per millilitre and 100 ul of this dilution was added to wells (96-well flat-bottomed, Nunclon). IL-1 standards were prepared at concentrations of 3, 10, 30, 100, 300, and 1000 pg/ml of rh IL-1ß. A 20-ul aliquot of these standards or supernatant from macrophage cultures was added to the wells and plates were incubated for 24 hr at 37°C in 5%CO_2. After 24 hrs plates were centrifuged and 100 ul of the supernatant was added to wells containing $1x10^4$ CTLL cells in 100 ul medium and incubated a further 24 hr. CTLL cells were routinely maintained in the same medium as NOB1 cells with the addition of IL-2. About 4 hr prior to harvest, wells were pulsed with 0.5 uCi [^{3}H]thymidine and cells, harvested onto glass fiber paper, and placed into scintillant, and incorporation was determined by counting discs in a Beckman ß scintillation counter. IL-1 concentration of the supernatant was determined by comparing

the [^{3}H]TdR incorporation of the macrophage supernatant samples with the IL-1 standards and then converting to pg/ml.

Neutralization of IL-1 activity was accomplished by preincubating 20 ul of macrophage supernatant and 8 ul of rabbit anti-human IL-1 antibody (Genzyme, Boston, MA) or normal rabbit IgG (Teknika, Westchester, PA) overnight at 4°C before adding to the assay. Rabbit anti-IL-1 antibody was preabsorbed with NOB1 cells at 4:1 vol:vol ratio at 4°C for 1 hr. this was repeated four times until no NOB1 toxicity was detected by trypan blue exclusion, similar to the normal IgG control.

2. TGF-ß Assay

The method of assay for TGF-ß was the CCL-64 mink lung growth inhibition assay[149]. CCL-64 mink lung epithelial cells maintained in DME (GIBCO) with 10% fetal bovine serum were trypsinzed, washed with aMEM and resuspended in DME, 0.2% fetal bovine plasma, 10 mM Hepes, penicillin (25 U/ml) and streptomycin, and plated at a concentration of $5x10^5$ cells per 0.5 ml in 24-well Costar dishes (Flow). Acid-activated supernatant was neutralized and added with or without anti-TGF-ß antibody (R&D Systems) 3 hr after cells were initially plated. Following 22 hr incubation, cells were pulsed with 0.25 uCi [^{125}I]IUdR for 2-3 hr at 37°C. Cells were fixed in 1

ml methanol-acetic acid[150] (vol/vol). After 1 hr at room temperature, wells were washed twice with 2 ml of 80% methanol. Cells were lysed in 1 ml of 1 N NaOH for 30 min at room temperature and [^{125}I]IUdR was counted on an LKB gamma counter. A standard curve of porcine TGF-β_1 was included and data were expressed as pg TGF-ß/10^6 splenocytes.

3. ACTH and Corticosterone Radioimmunoassay

Blood was centrifuged in EDTA and plasma collected and stored at -20°C pending assay. An RIA kit (Nichols Institute Diagnostics) was used for plasma ACTH determination according to the manufacturer's institutions. Corticosterone assay followed the method described previously[151].

4. Norepinephrine Assay

Frozen spleen was weighed and homogenized in 0.1 M perchloric acid containing 0.1 mM EDTA to yield a final tissue concentration of 20 mg per milliliter. Homogenates were centrifuged at 1500 rpm for 10 min and 1.0 ml of the supernatant was taken for alumina extraction using the ESA Plasma Catecholamine methodology with an internal standard. Samples were separated by HPLC using an ESA Model 5700 solvent delivery model with a CSC-S ODS2 5-um column. Analysis and

quantitation was accomplished with a Coulochem 5100 A electrochemical detector and Shimadzu CR601 Chromatopac Integrator. Data were expressed as nanogram NE per gram wet tissue weight.

Results

1. EXPERIMENT I

1.1. Plasma Corticosterone Level

Two hours after intraperitoneal injection of 5, 10 or 100 ng IL-1ß, a dose related increase of levels of plasma corticosterone was detected (Figure 2). Only 100 ng of IL-1 produced a significant stimulation of corticosterone secretion, whereas 5 ng and 10 ng of IL-1 produced small but not significant increases of the hormone compared to the saline control.

1.2. Splenic Macrophage IL-1 Secretion

Figures 3 and 4 show a dose related effect of intraperitoneal injection IL-1ß (5 and 100 ng) on splenic macrophage IL-1 secretion following stimulation by LPS in vitro. Two hours after injection of 5 ng of IL-1ß, secretion of IL-1 from splenic macrophages was increased (Fig. 3). In contrast, 100 ng of IL-1ß suppressed IL-1 secretion from splenic macrophages (Fig. 4), compared to the saline control. Relating these results to corticosterone induction by IL-1(Figures 2), IL-1 induced suppression of macrophages only at a dose which induced corticosterone secretion.

In this thesis, IL-1 values were expressed as pg/ml as this was identical to conversion of the data to pg/ml/ug

macrophage DNA or protein. No variation was detected in the total macrophage recovery from spleens following IL-1 ICV compared to the saline control group (data not shown).

2. EXPERIMENT II

2.1. Plasma ACTH and Corticosterone Release following ICV Interleukin-1ß

Figures 5 and 6 show the effect of 0.5, 5, 10, or 100 ng IL-1ß ICV on plasma ACTH and corticosterone two hours after injection. Both ACTH and corticosterone levels were markedly increased and 5, 10, or 100 ng doses had the greatest effect on both hormones. Figure 7 show that ACTH levels returned to control levels by 8 hours while corticosterone levels were still high at that time point. By 24 hours, both hormones were back to normal.

2.2. Macrophage IL-1 secretion following ICV injection of interleukin-1ß.

A marked and statistically significant suppression of splenic macrophage IL-1 secretion following stimulation by increasing doses of LPS was observed within two hours of ICV injection of 5 ng IL-1ß (Figure 8). This suppression appeared in the basal level of IL-1 released by nonstimulated

macrophages. 100 ng of IL-1ß ICV injection produced a similar suppressive effect on macrophage IL-1 secretion (data not shown).

Figure 9 shows that TGF-ß secretion from the same splenic macrophages as those producing IL-1 was not affected. This result indicates a differential regulation of IL-1 and TGF-ß by central signals induced by ICV IL-1ß.

The CTLL cells in our NOB1-CTLL IL-1 bioassay system is a IL-2 sensitive cell line. In order to distinguish between IL-1 activity and the IL-2 activity, we also tested and confirmed that there was no direct effect by macrophage supernatants from above experiments on CTLL cell proliferation by incubating the CTLL cells with the supernatant (Figure 10).

2.3. Effect of ADX and SNS on Macrophage IL-1 production after ICV Interleukin-1ß

Figure 11 show that, while having extremely low levels of plasma corticosterone, adrenalectomy reversed the suppression of macrophage and resulted in an increase in IL-1 secretion. This increase of IL-1 secretion in ADX rats was observed with or without LPS stimulation.

For sympathectomized rats, the splenic nerve was surgically sectioned 5 days prior to ICV injection of IL-1ß. A 85-95% of reduction in splenic norepinephrine content was

observed and sympathectomy of the spleen prevented ICV IL-1ß from suppressing macrophage IL-1 secretion despite significant increase in plasma corticosterone levels (Figure 12). There was no significant difference for macrophage IL-1 secretion between IL-1 ICV group and the control group.

Combining ADX and sympathectomy together, Figure 13 showed that there was a profound stimulation on macrophage IL-1 secretion following LPS treatment. This stimulation of IL-1 secretion was considerably greater than that seen with either ADX or sympathectomy alone.

2.4. Result of IL-1 Neutralization

Since a bioassay system was used to detect the IL-1 activity from the macrophage supernatant, a IL-1 neutralization experiment was conducted to confirm the IL-1 activity in the supernatant was due to IL-1. Supernatant were preincubated in either rabbit anti-IL-1 antiserum or normal rabbit Ig prior to assaying. All proliferative activity was neutralized by the antiserum (Figure 14).

3. EXPERIMENT III

After observing that both the corticosterone and the sympathetic innervation were involved in the negative control of splenic macrophage IL-1 secretion following IL-1 ICV

injection, we next examined whether those two pathways control the macrophage activity in the normal rat.

3.1. Enhancement of Splenic Macrophage IL-1 secretion following Splenic Nerve Section

5 days following sympathectomy, compared to sham controls, a significant enhancement of IL-1 secretion was observed from splenic macrophages stimulated by increasing doses of LPS in vitro (Figure 15). This Significant enhancement of IL-1 secretion from sympathectomized animals ($P < 0.01$) was seen following 48 hours of LPS stimulation (Figure 16).

These results provided direct evidence that sympathetic innervation of the spleen has an inhibitory control over splenic macrophage IL-1 secretion.

3.2. Suppression of Splenic Macrophage IL-1 Secretion following Subcutaneous Injection of Hydrocortisone

Hydrocortisone (40 mg per rat) was injected subcutaneously into rats. Two hours after injection, a significant suppression ($P < 0.01$) of splenic macrophage IL-1 production following LPS stimulation in vitro was observed (Figure 17). This result confirmed the well-known suppressive effect of corticosteroids on the macrophage function.

