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Role of Interleukin-1 in Stress Responses

A Putative Neurotransmitter

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Abstract

Recently, the central roles of interleukin-1 (IL-1) in physical stress responses have been attracting attention. Stress responses have been characterized as central neurohormonal changes, as well as behavioral and physiological changes. Administration of IL-1 has been shown to induce effects comparable to stress-induced changes. IL-1 acts on the brain, especially the hypothalamus, to enhance release of monoamines, such as norepinephrine, dopamine, and serotonin, as well as secretion of corticotropin-releasing hormone (CRH). IL-1-induced activation of the hypothalamo-pituitary-adrenal (HPA) axis in vivo depends on secretion of CRH, an intact pituitary, and the ventral noradrenergic bundle that innervates the CRH-containing neurons in the paraventricular nucleus of the hypothalamus. Recent studies have shown that IL-1 is present within neurons in the brain, suggesting that IL-1 functions in neuronal transmission. We showed that IL-1 in the brain is involved in the stress response, and that stress-induced activation of monoamine release and the HPA axis were inhibited by IL-1 receptor antagonist (IL-1Ra) administration directly into the rat hypothalamus. IL-1Ra has been known to exert a blocking effect on IL-1 by competitively inhibiting the binding of IL-1 to IL-1 receptors. In the latter part of this review, we will attempt to describe the relationship between central nervous system diseases, including psychological disorders, and the functions of IL-1 as a putative neurotransmitter.

Index Entries: Interleukin-1; stress responses; hypothalamo-pituitary-adrenal axis; interleukin-1 receptor antagonist; monoamine; corticotropin-releasing hormone; adrenocorticotropic hormone.

Central Stress Responses

Activation of the HPA Axis

Animals exposed to stressful stimuli exhibit responses causing adrenocorticotropic hormone (ACTH) secretion (Bilezikjian and Vale, 1983; Vale et al., 1983). ACTH is secreted in response to multiple stimuli by hypothalamic neuropeptides and neuronal monoamines, such as corticotropin-releasing hormone (CRH), vasopressin (VP), norepinephrine (NE), serotonin (5-HT), and so on. CRH is a major media-

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tor of ACTH release. ACTH enhances the secretion of glucocorticoids from the adrenal cortex (Bilezikjian and Vale, 1983; Vale et al., 1983). Glucocorticoids mediate many of the metabolic and immune aspects of the stress response, such as stimulation of hepatic gluconeogenesis, increase of metabolic rate, suppression of inflammation, and inhibition of immune reactions. Glucocorticoids also regulate the secretion of CRH and ACTH by an inhibitory feedback mechanism mediating type II glucocorticoid receptors, which are present at high concentrations in the hypothalamus, particularly in the CRH neurons (McEwen et al., 1968; Johnston et al., 1985).

In the case of animals exposed to sustained stress, the ACTH response manifests as a rapid increase followed by a decline toward normal levels despite the continuous presence of the stress (Keller-wood and Dallman, 1984; Hauger et al., 1988). It was suspected that a glucocorticoid feedback mechanism contributed to adaptation of the pituitary ACTH response in prolonged stress. However, the absence of glucocorticoids in adrenalectomized animals does not prevent the decline in ACTH secretion that normally occurs over a period of sustained stress (De Souza and Van Loon, 1982). Keller-wood and Dallman (1984) proposed that glucocorticoid feedback does not reduce ACTH responses during chronic stress. Such attenuation of the pituitary response in sustained stress may involve a number of mechanisms, including decreased hypothalamic secretion of CRH, exhaustion of the ACTH secretory capacity, and a decrease in pituitary receptors for ACTH regulators (Hauger et al., 1988). Hauger et al. (1988) reported that CRH receptor downregulation in the pituitary gland, after prolonged stress, results in partial desensitization of ACTH responses to CRH.

Further consideration of the finding that the absence of glucocorticoids in adrenalectomized animals does not prevent a decline in ACTH secretion during sustained stress (De Souza and Van Loon, 1982) is necessary, since this observation indicates that the glucocorticoid feedback mechanism is not the only system regulating this phenomenon. Whereas interaction between neural stimulatory effects on CRH and ACTH secretion and the inhibitory effects of glucocorticoids on these secretory activities have been investigated in considerable detail, questions persist as to what factors other than glucocorticoids are associated with decline of plasma ACTH levels toward normal levels, following a rapid increase, despite unabated stress. Gamma-aminobutyric acid (GABA) and opioid peptide have been suggested to participate in the inhibition of stress-induced CRH release, since these substances are known to be capable of inhibiting CRH release (Calogero et al., 1988).

On the other hand, in contrast with the participation of putative mechanisms for adaptation of the pituitary ACTH response to prolonged stress, ACTH responses to CRH or to novel stressful stimuli during prolonged stress were potentiated (De Souza and Van Loon, 1982). Although glucocorticoids downregulate their own receptors in many biological systems (Keller-wood and Dallman, 1984), this phenomenon occurs in a site-specific manner in the brain, namely in the hippocampus, septum, and amygdala but not in the hypothalamus or pituitary gland (Sapolsky et al., 1984). Hypothalamic-lesioned animals, in whom pituitary responsiveness to CRH is diminished by a glucocorticoid feedback signal, have been used to demonstrate that glucocorticoid-feedback inhibition can occur at the pituitary level (Jones et al., 1977). These findings suggest that the involvement of the negative feedback system is at the level of the hypophysis and the hypothalamus. The anatomical loci of such feedback inhibition and mechanisms to maintain the release of these hormones by overcoming the glucocorticoid negative feedback have been areas of major research interest.

Glucocorticoid Resistance Mechanism in the Hypothalamus

It has been suggested that VP may play a role in regulating pituitary responsiveness during chronic stress. VP was found not only to

stimulate ACTH release on its own but also to potentiate the stimulatory effect of CRH on ACTH secretion (Gillies et al., 1982). De Goeij et al. (1991) reported that the secretion of CRH and VP from the external zone of the median eminence are independently controlled during acute immobilization stress, and that chronic repeated stress leads to increased VP stores and colocalization in CRH nerve terminals. Although the ACTH releasing activity of VP is several-fold less potent than that of CRH (Spinedi and Negro-Vilar, 1983), levels of VP in the external zone of the median eminence after chronic immobilization stress increase twofold (De Goeij et al., 1991). This indicates that VP is not the only factor enhancing the ACTH release.

Spinedi and Negro-Vilar (1983) found that 5-HT potentiates ACTH releasing activity of VP in the rat anterior pituitary, whereas the potentiating activity of 5-HT was not seen in the presence of CRH. They also found that 5-HT directly affects the release of ACTH from anterior pituitary cells. Since the anterior pituitary does not have the capacity to decarboxylate 5-hydroxytryptophan to 5-HT, most of the 5-HT present in the gland is suspected to be formed in other tissues, especially the hypothalamus (Johnston et al., 1985). Past investigations have revealed a prominent serotoninergic innervation of CRH perikarya in the paraventricular nucleus (PVN) of the hypothalamus (Liposits et al., 1987). 5-HT also increases the secretion of CRH from hypothalamic fragments in vitro (Buckingham and Hodges, 1979), especially from the median eminence in vivo (Gibbs and Vale, 1983). Moreover, a 5-HT_{1A} agonist, 8-OH-DPAT, stimulates CRH secretion from isolated hypothalamic blocks in vitro (Calogero et al., 1989). These observations suggest that 5-HT enhances the release of CRH, resulting in potentiation of the secretion of ACTH, and that 5-HT in chronic stress enhances the action of VP, but not CRH.

On the other hand, NE has also been shown to play a role in overcoming negative feedback by glucocorticoids. NE can stimulate CRH release via the α_1-adrenergic receptor (Calogero et al., 1988), potentiate the release of ACTH by CRH (Lamberts et al., 1986), and exert a potent and highly specific stimulatory effect on ACTH release at the anterior pituitary level (Vale et al., 1978). Dexamethasone cannot completely abolish the ACTH release induced by the combined actions of CRH and NE (Bilezikjian and Vale, 1983; Giguere and Labrie, 1983; Lamberts et al., 1986), and depletion of hypothalamic NE enhances the dexamethasone negative feedback effect on ether-stress induced elevation of serum corticosterone (Feldman and Weidenfeld, 1991). Although there appears to be little information in the literature on the role of NE in glucocorticoid negative feedback during chronic stress, the few reports available provide further evidence that NE enhances the release of CRH resulting in potentiation of ACTH secretion.

