***In situ* Histochemical Localization of Type I Interleukin-1 Receptor Messenger RNA in the Central Nervous System, Pituitary, and Adrenal Gland of the Mouse**

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The cytokine interleukin-1 (IL-1) has a number of biologic activities, including pronounced effects on the nervous and neuroendocrine systems. In this study, *in situ* histochemical techniques were used to investigate the distribution of cells expressing type I IL-1 receptor mRNA in the CNS, pituitary, and adrenal gland of the mouse. Hybridization of ³⁵S-labeled antisense cRNA probes derived from a murine T-cell IL-1 receptor cDNA revealed a distinct regional distribution of the type I IL-1 receptor, both in brain and in the pituitary gland. In the brain, an intense signal was observed over the granule cell layer of the dentate gyrus, over the entire midline raphe system, over the choroid plexus, and over endothelial cells of postcapillary venules throughout the neuraxis. A weak to moderate signal was observed over the pyramidal cell layer of the hilus and CA3 region of the hippocampus, over the anterodorsal thalamic nucleus, over Purkinje cells of the cerebellar cortex, and in scattered clusters over the external-most layer of the median eminence. In the pituitary gland, a dense and homogeneously distributed signal was observed over the entire anterior lobe. No autoradiographic signal above background was observed over the posterior and intermediate lobes of the pituitary, or over the adrenal gland. This study therefore provides evidence for discrete receptor substrates subserving the central effects of IL-1, thus supporting the notion that IL-1 acts as a neurotransmitter/neuromodulator in brain. It also supports studies suggesting that IL-1-mediated activation of the hypothalamic-pituitary-adrenal axis occurs primarily at the level of the brain and/or pituitary gland.

Interleukin-1 (IL-1) is one of a growing number of cytokines that mediate important regulatory interactions between lymphocytes and many other cell types (see Dinarello, 1989). IL-1 has also been identified as a key mediator in the acute-phase

response, a generalized host reaction to microbial invasion, antigenic challenge, or large-scale tissue injury (see Dinarello, 1984). As its name suggests, the acute-phase response is rapid in onset and is characterized by marked alterations in metabolic, endocrinologic, immunologic, and neurologic functions (see Dinarello, 1984). Neurologic changes include the induction of fever (see Kluger, 1991), increased duration of slow-wave sleep (Opp et al., 1991), decreased appetite (McCarthy et al., 1985; Mrosovsky et al., 1989), activation of the hypothalamic-pituitary-adrenal (HPA) axis (Woloski et al., 1985; Besedovsky et al., 1986; Berkenbosch et al., 1987; Bernton et al., 1987; Sapolsky et al., 1987; see also Lumpkin, 1987; Bateman et al., 1989; Dunn, 1990), and inhibition of the hypothalamic-pituitary-gonadal axis (Rivier and Vale, 1989; Kalra et al., 1990), all of which are thought to be mediated by IL-1 (see Blatteis, 1988). In addition, centrally administered IL-1 has been found to elevate circulating levels of growth hormone and prolactin, while inhibiting the release of thyroid-stimulating hormone (Rettori et al., 1987; Dubuis et al., 1988). More recently, intracerebroventricular IL-1 has also been observed to inhibit both gastric acid (Ishikawa et al., 1990; Saperas et al., 1990; Uehara et al., 1990; Okumura et al., 1991; Shibasaki et al., 1991) and pepsin (Okumura et al., 1990, 1991) secretion, and to have profound analgesic properties (Nakamura et al., 1988).