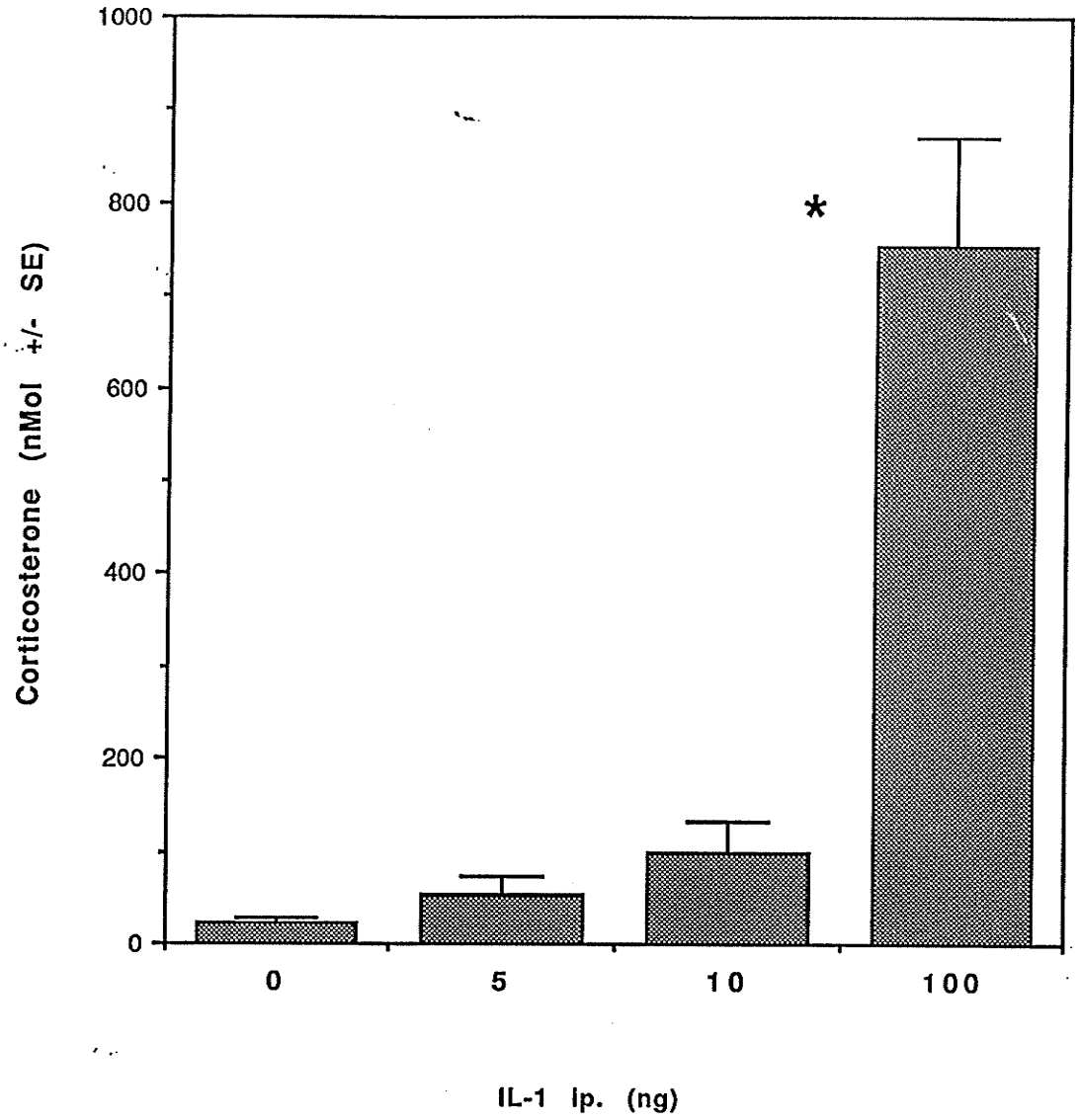

Figure 2. Effect of systemic injection IL-1ß on plasma corticosterone level, 2 hrs post-injection. * = $P < 0.01$ using Student's t test compared to saline control.

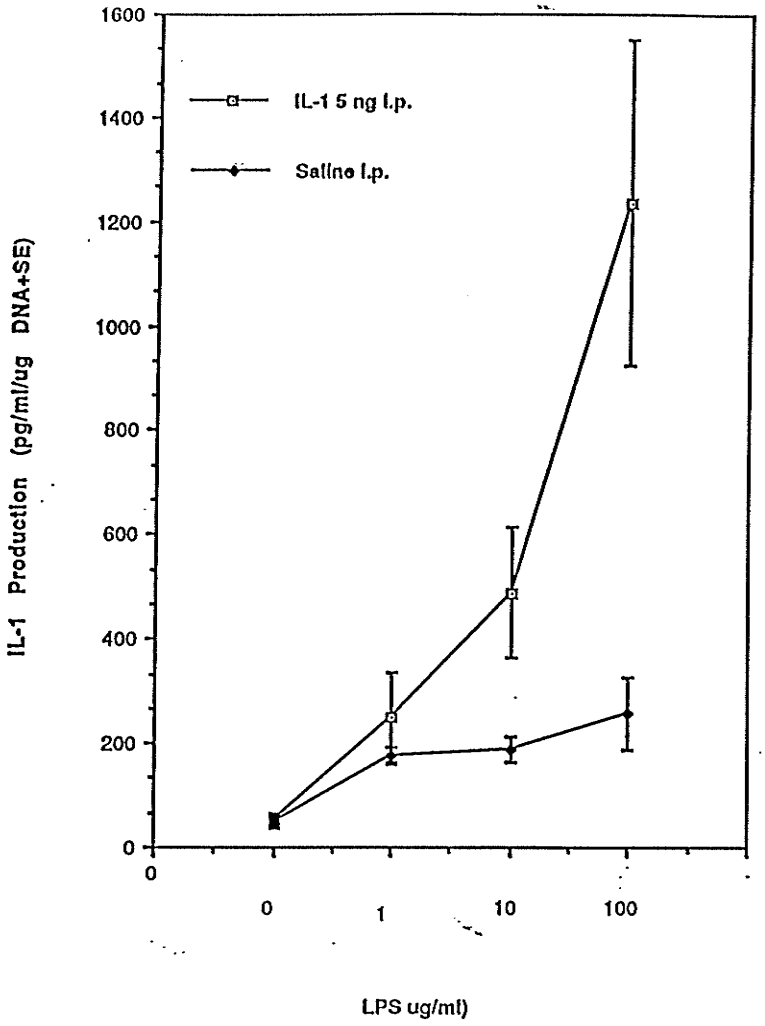

Figure 3. Effect of IL-1ß 5 ng systemic injection on splenic macrophage IL-1 secretion. Spleens were collected two hours postinjection.

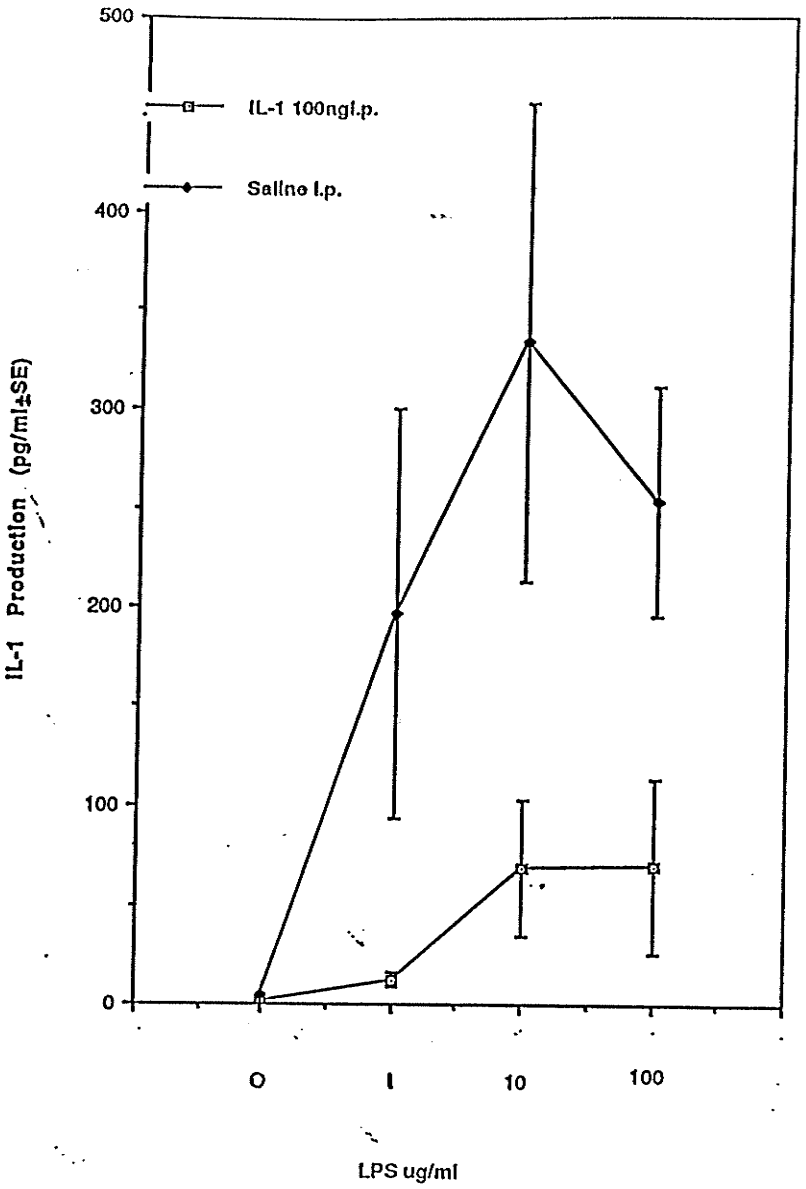

Figure 4. Effect of IL-1ß 100 ng systemic injection on splenic macrophage IL-1 secretion. Spleens were collected two hours postinjection.