Involvement of the Hippocampus in Suppression of the HPA Axis

The involvement of limbic structures has also been considered to facilitate basal pulsatile secretion of ACTH under nonstressful conditions (Mangili et al., 1966; Kawakami et al., 1968; Willoughby and Martin, 1978). Studies utilizing discrete foci of stimulation have shown that all hippocampal regions, with the exception of the CA1, inhibit the HPA axis (Dunn and Orr, 1984). Reports showing that hippocampal damage impairs the ability of rats to inhibit glucocorticoid secretion indicate a hippocampal capacity to appropriately terminate the stress response (Sapolsky et al., 1983). The hippocampus does appear to mediate glucocorticoid feedback inhibitory signals to the hypothalamus. However, its contribution to the HPA axis is limited, therefore the extent of hypercortisolism after hippocampal damage is moderate (Sapolsky and McEwen, 1988). Since glucocorticoid interaction with glucocorticoid receptors in the hippocampus is an obligatory first step, depletion of hippocampal glucocorticoid receptors without damaging the neurons of the hippocampus is speculated to activate the HPA axis (Sapolsky and McEwen, 1988). Actually, the hippocampus is preferentially

sensitive to such "downregulation" of all brain structures (Sapolsky et al., 1984), and sustained exposure to high concentrations of glucocorticoids produces an autoregulatory decline in glucocorticoid receptors (Sapolsky and McEwen, 1988). Thus, loss of hippocampal glucocorticoid receptors appears likely to be involved in activation of the HPA axis.

Activation of Monoamine Turnover in the Hypothalamus

Monoaminergic neuronal inputs to the hypothalamus, especially the PVN containing primarily CRH and participating in the regulation of pituitary-adrenocortical and sympathoadrenal outflows during stress, originate mainly from lower brain stem cell groups in the ventrolateral medulla (A1/C1 cell groups), around and within the nucleus or solitary tract (A2/C2 cell groups), and in the locus ceruleus (LC; A6 cell group) (Palkovits et al., 1980a,b). Since the parvocellular portion contains noradrenergic terminals on CRH-immunoreactive cells and is innervated mainly from A2/C2 cell groups, stress-induced CRH responses are believed to be partially controlled by A2/C2 cell groups (Nalai et al., 1986). The PVN receives dopaminergic fibers from A13 cells in the incerto-hypothalamic dopaminergic system (Bjorklund et al., 1975), and there are also relatively high concentrations of dopaminergic neurons in the medial and periventricular parts of the parvocellular division (Swanson et al., 1981). Furthermore, immunocytochemical studies have demonstrated that the PVN receives serotonergic innervation from the raphe nuclei, which are prominent in the parvocellular division, and that 5-HT-containing terminals form axodendritic and axosomatic synapses with CRF immunoreactive neurons (Sawchenko et al., 1983; Liposits et al., 1987). Neuronal efferents from the PVN project directly to the lower brain stem, specifically the intermediolateral cell column of the spinal cord that contains preganglionic sympathetic neurons, whereas the median eminence-pituitary stalk receives efferent fibers from the parvocellular portion of the PVN, and the neurohypophysis receives efferents from the magnocellular subdivision of the PVN (Carpenter, 1985; Pacak et al., 1992).

In order to identify stress-induced changes in central monoamine levels and to elucidate the anatomical organization of monoaminergic neurons during exposure to stressors, various techniques, such as immunohistochemistry, in vitro electrochemistry, and microdissection involving removal of individual brain nuclei, have been used. Recently, microdialysis has been used to determine the extracellular levels of monoamines and their metabolites in the brains of freely-moving, conscious rats. In general, the levels of NE in the hypothalamus and the brainstem have been reported to be decreased during acute immobilization stress, whereas the levels of 5-HT were either unaffected or increased, based on measurements of the levels of monoamines in the dissected rat brain (Richardson, 1984; Adell et al., 1988). Adell et al. (1988) reported that NE and 5-HT decreased, whereas 5-hydroxyindole-3-acetic acid (5-HIAA) was increased in most brain regions when acute immobilization was preceded by chronic restraint, as compared to the effects of chronic restraint alone. Sensitization of the monoamine response to an acute stressor by previous exposure to another stressor was also identified by Anisman et al. (1981) in an earlier study.

In recent reports using a microdialysis technique, however, it was revealed that acute immobilization stress produced rapid, proportionately similar increases in the extracellular levels of NE, dihydroxyphenylglycol (DHPG), and dihydroxyphenylacetic acid (DOPAC) in the rat PVN (Pacak et al., 1992), and that the increases in NE and DHPG did not differ significantly between acutely immobilized rats that had been subjected to repeated stress exposure and rats exposed to an acute stress alone (Pacak et al., 1992). Kvetnansky et al. (1992) reported that immobilization rapidly increases plasma levels of catecholamines, the catecholamine precursor DOPA, and metabolites of NE and DA. Moreover, using microdialysis,

5-HT and 5-HIAA levels in the lateral hypothalamic area were also observed to increase in acutely immobilized rats (Shimizu et al., 1992).

These findings suggest that immobilization rapidly increases NE, DA, and 5-HT in the hypothalamus, indicating rapid increases in the synthesis, release, reuptake, and metabolism of monoamines. Since tissue concentrations of NE and DOPAC in A1/C1, A2/C2, and LC cell groups have been shown to increase in response to immobilization (Laucher et al., 1991), and administration of 6-OHDA into the ventral noradrenergic bundle was demonstrated to significantly deplete levels of NE in the PVN and prevent an ether stress-induced increase in the serum ACTH concentration (Feldman and Weidenfeld, 1991), it is speculated that release of these monoamines in the PVN during stress is partially caused by activation of monoaminergic neurons in the brainstem.

Pathological and Behavioral Changes in Stress Responses

The majority of behavioral changes regarded as stressful are associated mainly with the CRH and LC-NE/autonomic systems, as well as their peripheral effectors, the HPA axis, and the sympathetic nervous system (Johnston et al., 1992). Although CRH contributes to the activation or coordination of metabolic, circulatory, and behavioral responses, CRH injected directly into the cerebroventricular system produces a number of effects that resemble responses to stress (Britton et al., 1982). In low doses, CRH produces increased changes in locomotion, sniffing, grooming, and rearing. In high doses, CRH produces bizarre behavior, including repetitive locomotion, irritability, or aggression (Koob and Bloom, 1985). In addition, CRH decreases food intake and inhibits the increases in food intake induced by NE and insulin, suggesting that CRH acts as a mediator of stress that suppresses appetite and/or food intake (Morely and Levine, 1982). Intracerebroventricularly administered ACTH appears to affect social behavior in the rat by reducing social interactions, decreasing aggression, and prompting excessive grooming behavior interrupted by bouts of stretching, but does not increase activity or exploration, which is in contrast to the effects of CRH (Johnston et al., 1992). Glucocorticoids, in addition to having a negative feedback effect on stress-activated neural circuits and metabolic processes, are known to play a role in perception and the coordination of circadian patterns of food intake and sleep (Johnston et al., 1992). Glucocorticoids are also known to engender euphoria acutely, whereas the chronic effects of glucocorticoids generally appear to be depressive (Bohus et al., 1983).

Along with the HPA axis, the LC-NE system is thought to be the other major effector of the generalized stress response (Johnston et al., 1992). The sympathetic nervous system, as illustrated by the "fight or flight" concept proposed by Walter Cannon in the early 1900s, is responsible for coordinating responses necessary to meet external stressors (Bohus et al., 1983). CRH itself is thought to function as a potent stimulus to the LC-NE system and both NE and epinephrine stimulate CRH release. Johnson et al. (1992) have proposed a functional interaction between the CRH and LC-NE systems, acting as the principal central biologic effectors of the generalized stress response.