A number of recent radioligand binding studies have used ¹²⁵I-labeled forms of IL-1 to localize receptors that might mediate the above-mentioned effects of the cytokine in the rodent CNS (Farrar et al., 1987; Katsuura et al., 1988; De Souza et al., 1989; Haour et al., 1990; Takao et al., 1990; Ban et al., 1991). These studies have, however, produced somewhat different results, with earlier studies in rat brain reporting a fairly widespread distribution (Farrar et al., 1987; Katsuura et al., 1988), and more recent studies in mouse brain (Haour et al., 1990; Takao et al., 1990; Ban et al., 1991) describing a relatively restricted localization particularly in the dentate gyrus of the hippocampus and choroid plexus. These latter studies also described diffuse binding over the anterior pituitary (Haour et al., 1990; Takao et al., 1990; Ban et al., 1991). In addition, marked species differences have been demonstrated in the pattern of central binding using human recombinant human IL-1 α in mouse, rat, and guinea pig (Takao et al., 1990). Finally, it is well known that such binding studies provide limited insight into the site of synthesis of putative receptors, since ligands may

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Figure 1. Dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the hippocampus following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (A). An adjacent Nissl-stained section is shown for reference (B). Note the dense autoradiographic signal over granule cells in the dentate gyrus (DG) and, to a lesser extent, over the pyramidal cell layer of the hilus and CA3 region (arrow). Note also the dense autoradiographic signal over endothelial cells of postcapillary venules (v). The autoradiographic signal over the CA1 region is comparable to background. *fi*, fimbria hippocampus; *Hb*, medial habenular nucleus.

bind to neuronal receptors on cell bodies, or at any point along dendrites, axons, or axon terminals. To address these issues, *in situ* hybridization of a ^{35}S -labeled antisense cRNA probe derived from a murine T-cell IL-1 receptor cDNA (Chiou et al., 1989) generated from a previously published sequence (Sims et al., 1988) was used to investigate the regional expression of type I IL-1 receptor mRNA in the CNS. Given the pronounced effects of IL-1 on the hypothalamic-pituitary axes, particularly the HPA axis, we also investigated the distribution of type I IL-1 receptor mRNA in the pituitary and adrenal gland.

Materials and Methods

Studies in this report were carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Animals. Seven to eight week old male C57BL/6 mice of the Harlan Sprague-Dawley strain (Indianapolis, IN) were used in all experiments. All animals were housed in a light- (12 hr on/12 hr off) and temperature-controlled environment, with food and water available.

Tissue preparation. All mice were deeply anesthetized with 35% chloral hydrate and perfused transcardially with 10–15 ml of 0.15 M NaCl, followed immediately by 400 ml of 4.0% paraformaldehyde in 0.1 M sodium tetraborate buffer, pH 9.5. The brains, pituitaries, and adrenal glands were removed and allowed to postfix for 6–8 hr in the final

perfusate with 10% sucrose added as a cryoprotectant. Five 1-in-5 series of 20- μm -thick sections were cut through the rostrocaudal extent of each brain using a sliding microtome. Alternating series were mounted onto lysine-coated slides to be processed for sense and antisense *in situ* hybridization as described below. Intervening series were mounted onto gelatin-coated slides and counterstained with either thionin or hematoxylin-eosin for reference. All sections processed for *in situ* hybridization histochemistry were similarly counterstained, although the quality of the staining was markedly decreased by proteinase K treatment. Series of 20- μm -thick sections were cut through the pituitaries and adrenal glands using a cryostat, and collected directly onto lysine-coated slides to be processed for sense and antisense *in situ* hybridization. All sections were counterstained with hematoxylin-eosin.

In situ Hybridization histochemistry. A previously characterized murine full-length T-cell IL-1 receptor cDNA was cloned into a pGEM plasmid vector (Chiou et al., 1989). ^{35}S -labeled sense and antisense cRNA probes were prepared by transcription with Sp6 and T7 RNA polymerase, respectively (Zoeller et al., 1989; Wada et al., 1990). Each radiolabeled cRNA probe was approximately 2 kilobases in length. Prehybridization, hybridization, and autoradiographic localization techniques have been described in detail elsewhere (Simmons et al., 1989; Wada et al., 1989). In brief, mounted sections were fixed in the above perfusate for 30 min, after which they were treated with proteinase K, acetylated, and dehydrated. ^{35}S -labeled cRNA probes ($5\text{--}10 \times 10^6$ cpm/ml) were applied in hybridization buffer, at 55°C overnight. Sections were then treated with RNase A to reduce background, and washed through progressively lower concentrations of saline-sodium citrate to reduce the salt content and increase the stringency of hybridization. Finally, sections were dehydrated, exposed to either Beta Max (Amersham) or Cronex (Du Pont) film for 2–4 d, dipped in Kodak nuclear emulsion NTB3, dried, exposed for 7–21 d, developed, and counterstained with either thionin (Nissl stain) or hematoxylin-eosin.