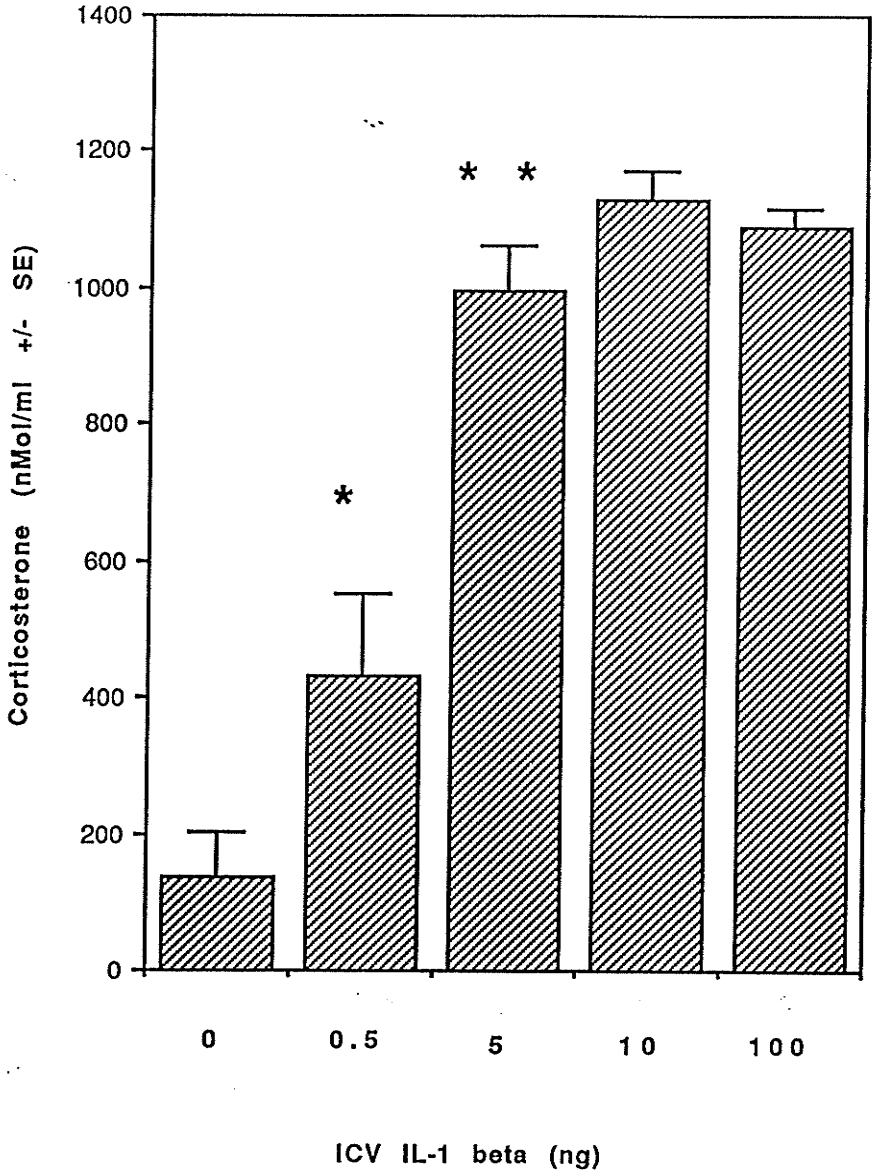

Figure 5. Dose response of 0.5, 5, 10, 100 ng IL-1ß or saline ICV on serum corticosterone level 2 hr postinjection. ** = P < 0.001 using Student's t test compared to vehicle injection. n = 5 rats/group.

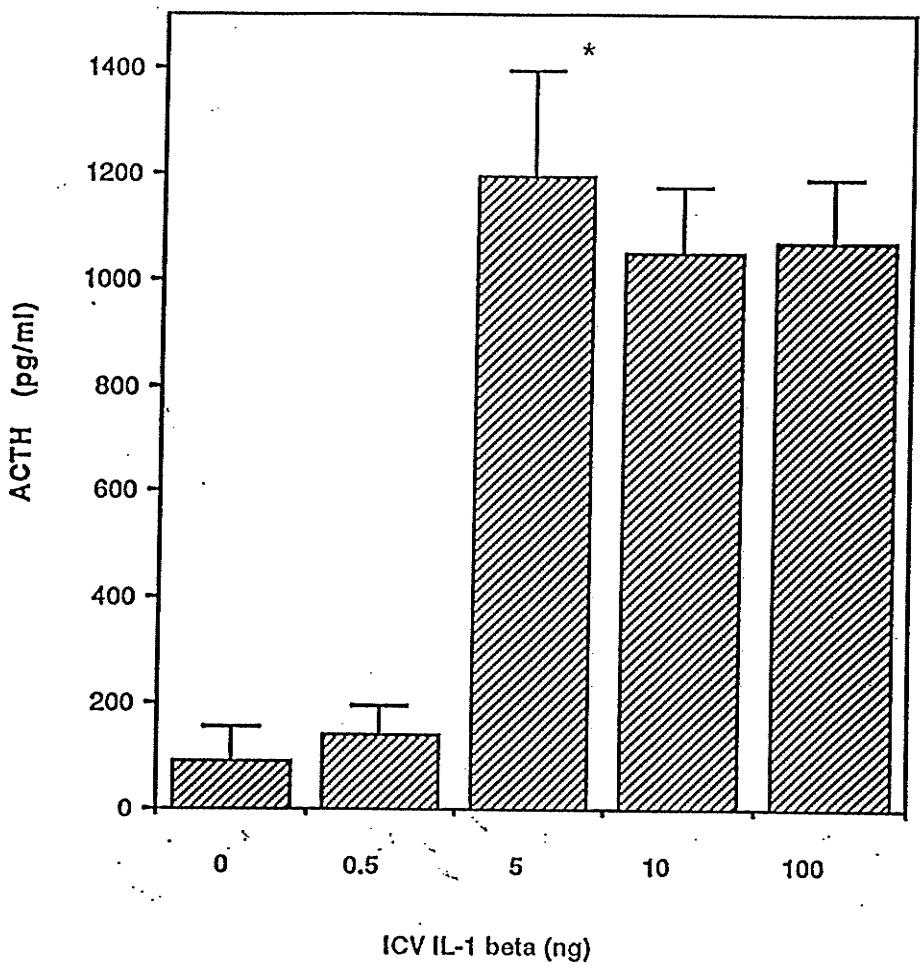

Figure 6. Dose response of IL-1ß on serum ACTH level. * = P < 0.001 using Student's t test compared to vehicle injection. n = 5 rats/group.

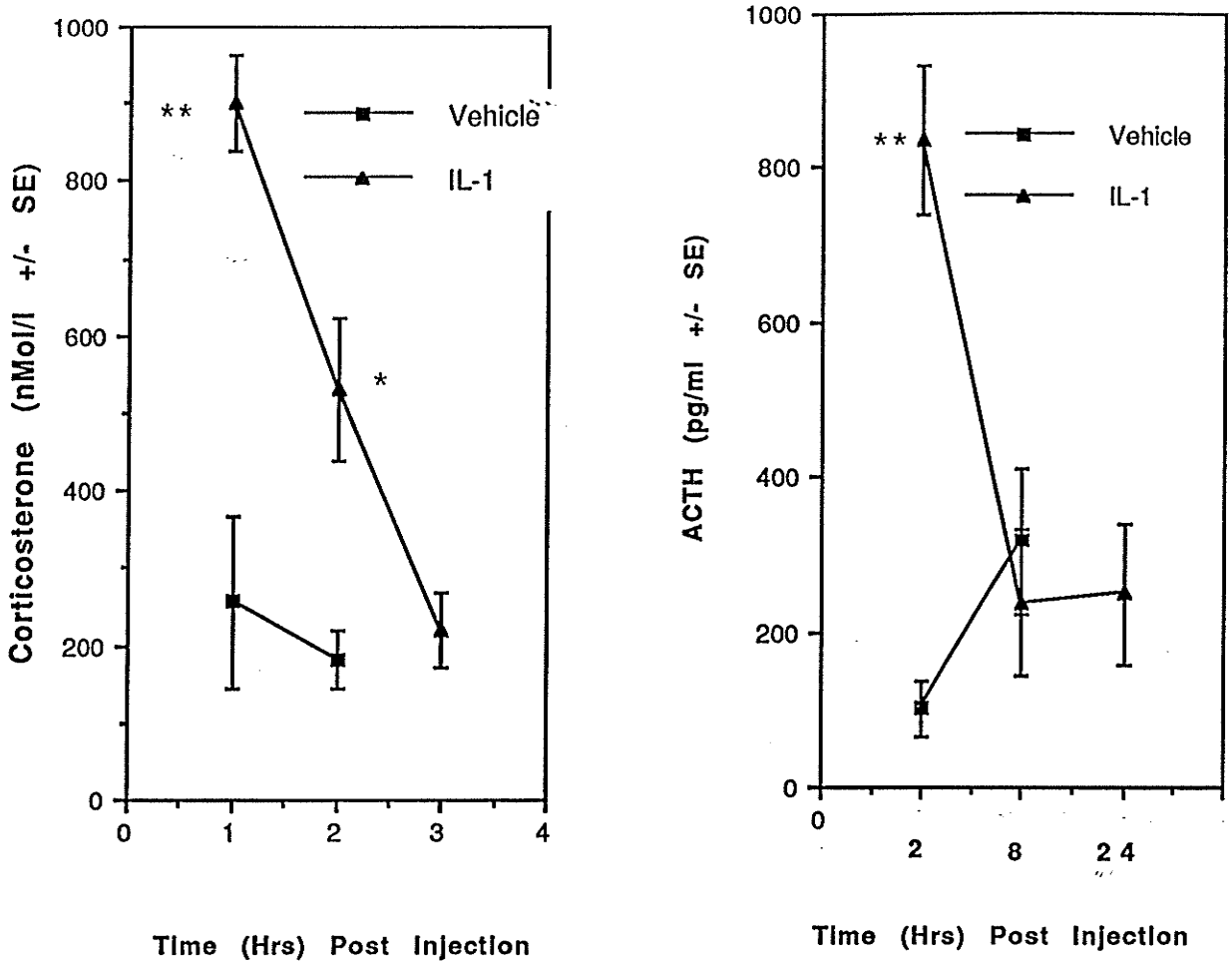

Figure 7. Time course of plasma ACTH and corticosterone on 2, 8, and 24 hr postinjection of 5 ng IL-1ß ICV. ** = $P < 0.001$, * = $P < 0.05$ using Student's t test compared to vehicle injection. n = 5 rats/group.