Stress and Interleukin-1 in Animal Models

Behavioral and Pathophysiological Similarity

There is similarity between the pathophysiological and behavioral changes observed in rats given IL-1 and those recognized as stress responses (Dantzer and Kelley, 1989; Weiss et al., 1989a). The hypothesized effect of IL-1 is supported by the observation that intracerebroventricular administration of IL-1 induces a stresslike reduction of exploratory behavior in mice (Spadaro et al., 1989), and alters the investigation of stimuli in a new environment (Spadaro and Dunn, 1990). IL-1 has also been

shown to exert a number of other behavioral effects: It can increase the sleeping time of rats (nonrapid-eye movement sleep), in relatively high doses IL-1 causes anorexia and decreases locomotion; it can disrupt operant response behavior, and has been shown to decrease the social exploration of juveniles (Dunn et al., 1991; Kent et al., 1992). Along with elevating body temperature, IL-1 administration elicits a number of behavioral responses in rats and mice, including anorexia, increased sleeping, decreased investigation of new objects and other animals, increased defensive withdrawal, and other behaviors characteristic of illness that also resemble behavioral changes typically observed in stress-exposed animals (Dunn, 1993). Since body temperature was reported to be transiently increased after 2.5 h of restraint but was significantly lower after 18 and 48 h of immobilization (Hauger et al., 1988), there is a possibility that the effects of IL-1 on thermogenesis may occur partially during the early periods of immobilization. IL-1 could thus account for many of the behavioral and pathological responses observed during stress exposure (Kent et al., 1992).

In addition to the above, IL-1 has also been reported to have a role in stress-induced immunosuppressive effects. Keller et al. (1981) showed that proliferation of T lymphocytes in response to mitogen stimulation was decreased in direct proportion to the intensity of the stressful event to which the animals had been exposed. Substeroidgenic doses of IL-1β administered intracerebroventricularly to rats resulted in depression of cellular immune responses, including IL-2 production, mitogen-induced proliferation, and natural killer cell activity (Sundar et al., 1989). This is in contrast to a report showing that injections of large amounts of IL-1 into the periphery promoted immune responses (Czuprynski and Brown, 1987).

Common Mechanism for the Actuation of Responses

In considering the mechanism responsible for the similarities between stress-induced pathophysiological changes and IL-1-induced effects, some portion of the peripheral changes occurring secondary to the central effects of IL-1 may be explained by responses causing secretion of CRH or synthesis of prostaglandins (PGs). Central injection of a CRF receptor antagonist or neutralizing antibody markedly inhibits IL-1-induced suppression of food intake (Uehara et al., 1989) and specific changes in behavior (Dunn et al., 1991), which resemble general stress responses. However, CRH may not be involved in the entire range of behavioral responses to IL-1, because α-helical CRH(9–41) did not reverse the disruptive effects of peripherally administered IL-1 on rats bar-pressing for a food reward (Bluthe et al., 1989). Inhibition of CRH by central injection of a CRH receptor antagonist or neutralizing antibody significantly inhibits the effects of IL-1 on fever and thermogenesis (Rothwell, 1991). However, one investigation has shown that IL-1-induced fever is not necessary for mediation of CRH actions (Bernardini et al., 1984). The involvement of CRH in fever induced by cytokines has not been fully elucidated. Furthermore, CRH does not appear to mediate the somnogenic effects of IL-1, and has been reported to inhibit sleep.

IL-1-induced behavioral changes are not apparently involved in synthesis of PGs, since the effects of IL-1 have been shown to be insensitive to a cyclooxygenase inhibitor (indomethacin) (Dunn, 1993). However, the effects of IL-1 on thermogenesis and fever do depend on the synthesis of PGs, since these actions can be attenuated by cyclooxygenase inhibitors (Rothwell, 1991). Fever traditionally has been ascribed to the actions of prostaglandin E_1 or prostaglandin E_2 within the hypothalamus. Since IL-1 induces synthesis of a number of different eicosanoids other than prostaglandin E, in the central nervous system, and prostaglandin F facilitates synthesis and/or release of CRH, which of these is the more potent generator remains controversial. The effects of IL-1 on sleep appear to be independent of eicosanoid synthesis (Rothwell, 1991). IL-1 induced nonrapid-eye movement sleep that appeared to be

independent of the effects of CRH or PGs. Its effects may be involved in $GABA_A$ receptor function, since femtomolar concentrations of IL-1 augment $GABA_A$ receptor function (Miller et al., 1991) and nonrapid-eye movement sleep has traditionally been reported in association with $GABA_A$ receptor function.

The similarities between responses to IL-1 injection and stress exposure on immunosuppression may also be explained in terms of responses caused by CRH secretion. Berkenbosch et al. (1987) proposed a model to show the relationship between the HPA axis and macrophage IL-1 production: Macrophage IL-1 is secreted in response to an antigen or other stimuli, enters the bloodstream, and reaches the central nervous system, thereby activating the HPA axis, possibly at the level of CRH release. However, several reports showing that the immunosuppressive effects of IL-1 would require not only HPA axis activation but also sympathetic activation have been published (Sundar et al., 1989; Weiss et al., 1989b; Brown et al., 1991). Sundar et al. (1989) suggested an autonomic pathway as the mediator of these immunosuppressive effects because of the absence of steroidogenesis and the rapidity of the observed effects following a 15 min postinfusion period. Weiss et al. (1989b) reported that intracerebroventricular administration of IL-1 to adrenalectomized rats elicited suppression of cellular immune responses, indicating that steroids are only partially responsible for the effects produced by IL-1. Furthermore, Brown et al. (1991) found that surgical interruption of the splenic nerve to eliminate autonomic innervation of the spleen also prevented suppression of splenic macrophage IL-1 secretion activity that had been induced by intracerebroventricularly administered IL-1β. They also proved that the combination of adrenalectomy and splenic nerve section resulted in a potent intracerebroventricular IL-1β effect on splenic macrophage IL-1 secretion that was greater than either adrenalectomy or splenic nerve section alone. These reports strongly suggest that both the HPA axis and the sympathetic nervous system mediate suppression of cellular immunity by means of the central action of IL-1β. Felten and coworkers (1984, 1985, 1987) have demonstrated extensive sympathetic innervation of the spleen with tyrosine hydroxylase positive and peptidergic nerve terminals distributed among fields of T lymphocytes and macrophages. They have also described synapse-like connections between nerves and lymphocytes in the spleen. Since NE is capable of blocking lipopolysaccharide (LPS)-induced IL-1 production in peritoneal macrophages (Koff et al., 1986), the immunosuppressive effects of IL-1 in the brain may mediate NE suppressive effects on macrophages within the splenic sympathetic autonomic system.

Interleukin-1 in the Central Nervous System

Source of IL-1

The origin of IL-1 in the brain has been investigated by several researchers. IL-1 synthesis has been identified in the brains of endotoxin-treated mice (Fontana et al., 1984). IL-1 is secreted from astroglia and C-6 glioma cells treated with LPS. Glial cells (astrocytes or microglia) do not include IL-1 under normal conditions, but can induce IL-1 synthesis in response to stimulation by pyrogens, such as LPS, as well as various physiological and pathological stimuli (Higgins and Olschowka, 1991; Benveniste, 1992). The IL-1β produced under these circumstances has been implicated in both tissue damage and repair. On the other hand, Breder et al. (1988) discovered the IL-1β like immunoreactive neuronal fibers of the human hypothalamus (*see* Fig. 1). These reports suggest that IL-1 is expressed in brain neurons under nonstimulated conditions. IL-1β immunoreactive fibers have been located in several areas, including the ventromedial nucleus of the hypothalamus, the posterior hypothalamus, the region of the hypophysial vessels of the median eminence, the subfornical organ, and the stria terminalis. Breder et al. (1988)