Results

Controls

A previously published Northern blot analysis of mRNA from the murine EL-4 T-cell line demonstrated hybridization of the type I IL-1 receptor cDNA used in the present study to a single species (Chiou et al., 1989). Autoradiographic signal specificity has been further documented by *in situ* hybridization histochemistry with sense probes in previous studies in hippocampus (Cunningham et al., 1991) and testis (Cunningham et al., 1992) and in the current study in pituitary and adrenal gland (not shown). In each case, the autoradiographic signal obtained with sense probes was comparable to background. In addition, McMahan and colleagues have used Northern blot analysis with cDNA probes directed against the type I and recently cloned type II IL-1 receptor mRNAs to demonstrate selective hybridization of the respective probes to a single mRNA species in a number of tissues, and to show that probes for the two IL-1 receptor subtypes do not cross-hybridize (McMahan et al., 1991). Hence, the possibility that the signal observed following *in situ* hybridization with the cRNA probe for the type I IL-1 receptor employed in the present study was due to cross-hybridization with mRNA species encoding the type II receptor, or other related sequences, would seem extremely unlikely.

CNS

A total of nine brains were processed for *in situ* hybridization histochemistry with antisense probes. The distribution of type I IL-1 receptor mRNA was identical in each case. It should be noted that an intense autoradiographic signal was observed over endothelial cells of postcapillary venules throughout the CNS, a result that is presented separately below (see Choroid plexus and postcapillary venules).

Forebrain. The strongest autoradiographic signal in the forebrain was found in the hippocampal formation (Cunningham et al., 1991), where an intense autoradiographic signal was observed over the granule cell layer of the dentate gyrus, and a weak to moderate autoradiographic signal was observed over the pyramidal cell layer of the hilus and the CA3 region (Fig. 1). The autoradiographic signal over other aspects of the hippocampal formation, including the CA2 and CA1 regions, the subiculum, and the entorhinal area, was comparable to background. A similar pattern was found at all rostrocaudal levels.

A weak to moderate autoradiographic signal was also observed over the anterodorsal nucleus of the thalamus (Fig. 2). The autoradiographic signal over other aspects of the forebrain, including the hypothalamic paraventricular nucleus (PVN, Fig. 3), was comparable to background. While the autoradiographic signal over most aspects of the median eminence was also comparable to background, scattered clusters of autoradiographic signal were consistently observed over its externalmost layer (Fig. 4). The quality of counterstaining in the median eminence following proteinase K treatment of frozen sections did not, however, allow for precise localization to either neurons or the small hypothalamic-hypophyseal portal vessels known to occupy this layer.

Brainstem. An intense autoradiographic signal was observed over all aspects of the midline raphe system (see Tork, 1985), including all subdivisions of the dorsal raphe (Fig. 5), the median raphe, the raphe pontis, the raphe magnus, the raphe obscurus, and the raphe pallidus (not shown). An autoradiographic signal above background was also observed over those portions of the pontine and medullary reticular formation known to contain extra-midline serotonergic neurons, but this signal was almost invariably associated with postcapillary venules. A moderate to dense autoradiographic signal was observed over sensory neurons of the mesencephalic trigeminal nucleus (Fig. 6). A weak to moderate autoradiographic signal was also found over the Purkinje cell layer of the entire cerebellar cortex (Figs. 6, 7). The autoradiographic signal over other aspects of the brainstem was comparable to background.

Spinal cord. Although a comprehensive analysis of all spinal levels was not performed, serial sections were examined through cervical level 3 or 4 in all animals. No autoradiographic signal above background was observed over any portion of the cervical spinal cord.