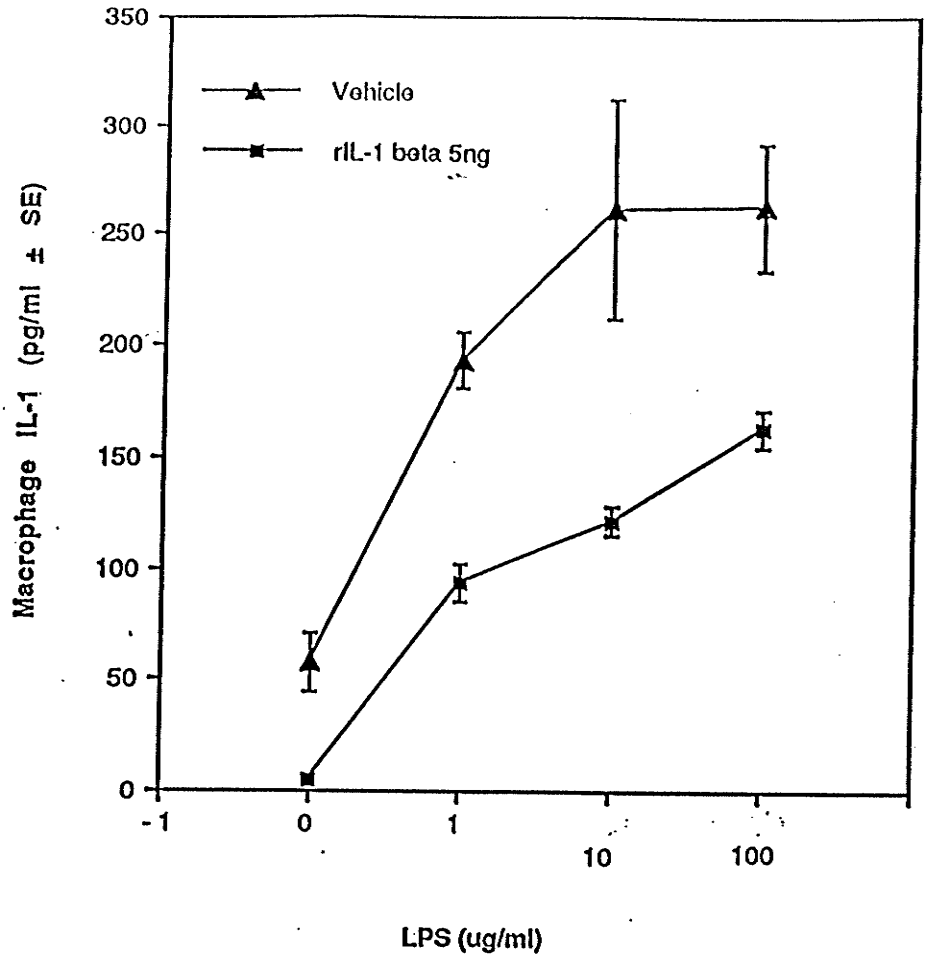

Figure 8. Effect of 5 ng IL-1ß ICV on splenic macrophage IL-1 production. (A) Suppression of splenic macrophage IL-1 production 2 hr postinjection. ($F(1.32) = 9.56$, $P < 0.01$). (B) TGF-ß production by macrophages from IL-1 ICV treated rats stimulated with 10 ng LPS.

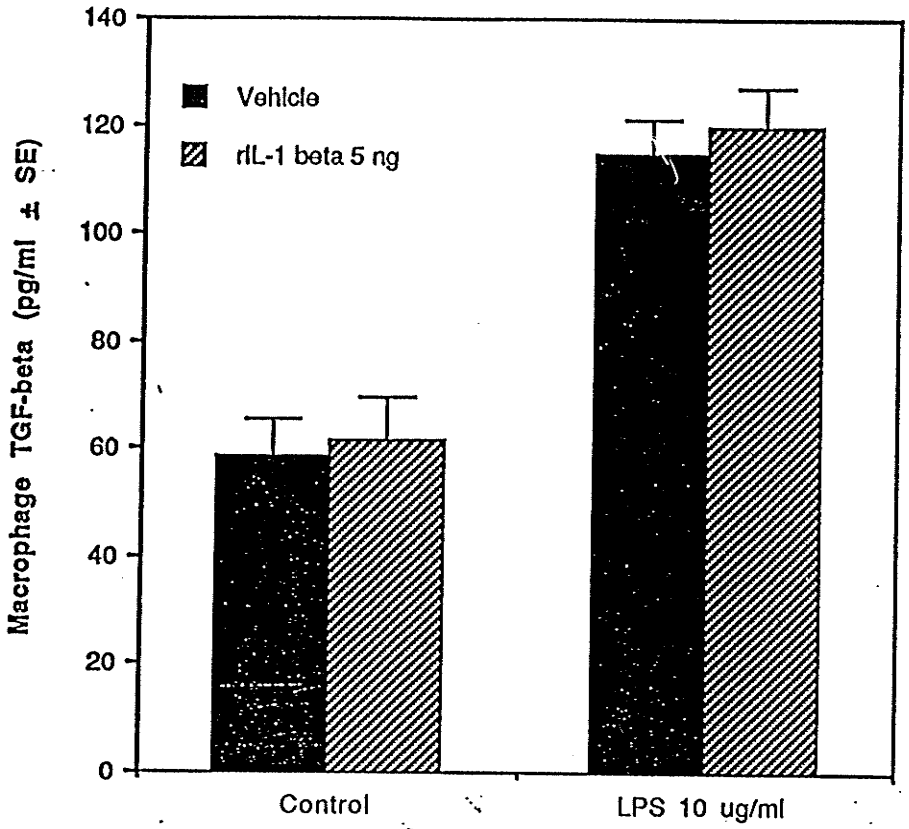

Figure 9. Effect of 5 ng IL-1 ICV on splenic macrophage TGF-ß production. Spleen were collected two hours postinjection.

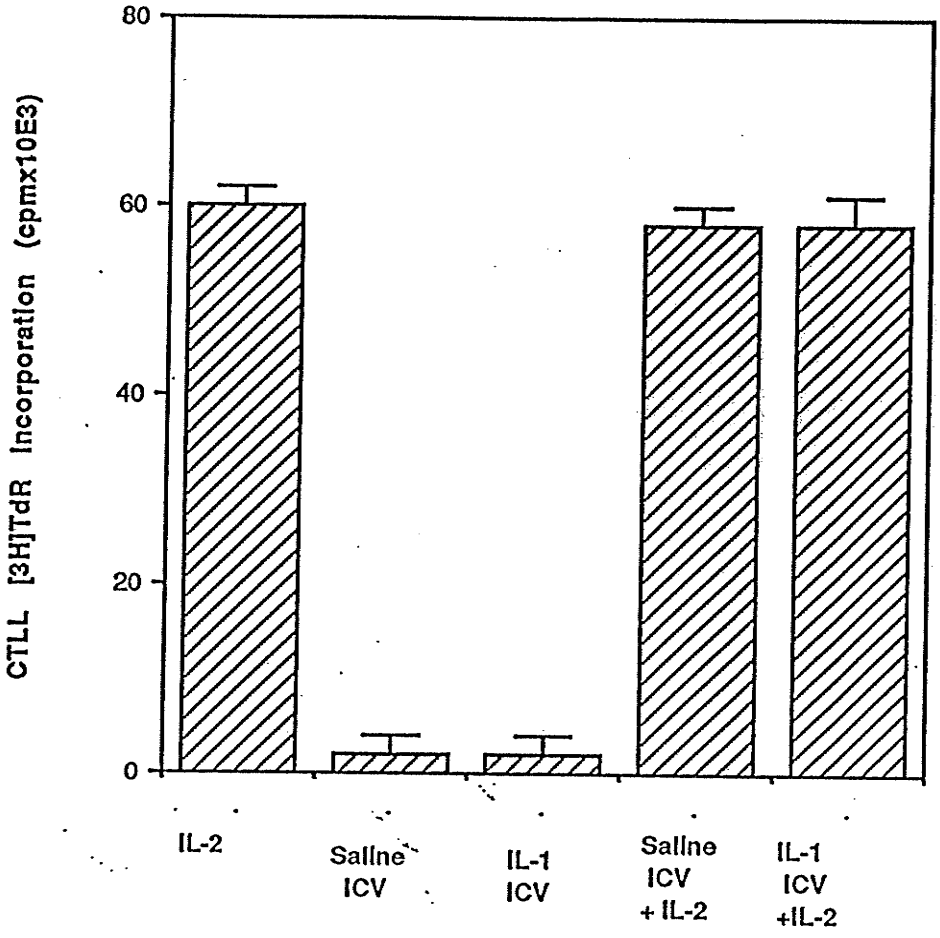

Figure 10. Effect of macrophage supernatant on CTLL cell proliferation. IL-2 or IL-2 plus each group were used as positive controls.