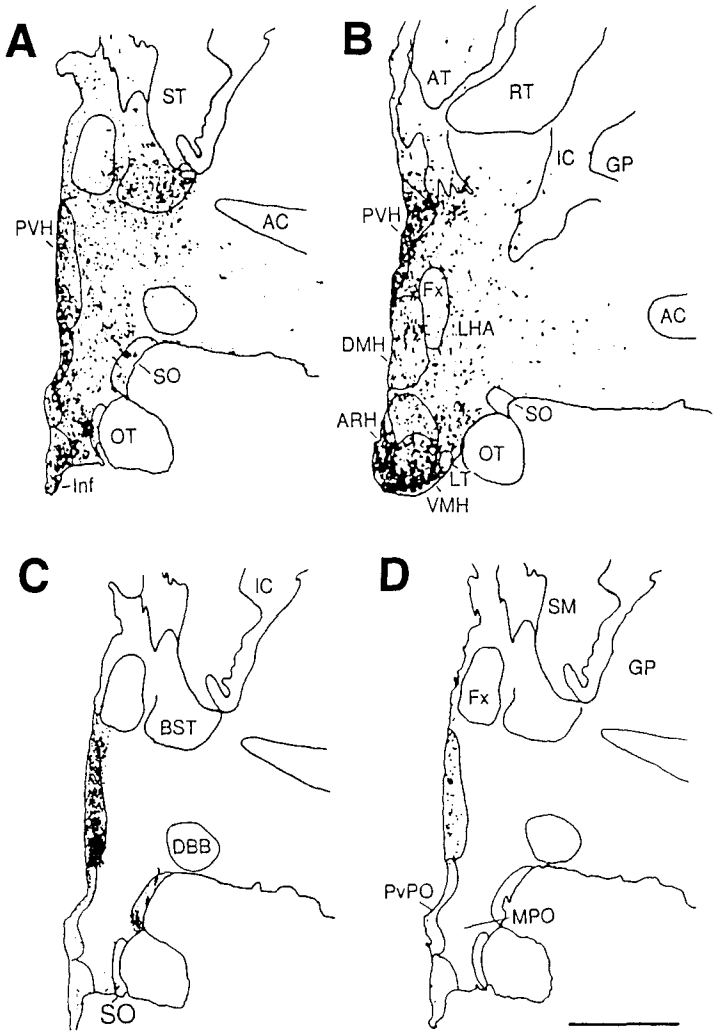

Fig. 1. Line drawings of sections through the human hypothalamus. (**A** and **B**) Pattern of IL-1β like immunoreactive fiber staining at the preoptic and tuberal levels of the hypothalamus, respectively. (**C** and **D**) Cell bodies at the preoptic level that were arginine vasopressin-like immunoreactive and CRH-like immunoreactive, respectively. The IL-1β like immunoreactive fibers innervate the key endocrine and autonomic areas of the hypothalamus, critical in elaborating the acute phase reaction, including the CRH and arginine vasopressin cell groups. Scale bar, 5 mm. (Breder et al., 1988). (Copyright 1988 by the American Association for the Advancement of Science).

Abbreviations: AC, anterior commissure; ARH, arcuate nucleus; AT, anterior thalamic nuclei; BST, bed nucleus of the stria terminalis; DBB, nucleus of the diagonal band of Broca; DMH, dorsomedial nucleus; Fx, fornix; GP, globus pallidus; IC, internal capsule; Inf, infundibulum; LHA, lateral hypothalamic area; LT, lateral tuberal nucleus; MPO, medial preoptic area; OT, optic tract; PVH, paraventricular nucleus; PvPO, periventricular preoptic nucleus; SM, stria medullaris; SO, supraoptic nucleus; ST, stria terminalis; VMH, ventromedial nucleus.

hypothesized that IL-1β-containing hypothalamic neurons form synapses with CRF immunoreactive neurons, and that IL-1β released from these neurons can facilitate CRH secretion. Lechan et al. (1990) also reported, on the basis of experiments using the rat brain, that a

high IL-1 concentration is present in several hypothalamic nuclei with fiber-like projections to other brain areas.

Whereas potential IL-1 origins exist within neuronal cells of the hypothalamus, the role of IL-1β as a neurotransmitter has not yet been clarified, because it is not known whether release is from neurons and the stimulants that induce release have not been identified. It is unlikely that IL-1β is packaged into secretory vesicles, because its precursor protein lacks a signal peptide. The actual process and mechanism of production and maturation of IL-1 within cells have been investigated using monocytes. IL-1α and 1β are first synthesized as large precursors, with a mol wt of 31,000. The mol wt of the mature forms is 17,500. Most of the IL-1α remains intracellular or is expressed on the surface of the cell membrane. It is cleaved subsequently by extracellular proteases into the mature form of IL-1α. On the other hand, the IL-1β precursor is cleaved by the IL-1β-converting enzyme (ICE) to its mature form within the cell, after which it is secreted (Dinarello and Wolff, 1993). Whereas both human and murine IL-1β precursors are ICE substrates, neither human nor murine IL-1α precursors are cleaved by ICE (Howard et al., 1991).

ICE purified from lysates of mononuclear phagocytes was shown to be a heterodimer of 20- and 10-kDa subunits (Thornberry et al., 1992). ICE represents a unique class of proteinases (Nett et al., 1992). Its activity was not reduced by treatment with leupeptin, pepstatin, or EDTA, suggesting that this convertase does not belong to the defined families of serine, aspartyl, or metallo-proteinases. Furthermore, although the convertase was inhibited by treatment with iodoacetate or *N*-ethylmaleimide, it was not sensitive to E-64, suggesting that it is not a typical cysteine proteinase (Black et al., 1989). It has been suggested that the ICE is capable of autoactivation (Thornberry et al., 1992), since the ICE itself undergoes maturation by three distinct proteolytic cleavage events and maturation is required for the acquisition of convertase activity (Nett et al., 1992). For regulation of ICE expression, treatment of murine peritoneal exudate cells with either LPS or recombinant interferon-γ increased the steady state level of convertase mRNA expression, whereas treatment with phorbol 12-myristate 13-acetate, TGF-β, IL-4, or dexamethasone exerted no altering effects on the basal level of convertase expression (Nett et al., 1992). It is speculated that the activation or expression of ICE may be a critical regulatory step controlling the generation of bioactive IL-1β.

Distribution of IL-1 Receptor

A type I receptor for IL-1 was identified as an 80-kDa protein that binds IL-1α and IL-1β with similar affinity and is present in many cell types, including central nervous system neurons (Dinarello and Savage, 1989; Higgins and Olschowka, 1991; Benveniste, 1992; Dinarello, 1992; Dower et al., 1992). Type II receptors exhibit a much higher affinity for IL-1β than for IL-1α (Higgins and Olschowka, 1991; Benveniste, 1992; Dower et al., 1992; Dinarello, 1992). Although ligand binding studies have demonstrated binding patterns in the brain, the results are not consistent with the immunocytochemical data (Farrar et al., 1987). Autoradiographic studies of the binding of radiolabeled IL-1 to rat brain have been performed to reveal specific binding sites, but do not always determine whether there are receptors in the brain (Katsuura et al., 1988a; Takao et al., 1990; Ban et al., 1991). These reports show that binding sites or receptors appear to be located on neurons, whereas receptors on glial cells were observed only after brain injury.

Whereas the hypothalamus is a major site of IL-1 action in the brain, whether or not IL-1 binding sites are present in the hypothalamus remains controversial. Katsuura et al. (1988a) have described an IL-1 receptor on hypothalamic membranes that binds IL-1β but not IL-1α. Takao et al. (1990) have also demonstrated the existence of a type I receptor on membranes prepared from mouse brain. However, Rothwell (1991) reported that these findings have not been corroborated by other investigators, and that they failed to detect mRNA for the type I receptor in the rat hypothalamus by poly-

merase chain reaction analysis. Although it is difficult to explain these discrepancies, there is a possibility that endogenous IL-1 present in abundance within neurons may prevent the binding of labeled IL-1 with its receptor. In a review, Rothwell (1991) suggested that the effects of IL-1 in the hypothalamus may be owing to the actions of type II receptor, because intracerebral injection of a monoclonal antibody to the type II receptor attenuates fever and suppresses food intake (but not corticosterone release) in the mouse. In any event, there is no firm evidence to show the existence of IL-1 receptors in the hypothalamus.

