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Regulation of interleukin-1 receptors on AtT-20 mouse pituitary tumour cells

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To study the cellular mechanisms of interleukin-1 (IL-1) in the pituitary corticotroph, we studied the properties of IL-1 receptors on a mouse pituitary ACTH-producing cell line, AtT-20. Scatchard plot analysis revealed a single type of receptor with a K_d (dissociation constant) of 93 pM, and 482 binding sites/cell. [^{125}I]IL-1α binding competed with IL-1α and IL-1β in an equimolar fashion. A 24 h pre-incubation with either CRH, epinephrine or nor-epinephrine increased the [^{125}I]IL-1α binding sites in the AtT-20 cells and conversely, a similar pre-incubation with either dexamethasone or tumour necrosis factor-α (TNFα) decreased them without affecting the affinity of the receptors in either case.

Interleukin-1, AtT-20 cell

1. INTRODUCTION

In recent years, it has been shown that IL-1, a cytokine originally isolated as a lymphocyte activating factor, has multiple biological activities, not only in regulating the immune system but also in modulating endocrine and metabolic functions [1–3]. In the hypothalamic-pituitary-adrenal (HPA) axis, it has been reported that IL-1 stimulates corticotropin-releasing hormone (CRH) synthesis [4] in and release [5,6] from the hypothalamus, ACTH synthesis [7] in and release [8,9] from the anterior pituitary, and glucocorticoid synthesis in the adrenal gland [10,11]. Although the cytokine is presumed to affect endocrine cells via a specific receptor on the plasma membrane as immune competent cells [12], information about the IL-1 receptor (IL-1R) on these neural or endocrine cells is quite limited.

We and others have previously reported that recombinant human IL-1 binds specifically to AtT-20 cells, a mouse corticotrophic cell line, and stimulates the synthesis and release of ACTH and β-endorphin through early activation of protein kinases [13–15]. In the present study, we further characterized the labeled IL-1α binding to AtT-20 cells and studied the effects of various cytokines, steroids, hypothalamic peptides and catecholamines on the IL-1α binding to AtT-20 cells.

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2. MATERIALS AND METHODS

2.1. *Cytokines and hormones*

Human IL-1α, human IL-1β (lymphocyte activating factor with an activity of 2×10^7 half-maximum U/mg protein) and human IL-2 (12×10^6 reference U/mg protein) were synthesized by recombinant DNA technology [16,17]. Human IL-6 (1×10^6 U/193 μg protein) was a gift from Ajinomoto Corp. (Kawasaki, Japan). Human tumour necrosis factor-α (TNFα) was donated from Dainippon Pharmaceutical Co. (Osaka, Japan). Arginine-vasopressin (AVP) and human/rat corticotropin-releasing hormone (CRH) were purchased from Peninsula Laboratories Inc. (Belmont, CA) and dexamethasone and dopamine were from Sigma Chemical Co. (St. Louis, MO). Epinephrine and norepinephrine were obtained from Sankyo Corp. (Tokyo, Japan) and Daiichi Pharmaceutical Co. (Tokyo, Japan), respectively.

2.2. *Cell line*

A subclone of AtT-20/D16v cells provided by Dr O. Midorikawa, Kyoto University, was used in this study. Cells were grown in Dulbecco's Modified Eagle's Medium (Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum at 37°C under a humidified atmosphere of 5% CO_2:95% air.

2.3. *Pituitary cell culture*

Anterior pituitaries from male Wistar rats or male New Zealand White rabbits, or whole mouse pituitaries from male Balb/c57 mice were enzymatically dispersed with collagenase type I (Worthington Biochemical, Freehold, NJ) and DNase (Sigma) as previously described [18]. The cells were incubated for 4 or 5 days in the same medium as AtT-20 cells before the binding study.

2.4. *Radioiodination of recombinant(r)IL-1α*

rIL-1α was labeled with ^{125}I using the chloramine T method and purified by Sephadex G100 (Pharmacia, Uppsala, Sweden) column chromatography. The specific activity of the iodinated rIL-1α was approximately 300 μCi/μg.

2.5. *Binding assay*

The binding study was performed as reported previously [19] with slight modifications. Briefly, cells harvested gently with a rubber po-

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liceman were washed twice with cold binding medium (RPMI 1640 containing 0.3% bovine serum albumin and 10 mM HEPES) and the viability of the cells was secured by the Trypan blue exclusion test. The same number of cells, approximately $1–5 \times 10^6$, was prepared in microcentrifuge tubes and the binding study was started by incubating the cells with [^{125}I]IL-1α at 4°C for 5 h. After the binding incubation, unbound radioactivity was separated from the cells by centrifuging the incubation mixture for 2 min at $1,000 \times g$ and discarding the supernatant. Radioactivity remaining in the cell pellet was measured by a gamma counter. Non-specific binding was determined in the presence of a 200-fold excess of unlabeled IL-1.

