Vol. 156, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1988 Pages 61-67

IDENTIFICATION OF A HIGH-AFFINITY RECEPTOR FOR INTERLEUKIN-1 *BETA* IN RAT BRAIN

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Received August 18, 1988

SUMMARY: A single type of high-affinity binding sites for IL-1 *beta* was identified in the rat hypothalamus (K_d=1.0 ± 0.2 nM) and cerebral cortex (K_d=1.3 ± 0.2 nM), but not in the pituitary. The maximum binding capacity (B_{max}) in the hypothalamus (B_{max}=75.4 ± 10.8 fmol/mg protein) was 4 times greater than in the cerebral cortex (B_{max}=17.2 ± 1.5 fmol/mg protein). Neither various neuropeptides nor IL-2 appeared to influence the binding of [^{125}I]IL-1 *beta* to the hypothalamic membrane preparations. The potency of unlabeled IL-1 *alpha* to replace the binding of [^{125}I]IL-1 *beta* to the hypothalamic membrane preparations was considerably less than that of unlabeled IL-1 *beta*. These findings indicate that IL-1 *beta* receptors are heterogeneously distributed in the central nervous system and that IL-1 *alpha* does not bind with IL-1 *beta* receptors in the brain

The neuroendocrine and immune systems are now recognized as being intimately linked and bidirectionally communicating (1). A prototypical immunotransmitter interleukin-1 (IL-1), produced by activated monocytes and macrophages which triggers various immune responses, has been reported as modulating the activities of the endocrine organs and the central nervous system (2-4). IL-1 stimulates adrenocortictropic hormone (ACTH) secretion from the pituitary via corticotropin releasing hormone (CRH) in the hypothalamus (5-7). Furthermore, IL-1 exerts other central effects such as induction of fever (2), slow-wave sleep (8), satiety (9) and analgesia (10). IL-1 is synthesized not only in monocytes and phagocytes, but also in astrocytes and glia (11, 12) and can be detected in the cerebrospinal fluid (13). Recent immunohistochemical study has

ABBREVIATIONS

Interleukin (IL), Adrenocorticotropic hormone (ACTH), Corticotropin releasing hormone (CRH), Growth hormone releasing factor (GRF), Somatostatin-14 (SS-14), Thyrotropin releasing hormone (TRH), Neuropeptide Y (NPY), Neurotensin (NT), Luteinizing hormone releasing hormone (LH-RH), Leu-enkephalin (L-Enk), Met-enkephalin (M-Enk), Angiotensin II (A-II), Vasoactive intestinal polypeptide (VIP)

0006-291X/88 $1.50

shown IL-1-like immunoreactivity in the neurons of the human brain (14) as well. These findings suggest that IL-1 has a physiological role(s) in the brain. If so, the brain tissues may have specific, high affinity receptors for IL-1. Our previous study indicated a clear-cut difference between IL-1 *alpha* and IL-1 *beta* in terms of their potency to stimulate ACTH release *in vivo* in rats (15). In the immune system, IL-1 *alpha* and IL-1 *beta* both exert similar activities and bind the same receptors (16-18); therefore, it is necessary to investigate to determine if that holds true for binding sites in brain tissue as well. The present study has been designed to address two questions: (1) Are there specific, high affiinity receptors for IL-1 in the hypothalamus, cerebral cortex and pituitary?; and, (2) Do both IL-1 *alpha* and IL-1 *beta* bind the brain receptors in a similar manner as they do in T lymphocytes?

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, 280-300 g BW, (Charles River Breeding Laboratories, Wilmington, MA) were housed at a constant room temperature of 23° C with a 12 h light/dark cycle (lights on at 0630 h). Food and water were available *ad libitum*.