Whereas the existence of IL-1 receptors in the hypothalamus remains controversial, many experiments using hypothalamus explants in vitro have proved the direct actions of IL-1 in increasing prostaglandin synthesis, and enhancing the secretion of monoamines and CRH *(see below)*. These findings would require the presence of IL-1 receptors on hypothalamic neurons. Mismatches of ligand binding with immunocytochemical data have been noted for many peptides (Herkenham, 1987). As information about IL-1 receptor families becomes available, the origin of this discrepancy will become increasingly clear. When cytokine families and their receptors have all been cloned, this mystery may be solved (Saper and Breder, 1992).

Effects of IL-1 on the HPA Axis

Several studies have shown that IL-1 stimulates secretion of CRH (Besedovsky et al., 1986; Berkenbosch et al., 1987; del Rey et al., 1987; Sapolsky et al., 1987; Uehara et al., 1987; Katsuura et al., 1988b; Saphier and Ovadia, 1990; Watanobe et al., 1991), and activates the HPA axis (Barbanel et al., 1990; Berkenbosch et al., 1991; Besedovsky et al., 1991). Whether or not IL-1 acts directly on the pituitary gland to enhance the secretion of ACTH has been discussed by several investigators. Woloski et al. (1985) were among the first to show that murine IL-1 stimulated ACTH release from murine ACTH secreting pituitary tumor cells (AtT-20), suggesting that IL-1 may be a CRH-like molecule. Bernton et al. (1987) also reported that IL-1 induces release of multiple hormones by exerting a direct action on pituitary cells. In a well-designed series of experiments, Sapolsky et al. (1987) demonstrated an increased concentration of CRH in the portal blood after 3 μg human IL-1 was intravenously administered to rats, but they failed to demonstrate a direct effect of human IL-1 on ACTH secretion by cultured rat anterior pituitary cells. Neither human IL-1α nor IL-1β stimulated ACTH release from normal rat pituitary cells in concentrations up to 10 m*M* (Hooghe et al., 1991). Whereas Tsagarakis et al. (1989) provided evidence that various doses of CRH-41, but not of IL-1, had an effect on ACTH release from dispersed pituitary cells, the findings of Beach et al. (1989) included an acute effect of IL-1 on ACTH release. In addition to the evidence that IL-1 enhances CRH release and the induction of CRH mRNA accompanied by changes in cytoplasmic CRF- and proopiomelanocortin mRNA levels, respectively, rat pituitary cells and AtT-20 cells also exhibit an increase in proopiomelanocortin content in IL-1 treated cells as compared to untreated cells (Brown et al., 1987; Suda et al., 1989).

Although the site of action for IL-1 in regulating ACTH secretion is still controversial, the fact that IL-1 can somehow stimulate ACTH release is not in doubt. As a result, IL-1-induced ACTH secretion leads to secretion of corticosteroids from the adrenal gland. IL-1 also acts directly on the adrenal to augment steroid synthesis (Roh et al., 1987; Dinarello, 1988; Gwosdow et al., 1990; Winter et al., 1990). The effects of IL-1 on the secretion of other hormones also remain to be clarified. The IL-1-induced increase in ACTH is highly specific in that blood levels of PRL (Uehara et al., 1987), MSH, GH, VP (Berkenbosch et al., 1987), and oxytocin (Sapolsky et al., 1987) are not affected. However, Bernton et al. (1987) reported that IL-1 can directly stimulate the release of TSH, GH, and LH from rat pituitary cells.

Since VP constitutes a family and acts synergistically with CRH in the release of ACTH, it

is possible that VP release may also be affected by IL-1, although the results of various studies have been somewhat contradictory (Sapolsky et al., 1987; Berkenbosch et al., 1989). Sapolsky et al. (1987) have observed an approximate twofold increase in VP concentrations in the portal blood when IL-1 was administered intravenously. However, they concluded that IL-1 has no stimulatory effect on VP secretion, since the rise in the VP concentration was not statistically significant. Berkenbosch et al. (1989) also reported that VP turnover in the zona externa of the median eminence was not changed, whereas CRH turnover was stimulated by intraperitoneal IL-1 injection in the rat. A recent report, however, showed that IL-1 stimulated arginine VP release from the superfused rat hypothalamoneurohypophysial complex independently of cholinergic mechanisms (Nakatsuru et al., 1991). Most VP is produced in the magnocellular neurons of the supraoptic nuclei and is then conveyed to the neurohypophysis, but a minor population of VP-secreting neurons is located in the parvocellular portion of the PVN (Silvermann and Zimmerman, 1983), which projects axons to the external layer of the median eminence, where VP is released into the pituitary portal circulation and is involved in the regulation of ACTH secretion (Zimmerman, 1976; Silvermann and Zimmerman, 1983). Therefore, the results observed by Sapolsky et al. (1987) or Berkenbosch et al. (1989) cannot be taken as evidence proving that IL-1 does not promote VP release from the neurohypophysis. Considering that VP may be associated with avoidance of the stress-induced ACTH response from dexamethasone suppression, the stimulatory effects of IL-1 on VP secretion are very intriguing. Since IL-1 may activate the adrenocortical axis, at least in part, by interfering with the negative feedback effect of circulating glucocorticoids (Weidenfeld et al., 1989a), the synergistic actions of both IL-1 itself and VP secreted by IL-1 may be involved in the breakthrough of dexamethasone suppression, as described in the Glucocorticoid Resistance Mechanism in the Hypohalamus section.

Because IL-1 administered systemically affects the central nervous system so as to stimulate the release of these hormones, it is likely that IL-1 enters the brain. However, as is true for most other large polypeptides, IL-1 is not likely to cross the blood–brain barrier (Dinarello et al., 1978). The blood–brain barrier is, however, absent in several small areas of the brain, including the median eminence, the organum vasculosum of the lamina terminalis of the hypothalamus (OVLT), the subfornical organ, the choroid plexus, and the area postrema at the base of the fourth ventricle (Pardridge, 1983). Katsuura et al. (1990) reported that IL-1 may penetrate the brain at areas outside the barrier, such as the OVLT. These results support the view that circulating IL-1 enters the brain and thereby elicits its central actions (Bateman et al., 1989). However, we speculate that peripheral IL-1 may elicit its central effects in an indirect manner, rather than by means of direct actions, by mediating other factors or neural pathways. This point will be discussed further in the next section.

Effects of IL-1 on Release of Monoamines

Kabiersch et al. (1988) showed that the effects of IL-1 seem to be specific to 4-hydroxy-3-methoxyphenylglycol (MHPG) elevation, since no comparable changes in brain contents of DA, 5-HT, or 5-HIAA were detected after administration of IL-1. On the other hand, Palazzolo and Quadri (1990) reported that IL-1β promotes in vitro release and prevents the blunting catecholamine (NE or DA) release from hypothalami in vitro. Dunn and Welch (1991) showed that intraperitoneal injection of IL-1 elevates brain tryptophan, possibly reflecting increased 5-HT release. Increased release of 5-HIAA and MHPG was observed following systemic administration of IL-1 (Mefford and Heyes, 1990). Carmelia et al. (1991) showed that intracranioventricularly administered IL-1β increased 5-HIAA levels in the anterior hypothalamus. IL-1β injected into the medial basal hypothalamus has been shown to exert biphasic

effects on the release of DA and DOPAC from the hypothalamus (Mohankumar et al., 1991). As described above, the effects of IL-1β administration on monoamine release are controversial, but the route of administration may be an important factor altering the appearance of IL-1 effects, as discussed by Dunn and Chuluyan (1992). This suggests that there are plural pathways actuating the direct and indirect effects of IL-1 in eliciting monoamine responses in the brain.