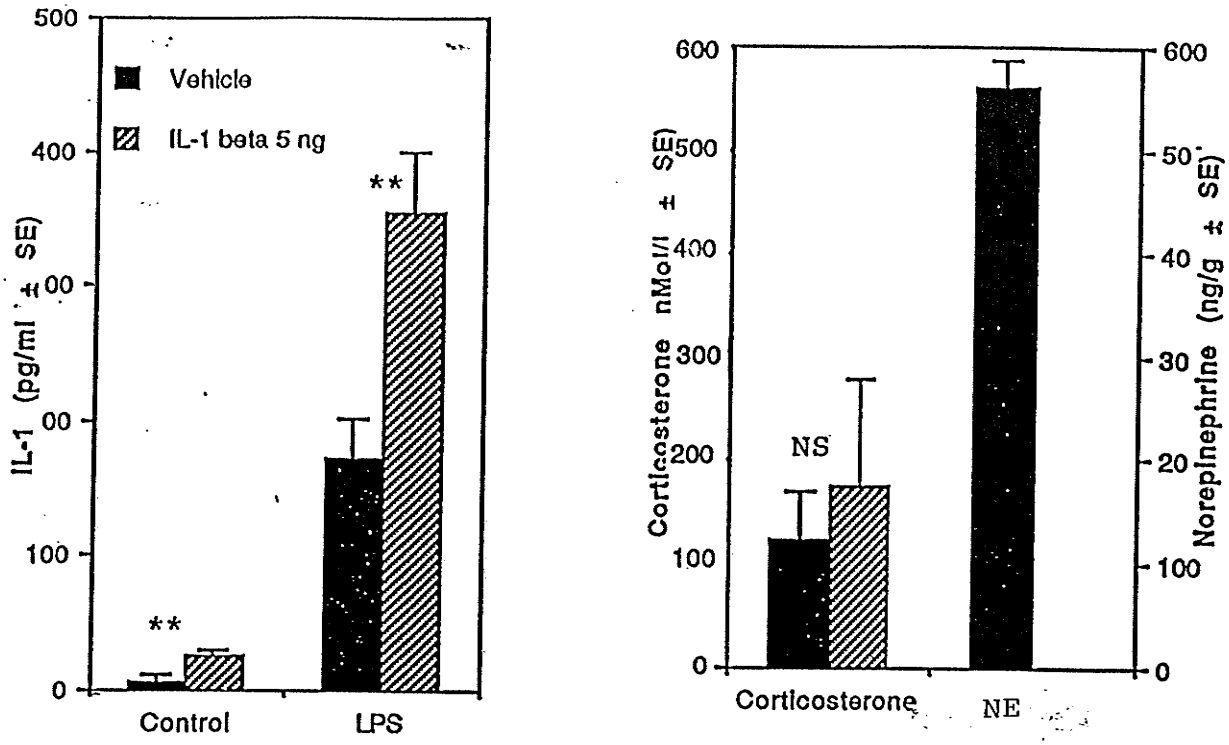

Figure 11. Effect of ADX on splenic macrophage IL-1 secretion following 5 ng of IL-1ß or saline ICV with or without LPS stimulation. * = $P < 0.001$ using Student's t test compared to vehicle injection. NS, not significant. n = 5-8 rats/group.

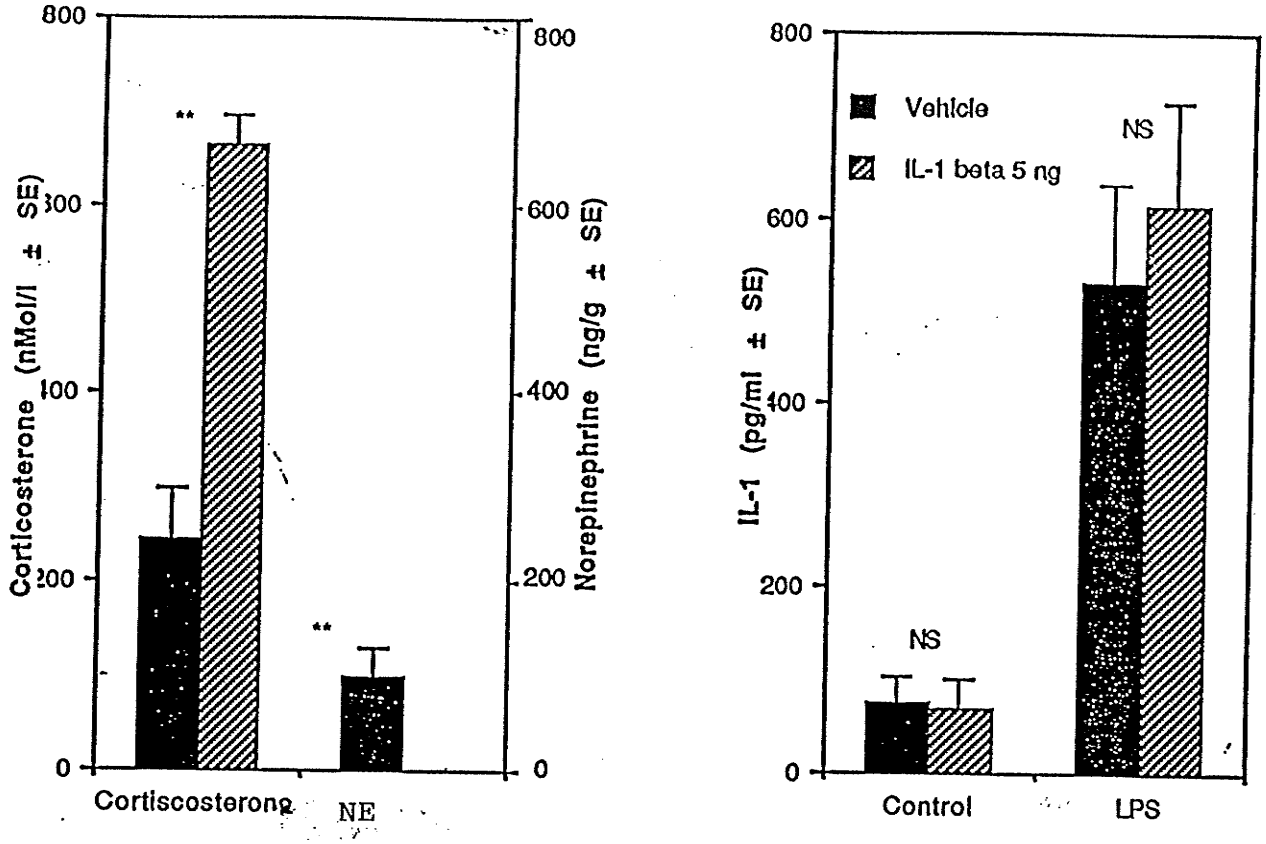

Figure 12. Effect of SNS on splenic macrophage IL-1 secretion following 5 ng of IL-1ß or saline ICV with or without LPS stimulation. * = $P < 0.001$ using Student's t test compared to vehicle injection. NS, not significant. n = 5-8 rats/group.

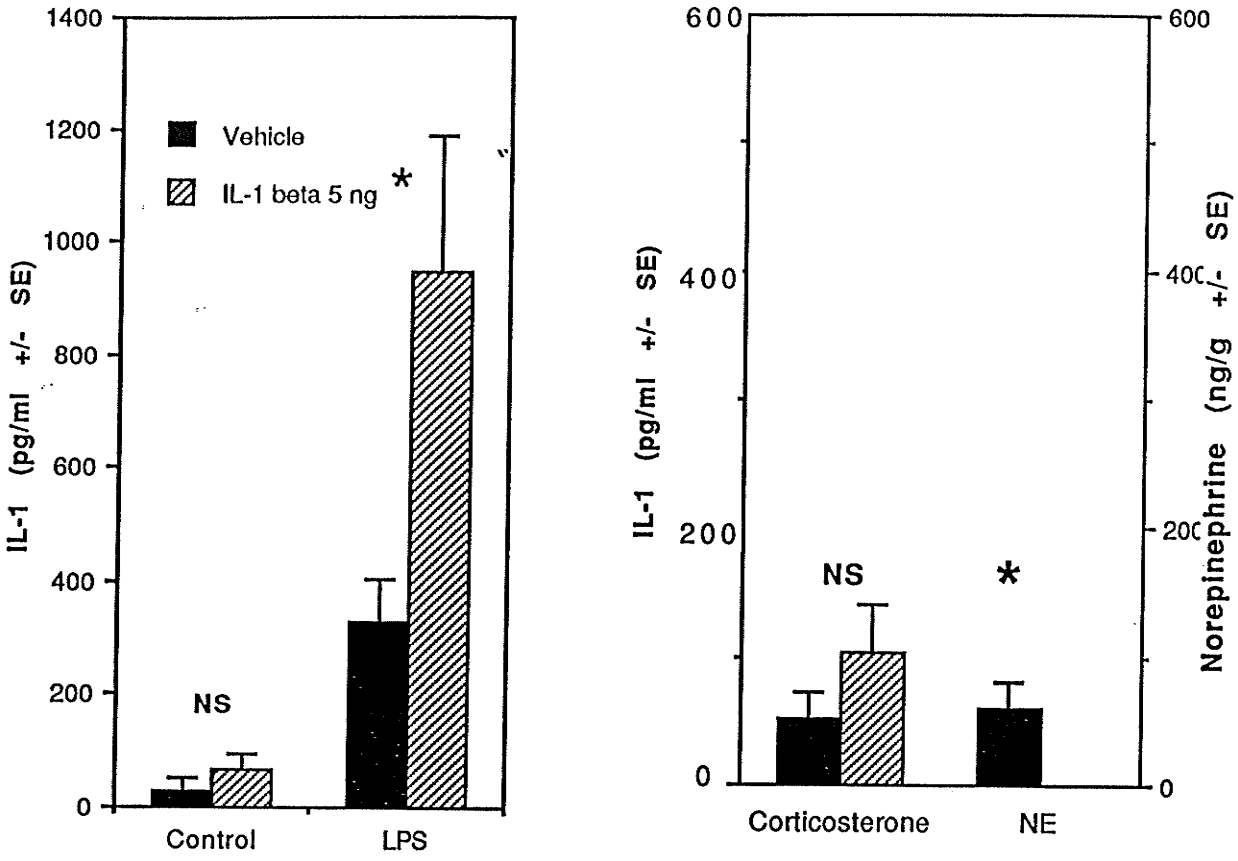

Figure 13. Effect of combining ADX and SNS on splenic macrophage IL-1 secretion following 5 ng of IL-1ß or saline ICV with or without LPS stimulation. * = $P < 0.001$ using Student's t test compared to vehicle injection. NS, not significant. n = 5-8 rats/group.