Choroid plexus and postcapillary venules. A dense autoradiographic signal was observed over the choroid plexus in the lateral, third, and fourth ventricles. While the intensity of the autoradiographic signal did not allow for localization to a specific cell type of the choroid plexus, densely labeled fronds of choroid plexus were often observed originating from venules, the endothelium of which was also densely labeled (Fig. 8).

An intense autoradiographic signal was found over endothelial cells of postcapillary venules throughout the CNS, both in the parenchyma and at the pial surface (Fig. 9). Such venules were readily recognized by their size (50–400 μm) and the absence of a muscular wall. An autoradiographic signal over arterioles at the pial surface (Fig. 9), and over capillaries throughout the CNS, was comparable to background.

Pituitary

A total of 12 pituitaries were processed for *in situ* hybridization histochemistry with antisense probes. A dense and homoge-

Figure 2. Dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the dorsal thalamus following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (A). An adjacent Nissl-stained section is shown for reference (B). Note the moderate autoradiographic signal over the anterodorsal nucleus (AD), as compared to the dense autoradiographic signal over granule cells in the dentate gyrus (DG), and over endothelial cells of postcapillary venules (v). Hb, medial habenular nucleus; sm, stria medullaris.

neously distributed autoradiographic signal was observed over the entire anterior lobe in each case. The autoradiographic signal over the posterior and intermediate lobes was comparable to background (Fig. 10). As with the median eminence, the quality of counterstaining following proteinase K treatment of frozen sections did not allow for localization to specific cell types within the anterior lobe.

Adrenal gland

A total of 16 adrenal glands from 12 animals were processed for *in situ* hybridization histochemistry with antisense probes. In each case, the autoradiographic signal over the medulla and all cortical layers, including the zona glomerulosa, the zona fasciculata, and the zona reticularis, was comparable to background (Fig. 11).

Figure 3. Dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the medial part of the hypothalamic PVN (PVN_m) following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (*A*). An adjacent Nissl-stained section is shown for reference (*B*). Note the absence of increased autoradiographic signal over the PVN_m , as compared to the dense autoradiographic signal over endothelial cells of an isolated postcapillary venule (*v*) in the lateral part of the nucleus. *V3*, third ventricle.

Discussion

In situ hybridization histochemistry was used to localize type I IL-1 receptor mRNA in the CNS, pituitary, and adrenal gland of the mouse. In the brain, an intense autoradiographic signal was observed over the granule cell layer of the dentate gyrus; over the entire midline raphe system; over the choroid plexus in the lateral, third, and fourth ventricles; and over endothelial cells of postcapillary venules throughout the neuraxis. A weak to moderate autoradiographic signal was observed over the pyramidal cell layer of the hilus and CA3 region of the hippocampus, over the anterodorsal thalamic nucleus, over sensory neurons of the mesencephalic trigeminal nucleus, over Purkinje cells of the cerebellar cortex, and in scattered clusters over the externalmost layer of the median eminence. In the pituitary gland, a dense and homogeneously distributed autoradiographic signal was observed over the entire anterior lobe. No autoradiographic signal above background was observed over the posterior and intermediate lobes, or over the adrenal gland. Together, these results provide evidence for discrete receptor substrates subserving the central effects of IL-1, and thereby support the notion that IL-1 acts as a neurotransmitter and/or neuromodulator in

brain. These results also support previous evidence suggesting that IL-1-mediated activation of the HPA axis occurs primarily at the level of the brain and/or pituitary gland.