Preparation of receptor membranes: Immediately following sacrifice of rats by decapitation, brains and pituitary glands were removed. Each brain was placed on a glass plate on ice, and the hypothalamus and cerebral cortex were dissected by the method described by Glowinski and Iversen (19). Dissected tissues were quickly frozen in crushed, solid CO_2, stored at -80° C and analyzed within 3 days. To prepare receptor membranes, tissues were homogenized in freshly prepared, ice-cold 10 mM Tris/HCl buffer (pH 7.5) containing 2.5 mM dithiothreitol with a glass-Teflon homogenizer. The homogenate was centrifuged at 1000 x *g* for 10 min, the pellet was discarded, and the supernatant was centrifuged at 48,000 x *g* for 30 min. The resultant pellet was resuspended and washed twice in Tris buffer. The pellet was then suspended in Tris buffer. All preparation procedures were performed at 4° C.

Iodination of IL-1 *beta*: Recombinant human IL-1 *beta* (Cistron Corporation, Pine Brook, NJ) was iodinated by the lactoperoxidase method and then purified by reverse-phase HPLC on a Vydac C18 column with a linear gradient of acetonitrile from 10 - 60% in 0.1% trifluoroacetic acid. The radioligand was stored at 4° C. Specific activity was 65 μCi/ug.

Binding assay: Binding assay was performed using a slightly modified method described by Srikant and Patel (16). Optimum conditions for radioreceptor assay for IL-1 *beta* were determined from the preliminary experiment. The binding assays were performed using approximately 250 μg protein/tube of membrane preparation for 75 min incubation at 37° C. Membrane homogenates (100 μl) were incubated with [^{125}I]IL-1 *beta* in 50 mM HEPES/KOH buffer (pH 7.5) (assay buffer) containing 5 mM $MgCl_2$, 200 KIU/ml Trasylol, 0.02 μg/ml Bacitracin and 0.02 μg/ml phenylmethylsulfonyl fluoride in 12 x 75 mm glass tubes. Final assay volume was 0.4 ml. Nonspecific binding was determined by the addition of 1 μg unlabeled IL-1 *beta* to assay tubes. The mixture was incubated for 75 min at 37° C in a shaking water bath at 100 strokes/min, unless otherwise indicated. After incubation, 1 ml of 0.125% Celite solution was added, the tubes were centrifuged at 2000 x *g* for 10 min, and the precipitate which contained membrane-bound radioactivity was measured (21). The following reagents were used to test competence in binding: IL-1 *alpha* (a gift of Dr. Steven Gillis, Immunex Corporation, Seattle, WA), IL-2 (Cetus Corporation, Emeryville, CA), growth hormone releasing factor (GRF), CRF, somatostatin-14 (SS-14), thyrotropin releasing hormone (TRH), neuropeptide Y (NPY), neurotensin (NT), luteinizing hormone releasing hormone (LH-RH), Leu-enkephalin (L-Enk), Met-enkephalin (M-Enk), angiotensin II (A-II) and vasoactive intestinal polypeptide (VIP) All neuropeptides were purchased from Bachem Bioscience Inc., Philadelphila, PA and were diluted in the assay buffer. Protein concentrations were determined using a Bio-Rad (Richmond, CA) assay kit.

RESULTS

The binding of [^{125}I]IL-1 *beta* to hypothalamic membrane preparations increased over time during the incubation at 37° C, and reached its maximum level after 75 min when it then began to