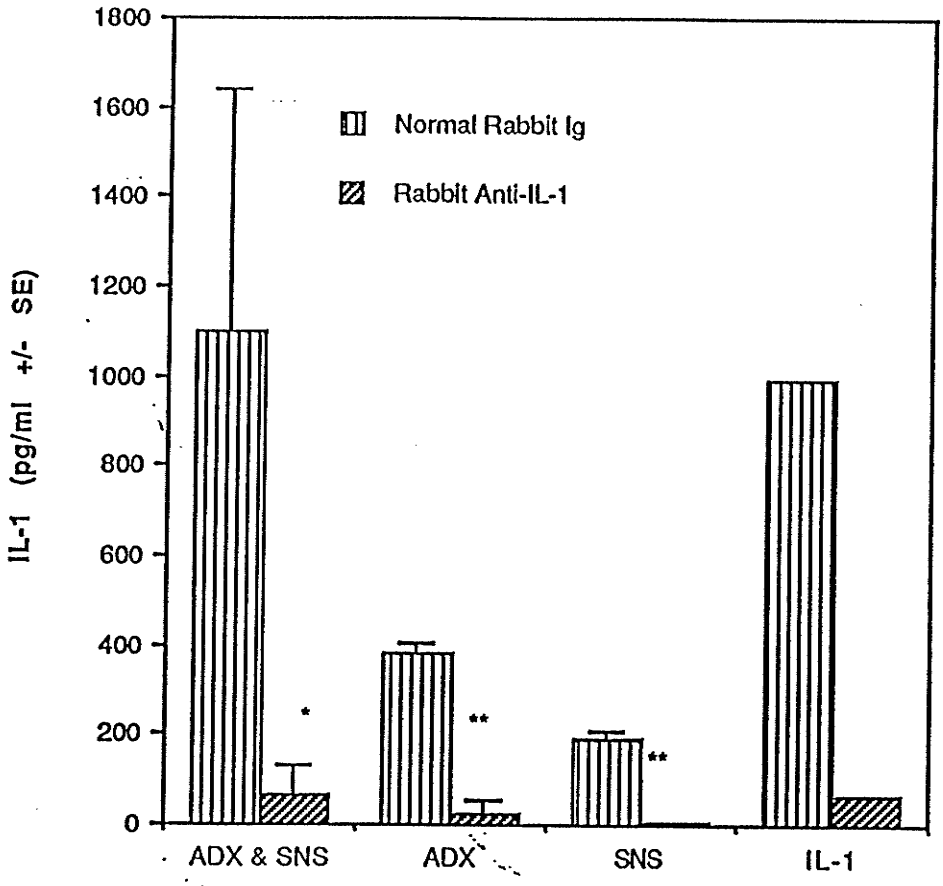

Figure 14. Neutralization of IL-1 in the supernatant of macrophages from IL-1 ICV treated ADX and SNS rats. Immunoreactive IL-1 was neutralized by rabbit anti-IL-1 antibody, but not control rabbit immunoglobulin. ** = $P < 0.01$, * = $P < 0.05$ using student's t test compared to control rabbit immunoglobulin; n = 5-8 rats per group.

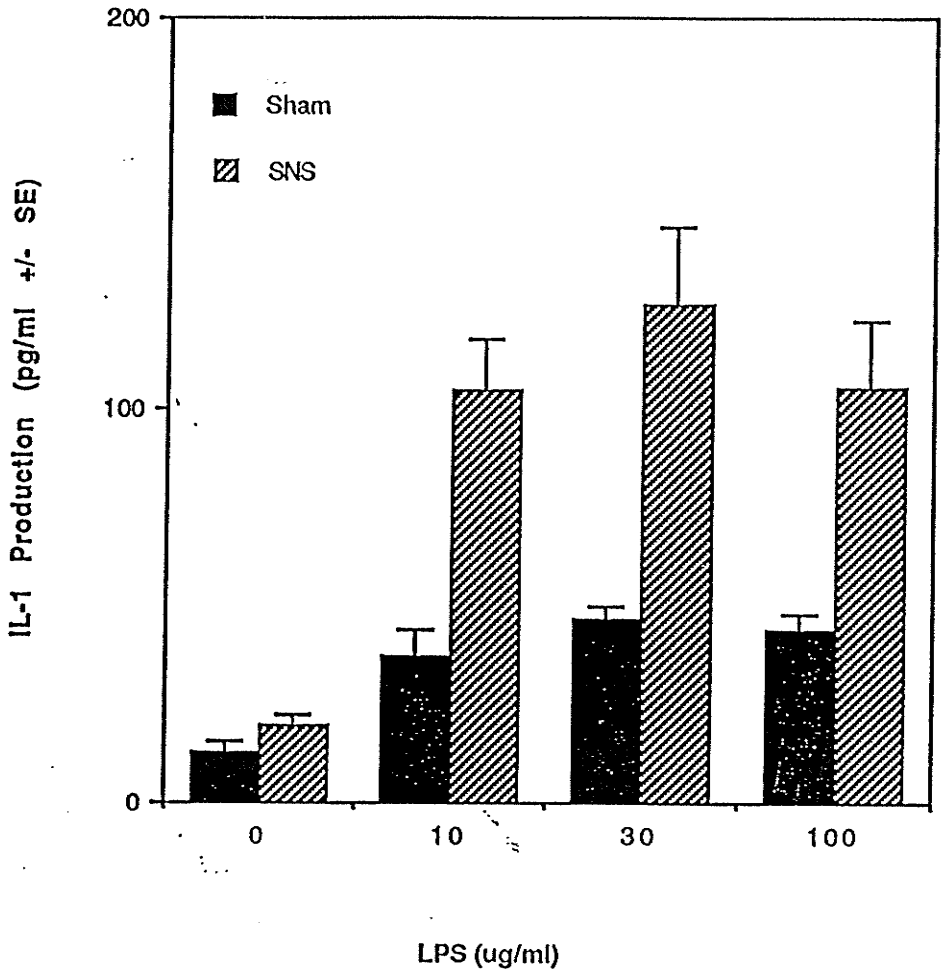

Figure 15. Effect of SNS alone on IL-1 secretion from splenic macrophages stimulated by increasing doses of LPS.