The results of the present study are in close agreement with high-affinity binding studies in brain and pituitary using human ^{125}I -recombinant IL-1 α in mouse (De Souza et al., 1989; Haour et al., 1990; Takao et al., 1990; Ban et al., 1991), although they fail to confirm previous reports of widespread central binding of murine ^{125}I -recombinant IL-1 α (Farrar et al., 1987) and human ^{125}I -recombinant IL-1 β (Katsuura et al., 1988) in rat. While such apparent species differences in the distribution of IL-1 receptors may be due to differential binding of the various recombinant forms of the cytokine when applied across species or under different experimental conditions, alternative explanations for the absence of more widespread IL-1 message must be considered, and include (1) the existence of multiple IL-1 receptors in the brain, a possibility strengthened by the recent identification (Bromsztyk et al., 1989; Chizzonite et al., 1989; Munoz et al., 1991) and cloning (McMahan et al., 1991) of a second, type II IL-1 receptor subtype; (2) that binding studies may detect putative receptors at sites far from the site of syn-

Figure 4. Dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the median eminence following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (*A*). The same section counterstained with hematoxylin-eosin is shown for reference (*A'*). Note the absence of increased autoradiographic signal over most of the median eminence, with the exception of scattered clusters of autoradiographic signal over its externalmost layer (arrows), a layer known to contain the small hypothalamic-hypophyseal portal vessels. V3, third ventricle; Ar, arcuate nucleus.

thesis, such as at axon terminals; and (3) that some areas that synthesize type I IL-1 receptor may do so at such a low level as to avoid detection with current *in situ* techniques.

The restricted localization of autoradiographic signal for type I IL-1 receptor mRNA to well-recognized cytoarchitectonic boundaries in the hippocampus, the thalamus, the raphe, the mesencephalic trigeminal nucleus, and the cerebellar cortex strongly suggests significant neuronal synthesis of the receptor. Additional support for neuronal expression of type I IL-1 receptor also comes from at least four lines of evidence. First, the most intense autoradiographic signal in the hippocampus was observed over the granule cell layer of the dentate gyrus, a layer virtually devoid of glial cells (Rickmann et al., 1987). Second, IL-1 binding in the hippocampus virtually disappears following application of the selective neurotoxins to this region (Takao et al., 1990; Ban et al., 1991). Third, IL-1 increases synaptic inhibition in rat hippocampal neurons *in vitro* (Zeise et al., 1991). And fourth, hippocampal neurons respond to IL-1 by increasing production of NGF, both *in vitro* (Friedman et al., 1991; Spranger et al., 1990), and *in vivo* (Spranger et al., 1990). It should be noted, however, that astrocytes cultured from both hippocampus (Friedman et al., 1991) and cortex (Gradient et al., 1990) have also been found to respond to IL-1 with an increase in the production of NGF.

Given the above-mentioned response of hippocampal neurons and glial cells to IL-1 *in vitro* and *in vivo*, it is perhaps not surprising that the cytokine has been suggested to promote neuronal survival and/or repair in this region, a notion fostered by the recent description of increased IL-1 production in hippocampal astrocytes following perferant path deafferentation, con-

current with the onset of cholinergic sprouting (Fagan and Gage, 1990). However, the hippocampus has also been suggested to be involved in thermoregulation, since it has been shown that hippocampal stimulation may directly influence thermoresponsive neurons in the preoptic area (Boulant and Demieville, 1977; Hori et al., 1982). Moreover, IL-1 might exert at least some of its central effects on the hypothalamic-pituitary axes at the level of the hippocampus, as has been postulated for glucocorticoids and regulation of the HPA axis (see Keller-Wood and Dallman, 1984; Jacobson and Sapolsky, 1991). This latter possibility seems particularly appealing given the relative absence of type I IL-1 receptor mRNA in either the PVN or median eminence (Fig. 12). It should be noted, however, that IL-1 has been shown to stimulate release of corticotropin-releasing factor (CRF) from perfused rat hypothalami (Tsagarakis et al., 1989; Navarra et al., 1991), and to produce an increase in plasma ACTH following microinjection into the median eminence *in vivo* (Sharp et al., 1989). One possible explanation for such apparently discrepant results may lie in the presence of a second IL-1 receptor subtype in the hypothalamus and/or median eminence, as discussed above (Bromsztyk et al., 1989; Chizzonite et al., 1989; McMahon et al., 1991; Munoz et al., 1991). A second possibility is that IL-1 receptors are synthesized outside the hypothalamus and transported to axon terminals in this region. Lastly, the type I IL-1 receptors observed in endothelial cells of postcapillary venules in the PVN (Fig. 3), or scattered in external layer of the median eminence (Fig. 4), possibly in endothelial cells of portal vessels, might somehow affect CRF and/or ACTH release.