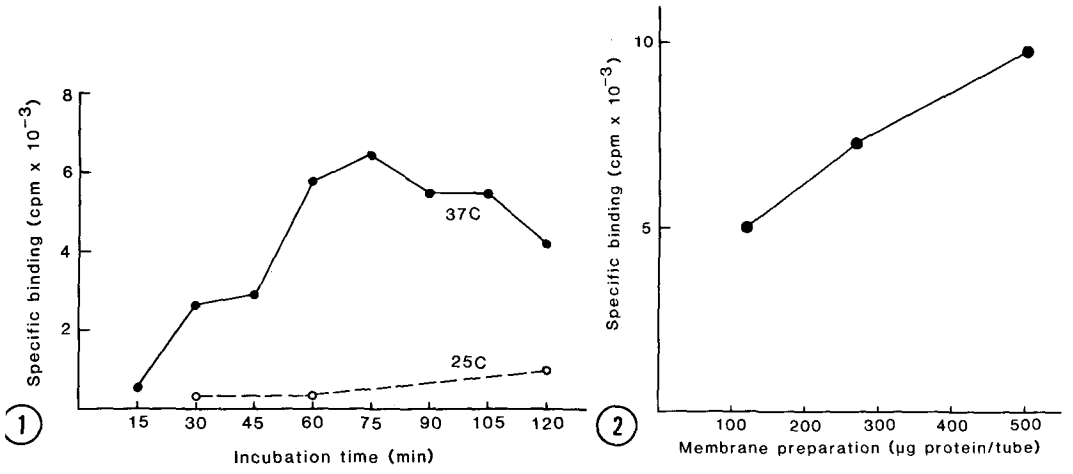

Fig. 1. Time course of [^{125}I]IL-1 *beta* binding to hypothalamic membrane preparations. The membrane preparations (approximately 250 μg protein/tube) were incubated with [^{125}I]IL-1 *beta* (1.8 nM) for the indicated time periods.

Fig. 2. Effect of protein concentrations in hypothalamic membrane preparations on [^{125}I]IL-1 *beta*. Membrane preparations at the indicated protein concentrations were incubated with [^{125}I]IL-1 *beta* (1.8 nM).

decrease. At 25° C the binding remained low for up to 2 h incubation (*Fig. 1*). Therefore, the following experiments were performed at 37° C for 75 min incubation. The binding of [^{125}I]IL-1 *beta* to hypothalamic membrane preparations increased proportional to the amount of membrane (*Fig. 2*). The binding assays were routinely carried out using approximately 250 μg of membrane protein per tube.

Fig. 3 shows specific binding of [^{125}I]IL-1 *beta* to membrane preparations of the rat hypothalamus, cerebral cortex and the pituitary as a function of ligand concentration, and the plot of Scatchard analysis for hypothalamus and brain cortex membranes. [^{125}I]IL-1 *beta* bound to the membrane preparations of the hypothalamus and cerebral cortex increased as the amount of ligand increased toward the level of saturation. Scatchard analysis yielded a single type of high-affinity binding site for IL-1 *beta* in these tissues. On the other hand, the pituitary membrane preparations did not show a dose-related increase in specific binding and therefore K_d value was not determined by Scatchard analysis. A similar study using hypothalamic and certebral cortex membrane preparations and results are shown in Table 1. K_d of [^{125}I]IL-1 *beta* was not different between the hypothalamus and the cerebral cortex. The maximum binding capacity (B_{max}) for IL-1 *beta* receptors in the hypothalamus was, however, 4 times greater than that in the cerebral cortex. Specificity of the binding of [^{125}I]-IL-1 *beta* with the hypothalamic membrane preparations was tested by incubation with 1 μg of IL-1 *alpha*, IL-2 and various neuropeptides. As shown in *Fig. 4*, none of the peptides tested other than IL-1 *beta* and IL-1 *alpha* affected the binding which indicated that the binding was specific to IL-1. In the next experiment, smaller and increasing doses of IL-1 *beta* or IL-1 *alpha* were incubated with [^{125}I]-IL-1 *beta* and the hypothalamic membrane preparation. Although both unlabeled IL-1 *alpha* and IL-1 *beta* decreased the binding of