Since it has been shown that systemically administered IL-1 can enter the brain (Banks et al., 1991), peripherally administered IL-1β may reach the hypothalamus. However, there are reports strongly suggesting that peripheral IL-1 cannot penetrate the blood–brain barrier (Coceani et al., 1988), and circulating IL-1 has not been found to diffuse into the brain (Hashimoto et al., 1991). Dinarello (1992) proposed the possibility that IL-1 may act on special endothelial cells of the periventricular organs where the blood–brain barrier is interrupted, or that arachidonic acid metabolites released from such endothelial cells may be involved in conveyance of its effects across the blood–brain barrier. If it is true that IL-1 administered systemically cannot enter the brain, how could it elicit central monoamine responses in the brain? We would have to assume indirect actions of IL-1 during the course of events. There appear to be at least two pathways by which peripheral IL-1 is able to act on the central nervous system to enhance monoamine release. One is the utilization of PGs as an intermediate, another is activation of ascending catecholaminergic neurons in the brain stem.

Many of the central effects of IL-1, such as fever, thermogenesis, ACTH release, and induction of nerve growth factor, depend on the synthesis of eicosanoids, since these actions can be attenuated by cyclooxygenase inhibitors (Rothwell, 1991). IL-1β increases prostaglandin E_2 in rat astrocyte cultures (Katsuura et al., 1989), and it has been clarified that IL-1 can induce the synthesis of a number of other eicosanoids (Rothwell, 1991). However, whether systemic administration of IL-1 enhances the release of monoamines in the brain through the actions of PGs has not been clarified. The increases in MHPG and 5-HIAA observed following systemic administration of IL-1 were antagonized by concurrent administration of indomethacin (Mefford and Heyes, 1990), and the effects of IL-1 on hypothalamic MHPG content and NE turnover were also blocked by indomethacin (Masana et al., 1990). These reports suggest that the increases in NE, DA, and 5-HT release induced by IL-1β in the hypothalamus are partially mediated by the actions of arachidonic acid metabolites. However, Dunn and Chuluyan (1992) reported that pretreatment of mice with indomethacin failed to prevent the elevation of hypothalamic MHPG that follows intraperitoneal administration of IL-1. This suggests that activation of monoamine release by IL-1 is not always caused by the effects of PGs.

In order to investigate this controversial point, we used a microdialysis probe equipped with a microinjection tube for administration of IL-1β into the same region into which the probe had been inserted (Shintani et al., 1993). IL-1β (1 ng) injected directly into the rat anterior hypothalamus elicited release of NE, DA, and 5-HT, as well as increases in their metabolites, MHPG, DOPAC, HVA, and 5-HIAA in the same region. Although the elevated levels of NE and DA persisted for more than 6 h after injection of IL-1β, the elevated levels of 5-HT were transient. We also found that direct administration of IL-1β into the anterior hypothalamus increases the levels of PGs, and that these increases are inhibited by indomethacin pretreatment. However, direct administration of IL-1β into the anterior hypothalamus can enhance the release of NE under conditions in which the formation of 6-ketoprostaglandin $F_{1\alpha}$ is inhibited by pretreatment with indomethacin, suggesting that IL-1β alone can directly enhance the release of NE without the involvement of prostaglandin production in the anterior hypothalamus. Furthermore, using in vivo microdialysis, we also found that IL-1β administered into the rat anterior hypothalamus may be capable of inducing the

release of monoamines directly without the involvement of CRH. These findings demonstrate that IL-1β in the hypothalamus may be capable of inducing the release of monoamine directly without involvement of PGs or CRH.

Nevertheless, a problem as to what mechanism makes it possible for peripheral IL-1 to induce prostaglandin synthesis in the brain across the blood–brain barrier remains to be resolved. Rothwell (1991) speculates that nitric oxide synthesis caused by IL-1 in the peripheral circulation may permit transduction of a signal across the blood–brain barrier, because IL-1 is known to induce nitric oxide synthase. Saper and Breder (1992) proposed the hypothesis that circulating IL-1 acts on cyclooxygenase containing neurons within the OVLT to produce local prostaglandin secretion, that PGs diffusing locally into the hypothalamic area act on IL-1 containing neurons in the same region, and that the IL-1 released from neuronal terminals then enhances release of monoamines. There is a possibility that peripheral IL-1 somehow activates the brain to induce its own synthesis in the brain, since IL-1 is known to have a self-inducing action. Furthermore, local prostaglandin may amplify the effect, induction, and synthesis of cytokines in other local neuronal cells (Saper and Breder, 1992).

On the other hand, it has been shown that noradrenergic neurons in the brain stem are involved in the actions of peripheral IL-1. Both 6-OHDA, injected intraventricularly or into the ventral noradrenergic ascending bundle, and the α_1-adrenergic antagonist, prazosin, injected intraventricularly, abolished elevation of ACTH and corticosterone in serum because of an intracerebral injection of IL-1 (Weidenfeld et al., 1989b). This suggests central adrenergic transmission, originating at the ventral noradrenergic ascending bundle and acting through α_1- adrenergic receptors, the activation of which is known to enhance CRH release in the PVN, is associated with the adrenocortical response to IL-1. Recently, Ericsson et al. (1992) performed a study to identify which cell groups possibly providing afferents to the PVN are involved in the induction of c-*fos* expression in the PVN in response to intravenous administration of recombinant IL-1β. They observed a sequential activation of brain stem catecholamine neurons in the nucleus of the solitary tract and in the ventrolateral medulla between 1 and 2 h after injection of IL-1β and found that transection of these ascending catecholaminergic pathways unilaterally at the level of the facial motor nucleus in the medulla, where the transection was capable of eliminating the influences of A1 and A2 noradrenergic neurons as well as those of the C1, C2, and C3 adrenergic neurons, prevented the IL-1β mediated induction of c-*fos* in the PVN on the side of the lesion. In contrast to immediate induction of c-*fos* with intraventricular injection of IL-1β, c-*fos* was initially detectable about 1 h after the intravenous injection, and the response was maximal at about 3 h. These results support the concept that A1/C1 and A2/C2 catecholaminergic cell groups, which project to the PVN, are necessary for the effects of intravenously administered IL-1β on CRH neurons to manifest. Moreover, the demonstration that IL-1 increases synthesis of substance P in sympathetic neurons indicates that neuronal afferents are capable of modifying central nervous system activity (Freidin and Kessler, 1991). The findings described above suggest that peripheral IL-1 may be capable of transmitting signals into the hypothalamus through an afferent pathway from the catecholaminergic neurons or autonomic nerves in the brainstem.

Interleukin-1 as a Putative Neurotransmitter

What does IL-I expressed constitutively in neurons do? Apart from the effects of IL-1 administered systemically, IL-1 has a direct action on the hypothalamus activating the secretion of monoamines (Palazzolo and Quadri, 1990; Shintani et al., 1993) and CRH (Cambronero et al., 1989; Tsagarakis et al., 1989; Bernardini et al., 1990), as shown in several explant experiments. We have also shown that IL-1 administered directly into the rat hypo-

thalamus is capable of stimulating the release of NE, DA, and 5-HT, as well as their metabolites, using an in vivo microdialysis technique (Shintani et al., 1993). Watanobe et al. (1991) showed the temporal profile of CRH secretion in the median eminence after intravenous administration of IL-1 to freely moving rats. Considering the similarity between the direct actions of IL-1 on these neurohormonal responses and stress-induced central reactions, we suspect that endogenous IL-1 that was preformed constitutively within the neurons of the brain, especially the hypothalamus, may somehow serve as an intermediate in the regulation of stress responses. A recent study showed that acute immobilization stress induces IL-1β mRNA in the rat hypothalamus (Minami et al., 1991). However, it has not been clarified whether biologically active IL-1 is generated in the brain by immobilization stress, or whether IL-1 is essential for inducing such acute stress responses. In order to assess this possibility, we examined whether acute immobilization stress enhances the levels of IL-1 activity in the rat hypothalamus. Moreover, to examine the role of hypothalamic IL-1 in immobilization stress-induced neurohormonal changes, we designed experiments involving the administration of interleukin-1 receptor antagonist (IL-1Ra) directly into the rat anterior hypothalamus. We thus investigated whether elevated NE release in the rat hypothalamus and increased plasma ACTH, in response to immobilization stress, are inhibited by IL-1Ra.