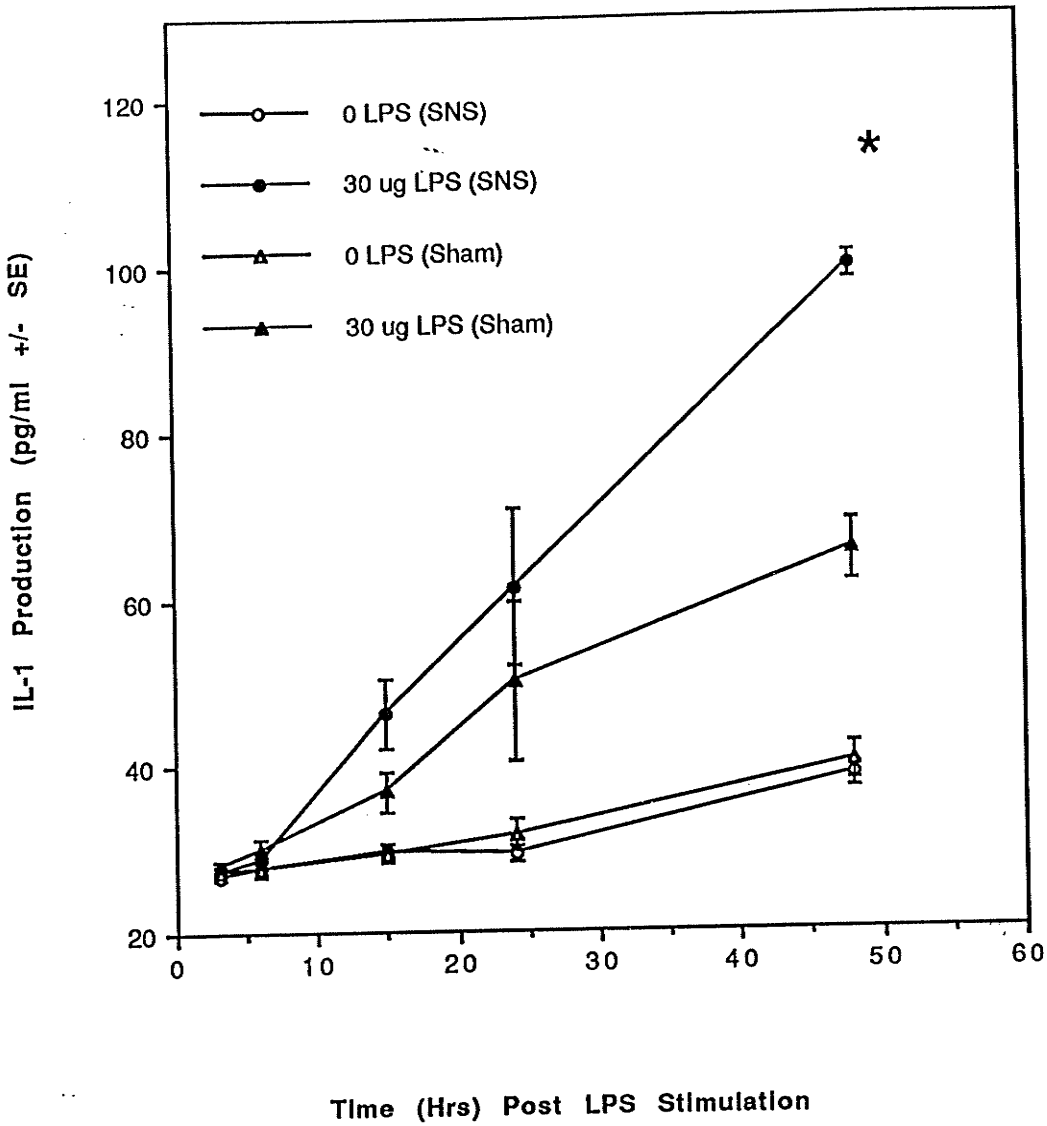

Figure 16. Effect of SNS alone on IL-1 secretion: time course after stimulation of macrophages with LPS.

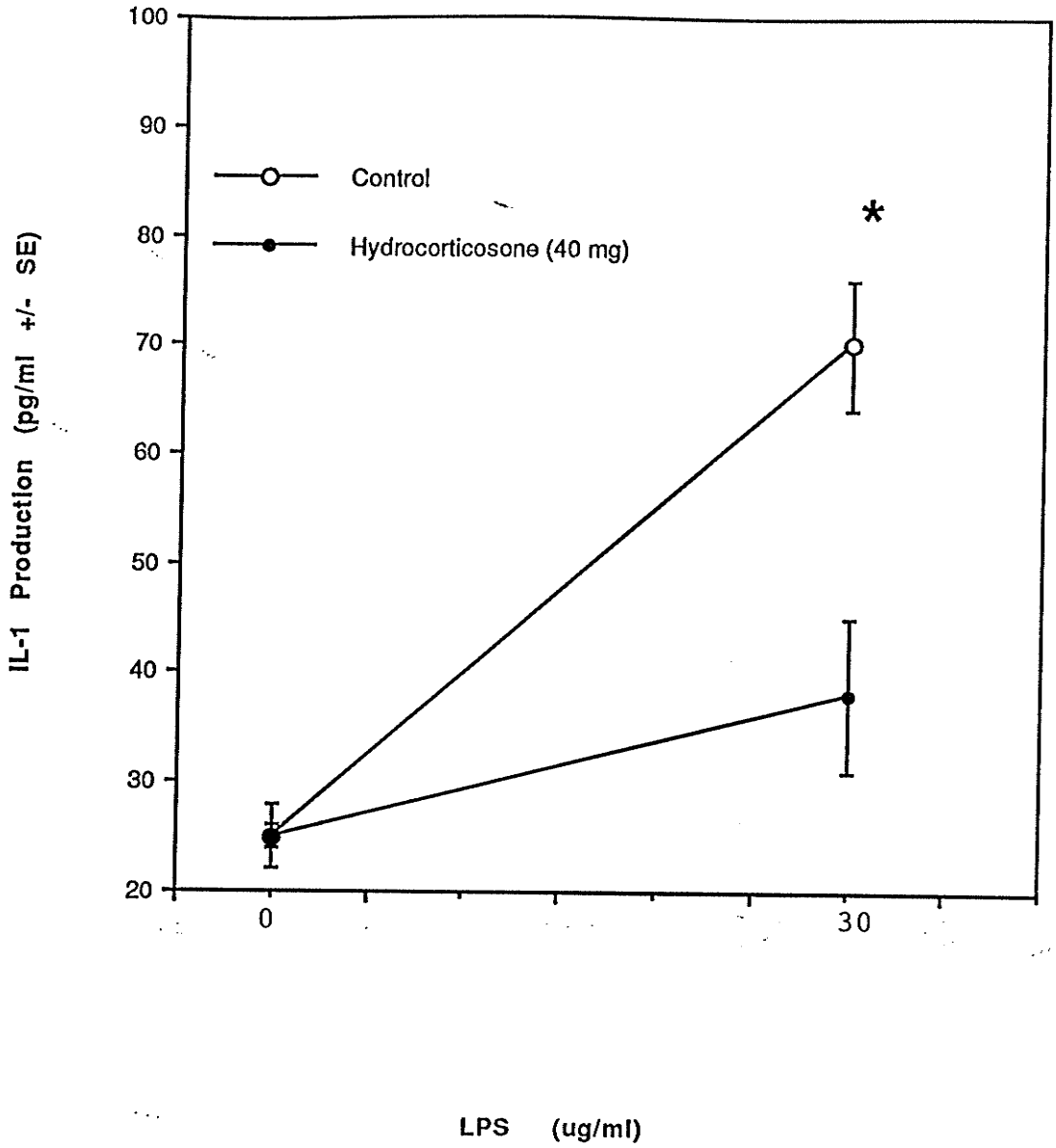

Figure 17. Suppressive effect of hydrocortisone in vivo on splenic macrophage IL-1 secretion.

Discussion

Some investigators have pointed out that there is a threshold for the CNS to respond to the immunological stimuli and the immune changes can only be detected when these changes reach a certain level[152],[9]. Using systemic IL-1 administration, we confirmed this hypothesis. In our experiments, higher intraperitoneal doses of IL-1 had a stimulating effect on blood corticosterone level and was accompanied by the suppression of splenic macrophage IL-1 secretion following stimulation by LPS in vitro. This result indicated the activation of the adrenal glands by peripheral IL-1 and is consistent with the findings by others[153],[154]. The suppressive effects of corticosterone on macrophage IL-1 production have been well documented[155],[153],[132]. Present results suggest a link between the peripheral IL-1 induced corticosterone secretion and the suppression of the splenic macrophage IL-1 secretion and supported the existence of a feedback circuit between the IS and the CNS. The results also show that 5 ng of IL-1 i.p. had no effect on the corticosterone secretion but did stimulate its own secretion from splenic macrophage. This result supports the idea that the immune system is autonomous[1], and IL-1 has a self-regulatory function[156]. It suggests further that the immune system can maintain homeostasis using autonomous networks when its response is below the threshold for stimulating the brain. The present experiments do not indicate how peripheral IL-1 entered the brain to affect brain functions, but other

evidence suggests that IL-1 can cross the brain-blood-barrier,[117] [157]. Therefore, it is reasonable to hypothesize that peripheral IL-1 has access to specific brain regions.

Activation of both the pituitary-adrenal function and the sympathetic nervous system was demonstrated by the effect of central IL-1 in our experiments. (1). First, the present study showed that ICV IL-1 increased both ACTH and corticosterone secretion. Since ACTH/corticosterone secretion is usually considered the results of the stimulation of the hypothalamic-pituitary-adrenal axis, it is reasonable to link the hypothalamic secretion of CRF with the central IL-1 induced ACTH/corticosterone secretion. Many studies have demonstrated that IL-1 induces secretion of corticosterone by stimulating CRF and ACTH secretion. For example, Sapolsky et al reported that human IL-1 activated the adrenocortical axis by stimulating the release of CRF from the hypothalamus and that immunoneutralization of CRF blocked the stimulatory effect of IL-1 on glucocorticoid secretion[132]. Ovadia et al reported that IL-1ß stimulated the secretion of ACTH/corticosterone in normal intact rats but failed to do so in rats after complete mediobasal deafferentiation. This suggested that IL-1 activated the HPA axis by a direct effect upon the brain, and that intact neural connections between the mediobasal hypothalamus and extrahypothalamic brain regions are essential for IL-1 induced HPA responses[158]. (2). It is known that

sympathetic nerves innervate the spleen[159]. In addition, it has been shown that central IL-1 can affect the metabolism of some central neurotransmitters[122],[160]. Recent studies by us[161](data not shown) indicate that ICV IL-1 increased the sympathetic activity in rat spleen by enhancing the rate of NE turnover. This is consistent with the recent findings that i.v. injections of IL-1 beta resulted in a dose-dependent increase in the activity of the splenic nerves[154]. Therefore, central IL-1 not only activates the HPA axis but also activates the sympathetic system.