2.6. *Data analysis*

K_d and number of binding sites were calculated according to the method of Scatchard [20]. Values shown are mean ± S.E. For comparison between means, data were analysed by the two-tailed Student's t test.

3. RESULTS

To assess the specificity of the binding, IL-1α, IL-1β, IL-2, IL-6, TNF, CRH, AVP, dexamethasone, dopamine, epinephrine or nor-epinephrine was incubated with [^{125}I]IL-1α. As shown in Fig. 1, only IL-1α and IL-1β competed in an equimolar fashion with the [^{125}I]IL-1α binding. The other materials tested had no effect on [^{125}I]IL-1α binding to AtT-20 cells.

Scatchard plot analysis of the binding data yielded an apparent K_d of approximately 9.3×10^{-11} M with approximately 482 binding sites/cell. Only one component of binding site was detected, as shown in Fig. 2.

Effects of hypothalamic peptides, catecholamines, dexamethasone and cytokines on the IL-1α binding to AtT-20 were next examined and the data are illustrated in Fig. 3, and Table 1. When AtT-20 cells were incubated for 24 h with various concentrations of CRH immediately before the binding experiment, a concentration-dependent increase in the IL-1α binding was observed, as shown in Fig. 3. Incubation for the same period with dexamethasone or TNFα decreased the IL-1 binding significantly. The same period of pre-incubation with either IL-2, IL-6, AVP or dopamine did not affect IL-1α binding to AtT-20 cells (data not shown). Although epinephrine and nor-epinephrine did not affect IL-1α binding under similar pre-incubation conditions, partial effects were observed when the pre-incubation media were repeatedly supplemented with fresh epinephrine or nor-epinephrine at 0, 2, 4, 6, 10 and 21 h during the 24 h pre-incubation period.

Kinetic studies revealed that significant modification of the IL-1α binding site by all substances tested were not apparent after a 24 h incubation (data not shown).

Changes in the IL-1α binding were characterized by Scatchard plot analysis. As shown in Table I, IL-1α binding sites in AtT-20 cells were increased by CRH, epinephrine and nor-epinephrine and decreased by dexamethasone and TNFα. K_ds were not significantly affected by any treatment.

As shown in Table II, pituitary cells obtained from several species of animals also demonstrated low numbers of specific IL-1α binding sites.

4. DISCUSSION

The results reported here show that the binding of [^{125}I]IL-1α to AtT-20 cells satisfies the criteria for a receptor; it is rapid, stable, saturable, of high affinity, low capacity and high specificity. [^{125}I]IL-1α appeared to bind to a single class of binding sites as demonstrated by the linearity of Scatchard plot, as reported previously [21,22]. It has been reported that a variety of cell types express IL-1R which can be divided into at least two classes of receptors, a large molecular weight type, p80, and a small type, p68 [23–28]. IL-1R found in T-cells, fibroblasts and many other cell types, binds

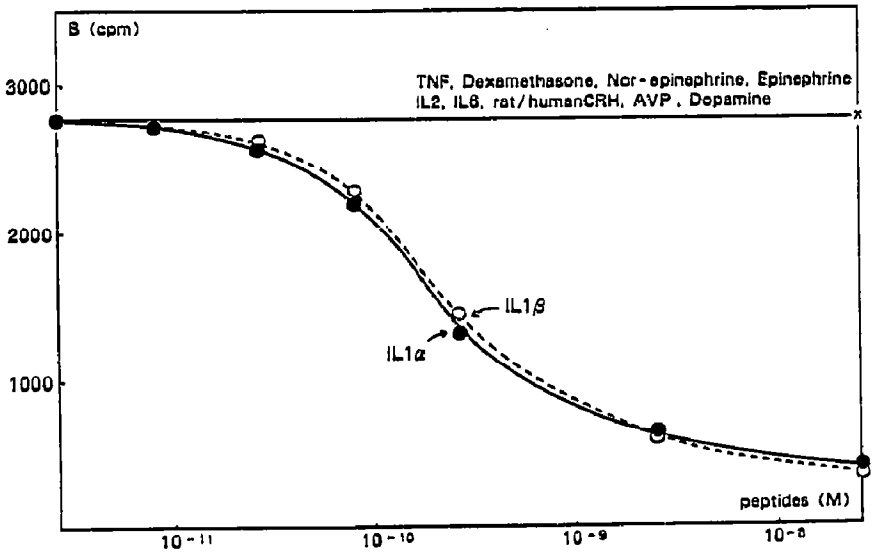

Fig. 1. Effect of unlabeled IL-1 and other materials on binding of [^{125}I]IL-1α to AtT-20 cells. Cells (10^6 cells/tube) were incubated with [^{125}I]IL-1α (23 pM) with unlabeled IL-1 and other materials at the indicated concentrations.