This study represents the first suggestion of IL-1 receptors in the midline raphe system, an intriguing finding for a number of

Figure 5. Dark-field photomicrographs of emulsion autoradiographs of equally spaced (approximately 1.5 mm) coronal sections through the dorsomedial midbrain following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (A–C). Adjacent Nissl-stained sections are shown for reference (A'–C'). Note the dense autoradiographic signal over all aspects of the dorsal raphe nucleus (DR). Aq, cerebral aqueduct; CG, central gray; mlf, median longitudinal fasciculus.

Figure 6. Dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the dorsal pons following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (*A*). An adjacent Nissl-stained section is shown for reference (*B*). Note the moderate to dense autoradiographic signal over sensory neurons of the mesencephalic trigeminal nucleus (*MeV*). Note also the weak to moderate autoradiographic signal over Purkinje cells of the cerebellar cortex (arrows; see also Fig. 7). *LC*, locus coeruleus; *bc*, brachium conjunctivum; *V4*, fourth ventricle.

reasons. First, the raphe system, particularly the rostral raphe, has long been implicated in the control of sleep and wakefulness, with increased activity having been correlated with synchronization of the electroencephalogram and enhancement of slow-wave sleep, presumably via projections to the cerebral cortex (see Wauquier and Dugovic, 1990). Second, 5-HT is well known to suppress appetite, most probably via hypothalamic projections (see Curson, 1990). Third, a number of lines of evidence have implicated the raphe and 5-HT in thermoregulation, including (1) the presence of thermosensitive neurons in the rostral raphe (Cronin and Baker, 1976; Hori and Harada, 1976); (2) enhanced firing of neurons in the preoptic area of the hypothalamus following rostral raphe stimulation (Bruck and Hinckel, 1980; Werner and Bienek, 1985), and (3) abolition of the thermoresponsiveness of preoptic neurons following lesions of the rostral raphe (Taylor, 1982; Werner and Bienek, 1985). Interestingly, the fever induced by administering bacterial pyrogens may be attenuated by raphe lesions (Kadlecova et al., 1977), or by depleting (Harvey and Milton, 1974; Kandasamy, 1977; Matuszek and Ishikawa, 1981) or antagonizing (Kandasamy,

1977) central 5-HT (see Myers, 1980). Fourth, 5-HT is known to activate the HPA axis (see Cowen et al., 1990), a finding of great interest given the description of direct serotonergic projections from the rostral and caudal raphe nuclei to the medial part of the PVN (Sawchenko et al., 1983), that part of the PVN known to contain the majority of CRF-containing neurosecretory neurons (Swanson et al., 1983). Moreover, it has recently been shown that electrical stimulation of the rostral raphe excites as many as 50% of PVN neurons (Saphier and Feldman, 1989), that chemical stimulation of the caudal raphe elicits a rise in circulating ACTH levels (Bereiter and Gann, 1990), and that elevated levels of ACTH following central IL-1 administration depend in part on intact afferent inputs (Ovadia et al., 1989). The serotonergic input from the raphe to the PVN might, therefore, provide an additional route for IL-1-mediated HPA activation. Rostral raphe stimulation has also been found to influence the secretion of hypothalamic growth hormone (Koibuchi et al., 1988) and pituitary luteinizing hormone (Morello and Taleisnik, 1985). Fifth, stimulation of the raphe, which sends dense serotonergic projections to the trigeminal nuclei and to

Figure 7. Low- (*A*) and high-power (*B*) dark-field photomicrographs of an emulsion autoradiogram of a coronal section through the cerebellar cortex following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA. Adjacent Nissl-stained sections are shown for reference (*A'*, *B'*); boxed region in *A'* shows area enlarged in *B* and *B'*. Note the weak to moderate autoradiographic signal over Purkinje cells (*arrows*), as compared to the dense autoradiographic signal over endothelial cells of postcapillary venules (*v*). Note also that the autoradiographic signal over other aspects of the cerebellar cortex is comparable to background.