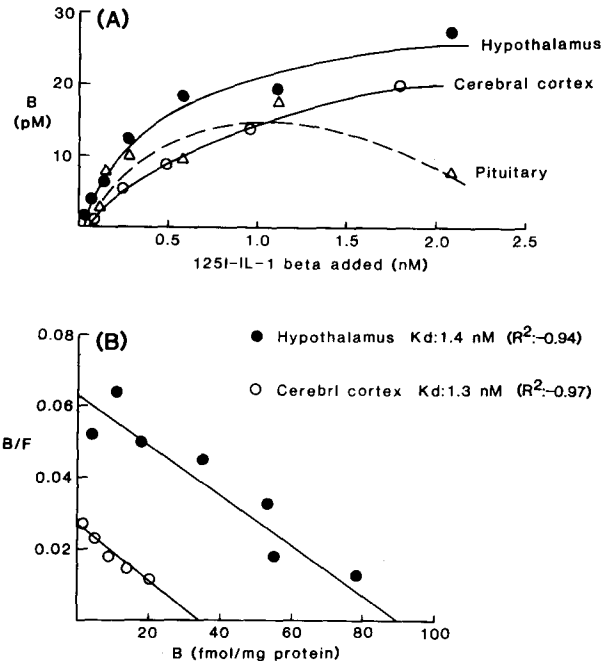

Fig. 3. Specific binding of [^{125}I]IL-1 *beta* as a function of ligand concentration to membrane preparations of the hypothalamus, the cerebral cortex and the pituitary (**A**), and Scatchard plot analysis (**B**). Membrane preparations were incubated with the indicated concentrations of [^{125}I]IL-1 *beta*. The data represent specific binding.

the radioligand with the membrane preparation in a dose-dependent manner, about 100 times greater amount of IL-1 *alpha* than IL-1 *beta* was needed to achieve suppression of binding to the same extent (*Fig. 5*).

DISCUSSION

The present study shows the presence of specific receptors for IL-1 *beta* in the brain tissue with K_d = 1.0 - 1.3 nM. The receptors appeared to be distributed heterogenously, about 4 times richer in the hypothalamus than in the cerebral cortex. On the other hand, no clear-cut saturation

TABLE 1.

Properties of IL-1 *beta* receptors in the hypothalamus and the cerebral cortex

Regions	No. of Exp.	Kd (nM)	Bmax (fmol/mg protein)
Hypothalamus	6	1.0 ± 0.2	75.4 ± 10.8
Cerebral cortex	5	1.3 ± 0.2	17.2 ± 1.5

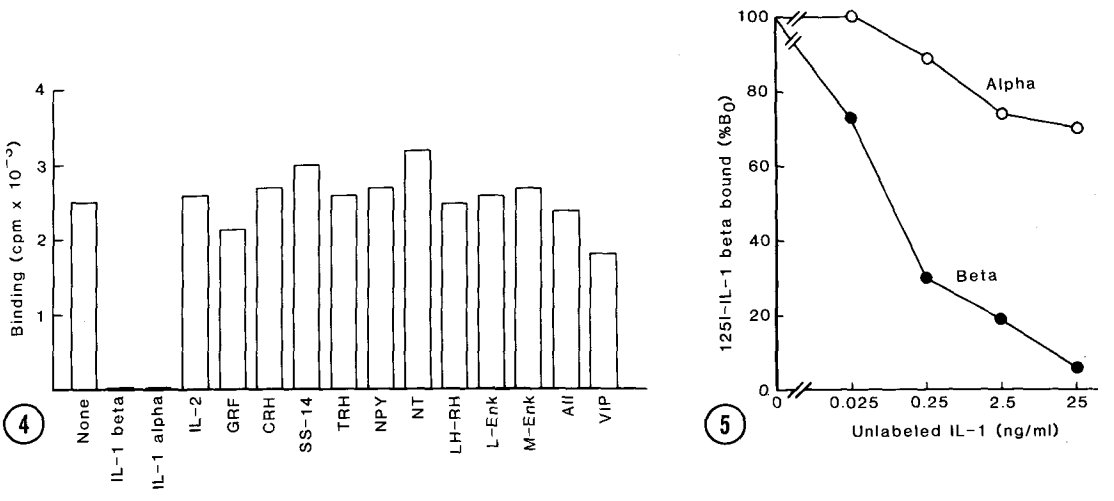

Fig. 4. Effect of IL-1 *alpha*, IL-2, and various neuropeptides on binding of [^{125}I]IL-1 *beta* to hypothalamic membrane preparations. Membrane preparations were incubated with [^{125}I]IL-1 *beta* (1.8 nM) with neuropeptides (2.5 μg/ml) and IL-2 (2.5 μg/ml). Each value represents specific binding in the presence of unlabeled IL-1 *beta* (2.5 μg/ml).