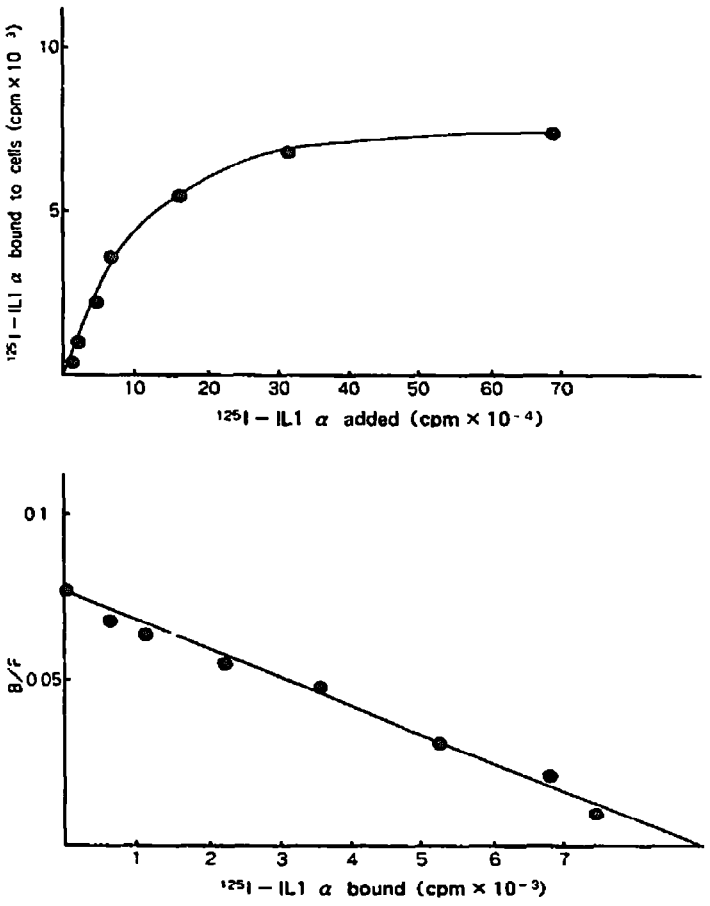

Fig 2 Specific binding of [^{125}I]IL-1α to AtT-20 cells and Scatchard plot analysis AtT-20 cells (10^6 cells/tube) were incubated with various dilutions of [^{125}I]IL-1α for 5 h at 4°C The data is representative of 5 experiments

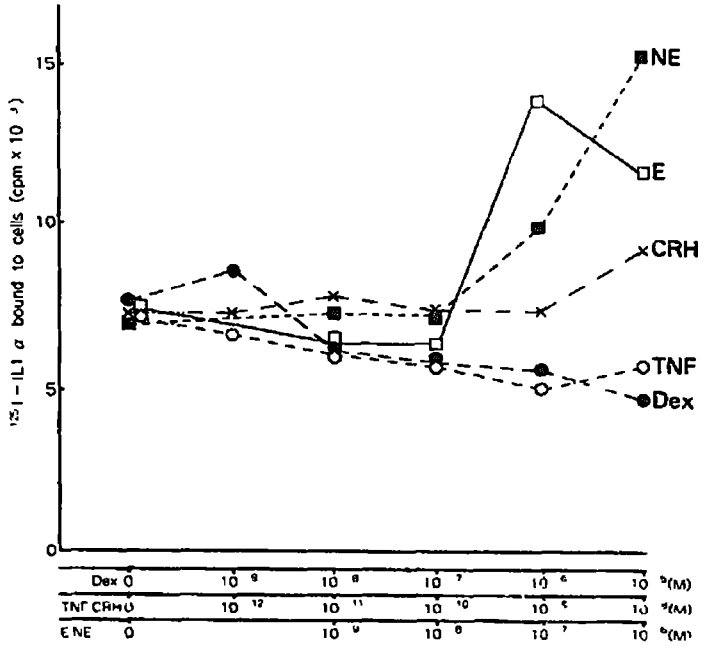

Fig 3 Dose-dependent effect of dexamethasone (Dex) TNF, CRH, epinephrine (E) and nor-epinephrine (NE) on IL-1αR expression AtT-20 cells were pre-incubated for 24 h with or without increasing concentrations of indicated materials At the end of the incubation the pre-incubated cells (5×10^6 cells/tube) were incubated with [^{125}I]IL-1α (100 pM) for 5 h Each point represents the mean of duplicate determinations

IL-1α and IL-1β indistinguishably, whereas the small molecular type receptor, expressed on B-cells and macrophages, discriminates between them. As shown in Fig 1, IL-1α and IL-1β are equally competitive with [^{125}I]IL-1α and the dissociation constant of 9.33×10^{-11} M is similar to the reported K_d of the T-cell type IL-1 receptor [26]. These data suggest that AtT-20 cells examined in our experiments possibly express T-cell type IL-1R and are compatible with a recent report which indicated a partial sequence of the AtT-20 IL-1 receptor molecule [29].