Figure 8. Dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the dorsal third ventricle following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (*A*). The same section counterstained with hematoxylin-eosin is shown for reference (*A'*). Note the dense autoradiographic signal over the choroid plexus (*CP*), and over endothelial cells of its supplying venules (*v*). Note also that the autoradiographic signal over the fimbria hippocampus (*fi*), the stria medullaris (*sm*), and the subfornical organ (*SFO*) is comparable to background.

the spinal cord (see Tork, 1985), elicits profound behavioral analgesia (Fields and Basbaum, 1978; Zieglansberger, 1986) of the type reported to follow central IL-1 administration (Nakamura et al., 1988). And sixth, the raphe densely innervates the dorsal vagal complex (see Tork, 1985), thus providing an indirect route for IL-1-mediated inhibition of gastric acid and pepsin secretion. In fact, recent studies have demonstrated a direct effect on gastric motility following stimulation of medullary raphe nuclei (McCann et al., 1989; Hornby et al., 1990). The presence of IL-1 receptors on raphe neurons would seem, therefore, to offer a particularly parsimonious explanation for many of the central effects of IL-1.

The physiological role of type I IL-1 receptors in the anterodorsal thalamic nucleus, a region best known as a limbic relay between the mammillary complex in the posterior hypothalamus and the cingulate cortex (Cruce, 1975; Beckstead, 1976), is at present unclear. Similarly, the effect of IL-1 on sensory neurons of the mesencephalic trigeminal nucleus, and on Purkinje cells of the cerebellar cortex, has yet to be addressed. With regard to localization of IL-1 receptor to sensory neurons, however, it should be noted that IL-1-like immunoreactivity has been described in unspecialized, perhaps sensory, nerve fibers in a number of peripheral tissues (Shultzberg et al., 1989).

The intense autoradiographic signal observed over the choroid plexus confirms the findings of the more recent high-affinity IL-1 binding studies in mouse (De Souza et al., 1989; Haour et al., 1990; Takao et al., 1990; Ban et al., 1991). However, given the intensity of the signal over the choroid plexus, it was not possible to determine whether the observed autoradiographic signal represented synthesis of type I IL-1 receptor by choroid plexus epithelial cells, postcapillary endothelial cells, or adherent macrophages. The finding of an autoradiographic signal over endothelial cells in supplying postcapillary venules (Fig. 8) might be taken, however, as a point in favor of endothelial expression in the choroid plexus.

The presence of type I IL-1 receptor mRNA signal over endothelial cells of postcapillary venules might, perhaps, have been predicted, particularly given the purported role of the cytokine in mediating the vascular changes that accompany infectious meningitis (Saez-Llorens et al., 1990), and its pivotal role in mediating inflammatory changes at postcapillary venules throughout the body (see Pober and Cotran, 1990). Interestingly, IL-1 has recently been reported to stimulate synthesis of amyloid β -protein precursor mRNA in peripheral endothelial cells (Goldgaber et al., 1989), a finding accented by a report of differential regulation of amyloid β -protein mRNA expression in

Figure 9. High-power dark-field photomicrographs of emulsion autoradiograms of sections through postcapillary venules (v) in the parenchyma (A) and at the pial surface (B) of the brain following *in situ* hybridization with an antisense probe for type IL-1 receptor mRNA. The same sections counterstained with hematoxylin-eosin are shown for reference (A', B'). Note the dense autoradiographic signal over endothelial cells (arrowheads). Note also that the autoradiographic signal over an adjacent arteriole (a) is comparable to background.

Figure 10. Low-power dark-field photomicrograph of an emulsion autoradiogram of a coronal section through the pituitary following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA (*A*). The same section counterstained with hematoxylin-eosin is shown for reference (*B*). Note the dense autoradiographic signal over the entire anterior lobe (*A*). Note also that the autoradiographic signal over the posterior (*P*) and intermediate (*I*) lobes is comparable to background.