The present studies show that ICV IL-1ß suppressed IL-1 release from LPS-stimulated splenic macrophages. Since the high levels of ACTH/corticosterone have been induced by 5 ng of ICV IL-1ß and the elimination of the suppressive effect of IL-1ß on macrophage IL-1 secretion has been observed following adrenalectomy, the activation of HPA axis may explain the suppression of IL-1 secretion. Sundar et al[162] [163] have reported the suppression of other cellular immune responses, including NK activity, T cell IL-2 activity and mitogen responsiveness following ICV IL-1 in rats. They found that ICV anti-CRF antibody could prevent the above suppression of cellular immune responses. However, the present results suggest that the regulation of macrophage IL-1 secretion following ICV IL-1 is not simple. (1) In our study, surgical section of the splenic nerve also blocked the suppressive effect of ICV IL-1ß on macrophage IL-1 secretion. This

indicates that sympathetic innervation mediates an additional inhibitory control of splenic macrophage IL-1 secretion. This result is supported by earlier pharmacological sympathectomy study[162]. Supporting evidence also comes from the observations by Felten and co-workers[65] [164] [34] that there is an extensive sympathetic innervation of the spleen with catecholamine and peptidergic nerve terminals are distributed among fields of T lymphocytes and macrophages. They also observed synapse-like connections between nerves and lymphocytes and macrophages in the spleen. (2) Following ADX , the suppressive effect of ICV IL-1ß injections on macrophage IL-1 secretion was not simply eliminated, but rather a stimulation of macrophage IL-1 secretion was observeed. This stimulation of macrophage IL-1 secretion was even observeed in animals without LPS stimulation in vitro. The origin of this stimulatory signal observed in ADX rats injected IL-1ß was not clear but it suggests multiple mechanisms for the regulation of the splenic macrophage function by the CNS. Since IL-1 also induced the secretion of other hormones or neuropeptides form the brain (see literature review), it is possible that some of these hormones or neuropeptides have a positive effect on splenic macrophage functions. For example, Substance P has a positive effect on macrophages, including enhancement of phagocytosis and production of H_2O_2 and O_2.[165]. Therefore, further studies of these multiple mechanisms of immunoregulation by the CNS are required. (3) The present results indicate that neither

the adrenal cortical hormone nor the sympathetic innervation was sufficient to induce macrophage suppression by itself. This was shown by the results that the prevention of the suppression of macrophage IL-1 secretion by SNS or ADX occurred in the presence of significant increases in blood corticosterone level or in the presence of intact sympathetic activity induced by IL-1 ICV. (4) Furthermore, following ICV IL-1ß injection, the combination of ADX and SNS resulted in higher level of IL-1 production by splenic macrophages than that seen with either ADX or SNS alone. This suggests a synergic effect for both HPA and sympathetic pathways and confirm that both pathways are involved in the suppression of macrophage function. Similar to our study, Besedovsky et al.[37] have described the effect of this ADX-SNS combination as " removal of restraint" when they found an increased ability of splenocytes to produce plaque-forming cells in response to SRBC immunization following surgical denervation of the spleen combined with ADX.

More direct evidence supporting the contribution of both corticosterone and sympathetic innervation to the negative control of splenic macrophage function comes from experiments of adrenalectomy and sympathectomy in animals without ICV IL-1ß administration. In recent years, the study of the sympathetic innervation to lymphoid organs has drawn more and more attention from many investigators[166]. Studies already

suggested an immunoregulatory role for the sympathetic system in some special situations, such as IL-1 administrations[162]. But, to our knowledge, there was no direct study addressing the question of whether surgical denervation has a regulatory effect on immune cells. Only pharmacological sympathectomy has been attempted. We observed a significant enhancement of IL-1 secretion from splenic macrophages stimulated by LPS following SNS. This result provided direct evidence to support the hypothesis that a negative control is exerted on splenic macrophage IL-1 production by the sympathetic nervous system. In our study, the drop of the splenic norepinephrine content has been the major index of the efficiency of SNS. Studies have showed that NE can block LPS-induced IL-1 production from macrophages[167]. A variety of neurotransmitters have been found to be co-localized at the sympathetic nerve terminals in the normal spleen[168] and the present study dose not answer the question of how different neurotransmitters in the spleen may play immunoregulatory roles. Nevertheless, from the above evidence, we hypothesize that the CNS maintains a continuing regulation on macrophages and this regulation is involved in the haemostasis of the IS. This regulation can become more active once the magnitude of the immune response is over the threshold for the CNS activation. The suppressive effect of corticosterone on macrophage IL-1 production has been well documented[169] [153] and was confirmed in our studies. It was known that corticosteroids act at the transcription and

translation levels of IL-1 production and a receptor-mediated mechanism has been well studied for this hormone[170] [171]. From the above discussion, it appears that multiple neural and endocrine factors act together on the macrophage, including corticosteroids and neurotransmitters derived from sympathetic nerve terminals. Therefore, future studies in this area should include the issue of how these different mediators act together on macrophages.

Taken together, the present study suggests that: (1) IL-1, is an important mediator in the communication between the CNS and the IS, and can not only transmit the signal from the IS to CNS but also can trigger the brain to modify the immunoregulatory function through both neuroendocrine hormones and sympathetic innervation. (2) The relationship between the CNS and macrophage IL-1 production is not as simple as the HPA model proposed by Besedovsky[172] [139] and the sympathetic nervous system is also involved in this regulation. Besedovsky and colleagues first proposed the activation of HPA axis by peripheral IL-1 and the suppressive effect of HPA axis on immune function[172] [139]. Felten and co-workers demonstrated the existence of sympathetic nerve terminals in lymphoid organs including the spleen[65]. Our study has linked both the HPA axis and sympathetic nervous system together in macrophage immunoregulation and has provided direct evidence that the sympathetic innervation of the spleen contributes to the

negative control of splenic macrophage IL-1 secretion. A recent study by Sundar et al[162] [163] noted that ICV IL-1ß had the suppressive effect on various cellular immune responses including NK activity, IL-2 production and T cell mitogen response. They called this phenomena " brain IL-1-induced immunosuppression" and observed the involvement of both the HPA axis and the sympathetic nervous system. Our results are consistent with this study and have confirmed the existence of a bidirectional mechanism for CNS-immune interaction.

Summary

1. Systemic injection of 100 ng IL-1ß resulted in the increase of blood corticosterone level and suppression of splenic macrophage IL-1 secretion, while 5 ng did not increase corticosterone level but rather has a stimulatory effect on macrophage IL-1 secretion.

2. Intracerebroventricular (ICV) injection of IL-1 beta produced a dose-dependent increase in plasma corticosterone and ACTH within 2 hr of injection. It also increase splenic NE content.

3. ICV injection of 5 ng IL-1 beta resulted in a suppression of splenic macrophage IL-1 secretion following stimulation by LPS in vitro. Macrophage TGF-beta was not affected.

4. Following ADX, the suppressive effect of ICV IL-1 was reversed and resulted in stimulation of macrophage IL-1 secretion.

5. Surgical interruption of the splenic innervation to the spleen also prevented the suppression of macrophage IL-1 after ICV IL-1.

6. The combination of ADX and SNS resulted in a potent stimulatory effect of ICV IL-1 on splenic macrophage IL-1 secretion.

7. Without IL-1 ICV, SNS alone resulted in the stimulation of splenic macrophage IL-1 secretion.

8. Subcutaneous injection of hydrocortisone induced suppression of splenic macrophage IL-1 secretion.

Conclusion

This study supports the concept that CNS plays a specific regulatory role in the regulation of the immune system and indicates that a bi-way mechanism, i.e., ACTH-corticosterone and sympathetic innervation is involved in the negative control of splenic macrophage IL-1 secretion.

Future Direction

1. The specific effect of central IL-1 on the brain, including the site of its effect, its target cells and mediators needs to be clarified.

2. The mechanism by which peripheral IL-1 produces its effects in the brain has to be clarified.

3. The balance between positive and negative signals from the brain to splenic macrophage has to be investigated. For example, what are the major stimulatory mediators for splenic macrophage function and how do macrophages maintain homeostasis via central control.

4. The specific contributions of different neurotransmitters to the regulation of macrophage function, including the cellular and molecular mechanisms should be studied in the future.

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EDUCATION

1978-1982: Degree in Medicine
Faculty of Medicine,
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1983-1986: Master of Science in Medicine
Department of Immunology & Microbiology,
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1988-1990: Research Associate
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1990-1992: Master of Science (Expecting graduation in the Fall of 1992)

Department of Immunology/Manitoba Institute of Cell Biology, University of Manitoba
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TEACHING EXPERIENCE

1985-1988: Lecturer in the Department of Immunology & Microbiology, ZunYi Medical College, P.R.China

SCHOLARSHIP AND AWARDS

1. Graduate Study Fellowship, DaLian Medical College, P.R.China 1983-1985
2. Awards for the excellent thesis, DaLian Association for Microbiology, P.R.China 1986

TECHNICAL SKILLS

1. Cell culture and the related techniques:
   (1). Sterile media preparation.
   (2). Tissue Process
   (3). Cell culture, such as NOB1, CTLL, 3T3, LBRM cell lines and splenic macrophages, alveolar macrophages, etc.
   (4). Maintenance and cryopreservation of cell lines
2. Bioassay systems:
   (1). IL-1 bioassay system.
   (2). IL-2 bioassay system.

(3). NK Assay.

3. Radioimmunoassay:

   Such as corticosterone assay.

4. Plaque forming cell assay for B lymphocyte antibody production in spleen cells.

5. T lymphocyte mitogen response assay.

6. Handling of laboratory animals including rats, mice, guinea pigs, rabbits.

7. Immunofluorecent techniques.

8. Drug administrations for animals:

   (1) Intracerebroventricular injection.

   (2) Intraperitoneal injection.

   (3) Subcutaneous injection.

9. Surgical procedures:

   (1) Cerebroventricular cannulation

   (2) Removal of spleen, adrenal, thymus from animals.

   (3) Spleen nerve section.

10. Computer applications:

   (1) Wordperfect program.

   (2) Sigmaplot program.

   (3) NCSS statistical program.

   (4) Computerized measurement of hydrogen peroxide.

   (5) Some UNIX system knowledge.

11. Other biomedical and immunological techniques learned in graduate and undergraduate lab. courses including ELISA, electrophoresis, etc.

PUBLICATIONS

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In Preparation:

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12. Khalil, N., Zuo, L. and Greenberg, A.H. Differential

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