Hormonogenesis in the corticotroph is controlled mainly by CRH, vasopressin and glucocorticoid hormones, but is also affected by many other hormones. AtT-20 cells maintain considerable responses to these factor. Among these factors, CRH, AVP, IL-1, IL-2, IL-6, TNFα, epinephrine, nor-epinephrine and dopamine are stimulatory [13,30] and dexamethasone is inhibitory [31] to AtT-20 cells. In this study, we showed that 10 nM of CRH, 1 μM of epinephrine and 1 μM of nor-epinephrine increased the number of IL-1R on AtT-20 cells, whereas 10 μM of dexamethasone and 10 nM of TNFα decreased it without apparent changes of their dissociation constants, suggesting that these changes are due to IL-1R induction and reduction on the AtT-20 cells. Although it is known that TNFα is cytotoxic for some tumour cells in culture [32], we used the same number of cells in TNFα-treated and non-treated vials after confirming cell viability and are confident that the changes are due to cytotoxic effect even in the TNFα-related experiments.

As all the changes in IL-1R binding observed in this study required at least 24 h of incubation, the effects may require a gene expression and subsequent protein synthesis related to IL-1R, as platelet-derived growth factor (PDGF) induces IL-1R in Balb/3T3 cells [33]. The cell proliferation cycle of the AtT-20 cells was also

Table 1

Regulation of the IL-1αR on AtT-20 cells

	Sites/cell	K_d (10^{-11} M)
Control	482 ± 27	9.3 ± 1.7
CRH (10^{-8} M)	711 ± 87*	14.4 ± 3.8
Epinephrine (10^{-6} M)	758 ± 99*	16.6 ± 0.4
Nor-epinephrine (10^{-6} M)	603 ± 61*	12.8 ± 0.7
Dexamethasone (10^{-5} M)	334 ± 24*	17.6 ± 3.0
TNF (10^{-8} M)	347 ± 25*	14.9 ± 2.3

In each reacting tube, 5×10^5 cells treated with the indicated materials, were incubated with [^{125}I]IL-1α (100 pM) for 5 h *$P<0.05$ ($n = 3$)

Table II

IL-1α binding to normal pituitary cells

	[^{125}I]IL-1α	Unlabeled IL-1α	Counts (cpm)
Rat anterior pituitary cells	+	–	132
	+	+	54
Mouse whole pituitary cells	+	–	739
	+	+	85
Rabbit anterior pituitary cells	+	–	721
	+	+	63
Total			193222

Normal pituitary cells (1.25×10^5 cells/tube) were incubated with [^{125}I]IL-1α (100 pM) Non-specific binding was determined with 100-fold molar excess of unlabeled IL-1α

possibly modified by these various agents and might contribute to the changes in these experiments. Although several reports have been published concerning the modulation of IL-1R expression [34–37], detailed mechanisms resulting in these changes, including ours, remain to be elucidated.

A variety of interactions among heterogenous receptor systems in the endocrine, immune and nervous systems have been reported. In the field of cytokine–neuroendocrine interactions, only a few reports concerning these interactions have been published, i e. IL-1 increased vasopressin effect on ACTH release from AtT- 20 cells [38], IL-1 preserved catecholamine effects on ACTH release from rat pituitary monolayer cells [39], and glucocorticoid induced IL-1R on human peripheral blood lymphocytes [37]. Our data shown here not only confirm the previous observations about CRH [40] but also extend the number of substances which modify IL-1R on the corticotrophic cell line and suggest the existence of the bi-directional interactions between cytokines and hormones on the surface of an endocrine cell.

It is still controversial whether IL-1 directly affects ACTH release from normal pituitary cells [8,9] We found low numbers of considerably specific IL-1 binding sites in normal rat, mouse and rabbit pituitary cells in this study. Although it is hard to assess the physiological significance of our study concerning AtT-20 cells, and the specific binding in these normal pituitaries were too small to be further characterized, this information, combined with the results using AtT-20 cells, favours the notion that human IL-1α acts directly on the pituitary cells.

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