Figure 11. Low- (*A*) and high-power (*B*) dark-field photomicrographs of an emulsion autoradiogram of a section through a whole adrenal gland following *in situ* hybridization with an antisense probe for type I IL-1 receptor mRNA. The same section counterstained with hematoxylin-eosin is shown for reference (*A'*, *B'*; the boxed region in *A'* shows area enlarged in *B* and *B'*). Note that the autoradiographic signal over the entire adrenal gland is comparable to background, including the capsular layer (*c*), the zona glomerulosa (*zg*), the zona fasciculata (*zf*), the zona reticularis (*zr*), and the medulla (*m*).

Figure 12. A schematic summary of the relationship between the hippocampus and the HPA axis illustrating the possible sites of action of interleukin-1 (shading). Only the hippocampus and the anterior pituitary were found in the present study to express high levels of type I IL-1 receptor (*). For details of HPA regulation, see Keller-Wood and Dallman (1984) and Jacobson and Sapolsky (1991). DG, dentate gyrus.

hippocampal neurons in the brains of patients with Alzheimer's disease (Higgins et al., 1988), and more recently of increased IL-1-like immunoreactivity in the brains of patients with Alzheimer's disease and Down's syndrome (Griffin et al., 1989). It could be, therefore, that the characteristic buildup of amyloid in these disorders is the result of chronic or repeated IL-1 stimulation (see Vandenabeele and Fiers, 1991). While the origin of centrally acting IL-1 is at present unclear, both glia (Fontana et al., 1982; Nieto-Sampedro and Berman, 1987; Giulian et al., 1988; Fagan and Gage, 1990) and neuronal (Breder et al., 1988) sources have been suggested. In addition, it should be noted that IL-1 has been found to cross the endothelial blood-brain barrier much faster, on average, than may be accounted for by leakage alone (Banks et al., 1989; Banks and Kastin, 1991), suggesting carrier-mediated active transport, and possibly providing circulating IL-1 increased access to central receptors.

The presence of dense and homogeneously distributed type I IL-1 receptor mRNA over the anterior pituitary confirms previous IL-1 high-affinity binding studies using whole mouse pituitary glands (De Souza et al., 1989; Haour et al., 1990; Ban et al., 1991) and AtT-20 cells (De Souza et al., 1989). In addition, a recent study employed Northern blot analysis to demonstrate that AtT-20 cells express low levels of type I IL-1 receptor

mRNA (Bristulf et al., 1991). Taken together, these studies lend support to the numerous reports of HPA activation occurring at the level of the anterior pituitary (Fig. 12; Woloski et al., 1985; Bernton et al., 1987; Fagarason et al., 1989, 1990; Fukata et al., 1989). It should be noted, however, that some groups have been unable to observe a direct IL-1-induced release of ACTH (Sapolsky et al., 1987; Uehara et al., 1987; see Lumpkin, 1987; Bateman et al., 1989; Dunn, 1990).

Lastly, we did not observe type I IL-1 mRNA in mouse adrenal glands, a finding at odds with some reports describing a direct effect of IL-1 on adrenal cortical cells (Roh et al., 1987; Whitcomb et al., 1988; Andreis et al., 1991). However, two recent studies have failed to find any acute stimulatory effect of IL-1 on glucocorticoid secretion at the level of the adrenal gland (Naito et al., 1990; Harlin and Parker, 1991). While the apparent discrepancy may again be due to use of various forms of the cytokine under different experimental conditions, and our inability to demonstrate IL-1 receptor mRNA may be due to either the presence of a second IL-1 receptor subtype or inadequate sensitivity, the results of the present experiment would tend to support the currently popular contention that IL-1-mediated HPA activation occurs primarily at the level of the brain and/or pituitary (Fig. 12; see Lumpkin, 1987; Bateman et al., 1989; Dunn, 1990).

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