Fig. 5. Effect of unlabeled IL-1 beta and *alpha* on binding of [^{125}I]IL-1 *beta* to hypothalamic membrane preparations. Membrane preparations were incubated with [^{125}I]IL-1 *beta* (1.8 nM) in the binding assay with unlabeled IL-1 *beta* and IL-1 *alpha* at the indicated concentrations. Inhibition of [^{125}I]IL-1 *beta* binding was calculated as percentage of total binding in the absence of competitor.

curve of binding of [^{125}I]-IL-1 *beta* could be obtained using the pituitary membrane preparation, suggesting the absence of high affinity binding sites in the pituitary gland. This is comparable to our previous finding and others that IL-1 *beta* failed to stimulate ACTH release *in vitro* when the monokine was added to monolayer cultures of the rat anterior pituitary cells (6, 7, 22). On the other hand, IL-1 *beta* did stimulate ACTH release *in vivo*, probably by stimulating the release of endogenous CRF from the hypothalamus since IL-1 *beta*-induced ACTH release was completely blocked by prior administration of antiserum against CRH (5-7). It has also been reported that injection of IL-1 increased CRH concentration in the hypophysial portal blood in rats (6).

The present finding may substantiate the view that, in terms of ACTH release, the primary site of action of IL-1 *beta* is the brain. K_d (1.0-1.3 nM) for hypothalamic IL-1 *beta* receptors, however, is at least 5 times greater than that reported for these receptors on lymphocytes or fibroblasts (K_d = 8 - 200 pM)(16, 23, 24). The binding sites in the brain tissue are specific to IL-1 since various neuropeptides as well as IL-2 failed to affect the binding of [^{125}I]-IL-1 *beta* with the hypothalamic membrane preparations. It is conceivable that the IL-1 receptors in brain tissue are different from those in lymphocytes or fibroblasts. A higher K_d in brain tissue suggests that a greater concentration of IL-1 *beta* is necessary for the brain cells to respond to the monokine than for lymphocytes or fibroblasts. It is possible that the concentration of the monokine in certain loci in the brain reaches the nM range by some concentrating or accumulating process for circulating IL-1 or local production of the monokine. Some neurons in the human brain appear to contain IL-1-like substance (14). Astrocytes and glia also synthesize IL-1 (11, 12).

It is interesting that the hypothalamic membrane preparations bind IL-1 *beta* much more efficiently than IL-1 *alpha*. Approximately 100 times greater a concentration of IL-1 *alpha* than IL-1 *beta* was necessary to replace the [^{125}I]-IL-1 *beta* bound with the hypothalamic membrane preparations to the same extent. Such a high concentration of IL-1 *alpha* might be unlikely to occur in the brain tissue, even though it is synthesized and released in a limited area in the tissue. Most reports on IL-1 binding sites in lymphocytes indicated common receptors for IL-1 *alpha* and IL-1 *beta* (16-18), though some showed that IL-1 *beta* has a different affinity for the receptors on immune cells and a different biological activity, such as production of prostaglandin E_2 and IL-2 from IL-1 *alpha* (23-25). Our study suggests that IL-1 *beta* receptors in the brain are different from those for IL-1 *alpha*. This view is in agreement with our previous demonstration that IL-1 *beta*, administered either intravenously or intracerebroventricularly, stimulated ACTH release, whereas IL-1 *alpha* produced little effect (15, 26). The receptors for IL-1 *alpha* in the brain, if present, may have different actions from IL-1 *beta* in the central nervous system.

ACKNOWLEDGMENT

We wish to thank Cistron Corporation, Immunex Corporation and Cetus Corporation for their gifts of interleukin preparations. We would like to gratefully acknowlege the editorial assistance of Mrs. Linda Powell. This study was supported in part by NIH grant DK 09094 and a grant from Shionogi Co. Ltd., Japan.

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