First, rats were immobilized for periods of 30–360 min by placing them in plastic restrainers. The IL-1 levels in the supernatants of homogenates of the dissected rat hypothalami were determined with a bioassay using thymocyte proliferation. After the start of immobilization stress, the levels of IL-1 activity in the hypothalamus reached a maximum at 60 min, and remained significantly increased at 240 min. The IL-1 activity gradually decreased thereafter, and returned to the preimmobilization stress levels at 360 min despite the continuance of immobilization stress (Fig. 2). IL-1 activity was approx 2.4 pg/mg wet hypothalamic weight in the preimmobilized rats, but a recent investigation using an ultrasensitive highly specific, enzyme amplified, immunometric assay showed that IL-1 concentrations higher than about 15 pg are present in the normal rat hypothalamus (Hillhouse and Mosley, 1993). Since tissue homogenates could contain pro-IL-1, and pro-IL-1 has also been reported to be biologically active (Rosenwasser et al., 1986; Jobling et al., 1988), measurement of IL-1 using the bioassay may demonstrate levels higher than those determined using the ultrasensitive highly specific immunometric assay.

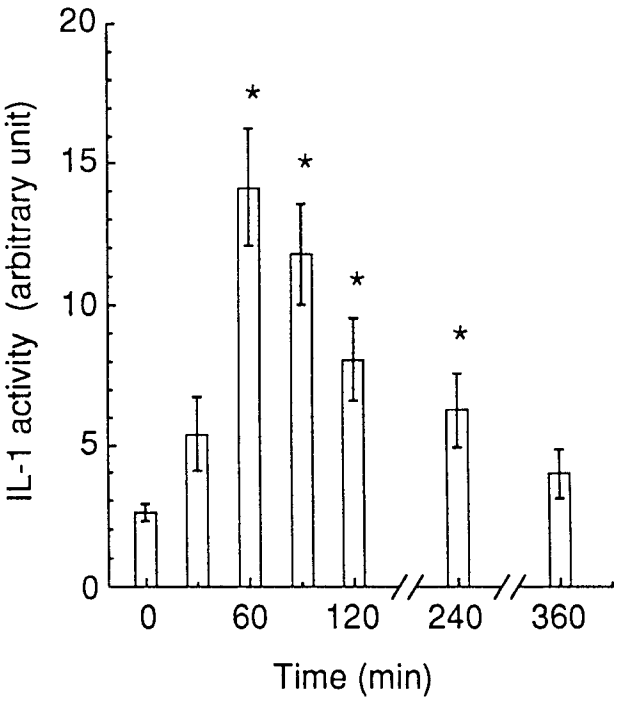

Fig. 2. IL-1 levels in the hypothalami of the rats immobilized for periods of 0–360 min by placing them in plastic restrainers. The IL-1 levels in the supernatants of homogenates of the dissected rat hypothalami were determined using a bioassay. One unit on the ordinate is equivalent to 1 pg hr IL-1β per 1 mg wet hypothalamic weight. Bars and vertical lines represent mean values and standard error of means, respectively. $*p < 0.05$ vs 0 time. (Redrawn from Shintani et al., 1995).

Second, in order to assess whether elevated IL-1 activity plays a role in stress responses, we administered IL-1Ra directly into the rat hypo-

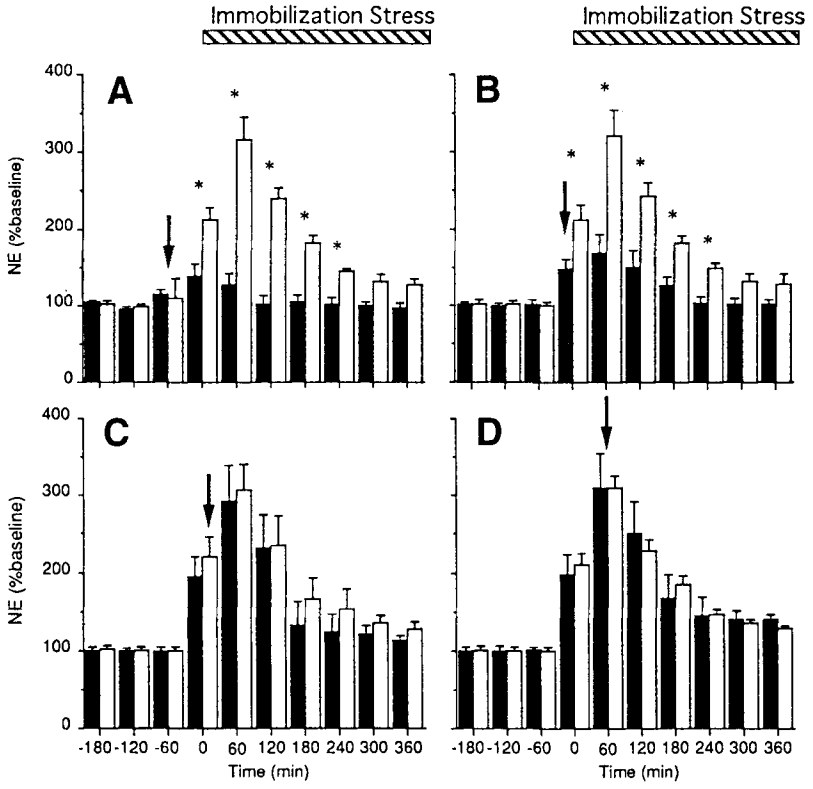

Fig. 3. Effects of IL-1Ra administration on NE levels in dialysate perfusing the anterior hypothalamus of immobilized rats. IL-1Ra was administered directly into one side of the rat anterior hypothalamus through a microinjection tube 60 min **(A)** and 5 min **(B)** before starting immobilization stress, or at 5 min **(C)** and 60 min **(D)** after the start of the immobilization stress, and vehicle was simultaneously administered into the contralateral side. Arrows represent the timing of IL-1Ra (solid bars) or vehicle (open bars) administration. Horizontal hatched bars represent the period of immobilization stress. *Open bars vs solid bars; $p < 0.05$, $n = 8$. (Redrawn from Shintani et al., 1995).

thalamus to block the effects of IL-1. Moreover, in order to exclude different responses resulting from immobilization stress among individuals, we compared the levels of NE between the side of the hypothalamus treated with IL-1Ra and the contralateral side treated with vehicle in the same rat. To determine whether the administered doses of IL-1Ra inhibited IL-1 effects in the hypothalamus, microdialysis experiments were carried out using human recombinant IL-1β activated NE release as an index of IL-1 effects, and the administered doses of IL-1Ra were confirmed to be adequate to inhibit the effects of IL-1. As shown in Figs. 3 and 4, the stress-induced elevations of both hypothalamic NE levels and plasma ACTH levels were significantly inhibited by IL-1Ra preadministered directly into the hypothalamus at 60 min before immobilization had been started (Figs. 3A and 4A). In addition, whereas IL-1Ra, which had been administered at 5 min before the start of immobilization, inhibited the elevation of NE or ACTH (Figs. 3B and 4B), IL-1Ra did not inhibit the elevations at approx the time when the NE or ACTH level had peaked (Figs. 3D and 4D), even 5 min after the start of immobilization (Figs. 3C and 4C).

These results suggest that the IL-1 effects responsible for NE release and ACTH secretion were completed no later than 5 min after the start of immobilization stress within the time range of neurotransmission. Since the elevated

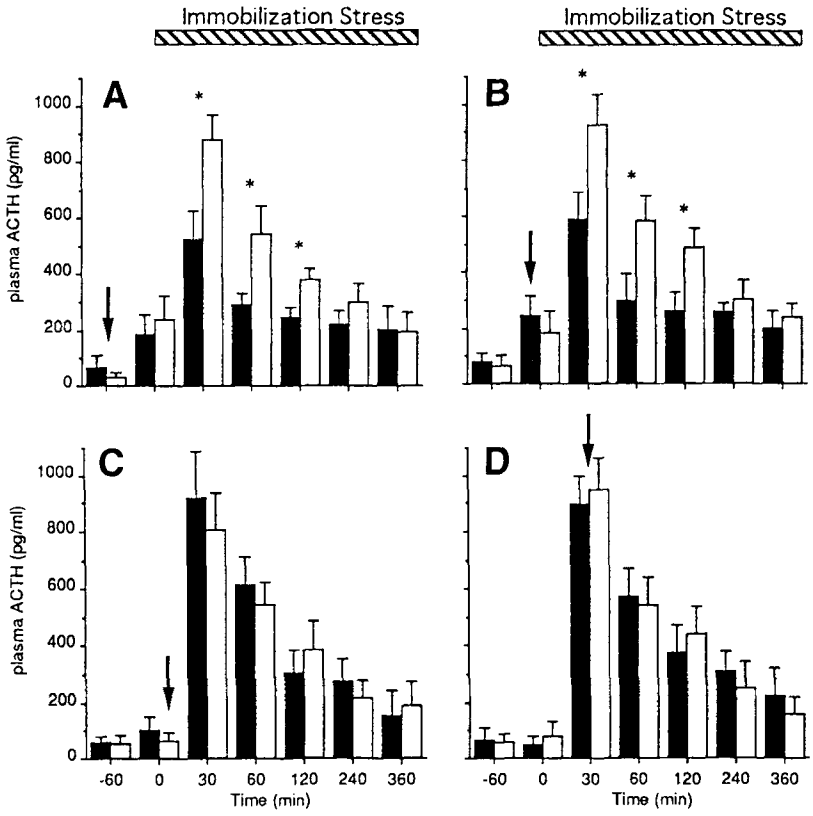

Fig. 4. Effects of IL-1Ra on plasma ACTH levels in response to immobilization stress. IL-1Ra or vehicle was administered directly into the anterior hypothalami at 60 min **(A)** and 5 min **(B)** before starting IS, or at 5 min **(C)** and 30 min **(D)** after the start of immobilization stress. Arrows represent the timing of IL-1Ra or vehicle injection. Horizontal hatched bars represent the period of immobilization stress. *Open bars vs solid bars, $p < 0.05$, $n = 8$. (Redrawn from Shintani et al., 1995).

levels of hypothalamic NE and plasma ACTH were still observed from 60–240 min after the initiation of immobilization, it is suggested that initial release of IL-1 triggers the sustained release of these monoamines and/or hormones. Since the blocking action of IL-1Ra on stress responses occurred immediately, this appears to be consistent with the release of IL-1, which probably preexisted within the neurons of the hypothalamus, elicited by immobilization stress. Furthermore, this raises a question as to why the attainment of maximal IL-1 levels is delayed irrespective of early exertion of its effects. This observation may be explained by the possibility that early release of IL-1 enhances induction of its own synthesis or other as yet unidentified factors, for example PGs, so as to enhance the synthesis of IL-1 in the hypothalamus during the late periods. This concept is supported by the observation that IL-1 can elicit its own synthesis in the brain (Spranger et al., 1990), and by the possible local amplifying action of prostaglandin on cytokine effects (Saper and Breder, 1992).

IL-1, especially IL-1β, likely satisfies several criteria for a neurotransmitter. The existence of IL-1 within neurons suggests that the synthetic enzyme is present in the relevant neurons. IL-1 appears to be released and exogenously applied IL-1 triggers the effects similar to stress exposure. Decay of the released IL-1 by nonspecific proteases would be conceivable. However, the existence of IL-1 receptors in the hypothalamus remains controversial. Further investigations

on the releasing mechanisms of IL-1, including blockade of the responses to stress exposure by inhibition of ICE, will be necessary.

Stress, IL-1, and Human Disease

Major Depression

Among the many biological findings of the patient with major depression, especially melancholia, an abnormal response to the dexamethasone suppression test (DST) has been reported most consistently. Several hypotheses concerning the mechanisms underlying this abnormal response to the DST have been proposed. Overcoming the suppression induced by dexamethasone is thought to be involved in interrupting the suppression of ACTH secretion. This may be owing to inadequate secretion of VP, as described in the Glucocorticoid Resistance Mechanism in the Hypothalamus section. Patients with major depression, who do not show suppression by dexamethasone, have a higher level of NE as well as increased cerebrospinal fluid and plasma levels of NE metabolites (Barnes et al., 1983; Gold et al., 1988). Feldman and Weidenfeld (1991) hypothesized that hyperfunction of the NE system may be responsible for the decrease in the negative feedback exerted by dexamethasone. Furthermore, the abnormal DST may be induced by central CRH hypersecretion (Nemeroff et al., 1984; Gold et al., 1987). CRH concentrations are elevated in the cerebrospinal fluid of depressives (Nemeroff et al., 1984). There are reports to suggest that this hypersecretion may result either from primary hyperserotonergic or hypercholinergic neurotransmission (Janowsky and Risch, 1984; Maes et al., 1991), or from attenuated hippocampal hypothalamic negative feedback control (Sapolsky and McEwen, 1988). Recently, Maes et al. (1993) reported that statistically significant positive correlations between mitogen-stimulated IL-1β production by peripheral blood mononuclear cells and post-DST cortisol values were found in patients with major depression. Our results, as described in the Interleukin-1 as a Putative Neurotransmitter section, also indicate that hypothalamic IL-1 may be involved in the escape of suppression by dexamethasone. Breder et al. (1988) showed that the IL-1β immunoreactive neurons of the human hypothalamus innervate the important endocrine areas of the hypothalamus, including CRH and VP cell groups. Moreover, considering that IL-1 and IL-1 receptors that are especially abundant in the hippocampus (Lechan et al., 1990; Ban et al., 1991), and also in the limbic system, play an important role in regulation of the HPA axis, involvement of central IL-1 in the pathogenesis of major depression is highly plausible. In light of our findings and those of other investigators, we hypothesize that central IL-1, likely released form neurons in response to stress, may be associated with affective control.

Alzheimer's Disease

Chronic neuropathological changes associated with neurodegenerative disease have recently been associated with cytokine release. For example, increased levels of IL-1β are found in the brains of patients with Alzheimer's disease (AD) (Griffin et al., 1989). IL-1β appears to induce expression of the amyloid precursor protein (APP) mRNA in endothelial cells (Goldgaber et al., 1989), and APP may be responsible for deposition of the β/A4 protein in the extracellular amyloid plaques and vasculature in AD (Higgins and Olschowka, 1991). Differential expression of the APP gene has been suggested to contribute to this amyloid deposition (Higgins et al., 1988). It has been postulated that increases in IL-1β levels are an initial event in the disease process. On the other hand, a hypercortisol state, including both hypersecretion of cortisol under basal conditions, as well as dexamethasone resistance, has been found in AD (Raskind et al., 1982; Spar and Gerner, 1982). Approximately 50% of AD patients show some form of hypercortisolism (Sapolsky and McEwen, 1988). The possibility that the hypercortisolism of some AD patients arises from hippocampal damage has

been suggested (Sapolsky and McEwen, 1988). As discussed in the Involvement of the Hippocampus in HPA Axis Inhibition section, the limbic system including the hippocampus is thought to be an important region regulating the HPA axis, and playing roles in brain functions including memory, feeling, affection, and so on.

Although induction or synthesis of IL-1 in the central nervous system is known to occur in response to injury (Dinarello and Savage, 1989; Woodroofe et al., 1991) or ischemia (Minami et al., 1992), inflammation, and infection, these factors do not appear to be related to the etiology of AD. We speculate that increased IL-1 levels in AD may result from excessive release of IL-1 in response to repetitive stress exposure. Moreover, once IL-1 is released from neurons, possibly thereby exerting an effect so as to amplify its own synthesis and that of other cytokines, there may be latent fragility of protective feedback mechanisms to overcome stress-induced IL-1 release in AD. Thus, IL-1 induced by various physical stresses may contribute to a variety of neuropathological changes associated with neurodegenerative disease. Therefore, an excessive IL-1 response and fragility of the counter-mechanisms against stress exposure, rather than the strength of the stress factor itself, may trigger the progression of neurodegenerative diseases, including AD.

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