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INTERLEUKIN-1 AND PHORBOL MYRISTATE ACETATE MODULATE THE PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR IN LYMPHOCYTES AND GLIAL CELLS

PAUL N. MOYNAGH,* LUKE A. J. O'NEILL and D. CLIVE WILLIAMS
Department of Biochemistry, Trinity College, University of Dublin, Dublin 2, Ireland

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Abstract—High affinity "peripheral-type" benzodiazepine binding sites were detected in an interleukin-1 (IL-1) responsive murine thymoma cell line EL4.NOB-1. Exposure of these cells to IL-1 over a period of at least 24 hr resulted in down-regulation of the binding sites. This effect was inhibited by the IL-1 receptor antagonist (IL-1RA) which in these cells inhibits IL-1 binding to the type I IL-1 receptor. Phorbol myristate acetate (PMA), another activator of EL4.NOB-1 cells, had an opposite effect to IL-1 in that it increased binding site expression dramatically suggesting different mechanisms of action for these two effectors. IL-1 produced a similar response in the rat glioma cell line C6 whereas PMA was ineffective. Such modulation of the peripheral-type benzodiazepine receptor may provide an insight into its physiological role and its possible participation in IL-1 actions in different cells.

Interleukin-1 (IL-1†) is a 17.5 kDa polypeptide hormone produced by many cell types including macrophages, keratinocytes and brain glial cells [1–3]. The ubiquitous distribution of IL-1 in diverse tissues has accordingly suggested an equally pleiotypic range of biological activities. The most rigorously studied biological effect of IL-1 has been its proposed role in the immune system as a pro-inflammatory molecule [4] and thus an important mediator of host responses to microbial and inflammatory diseases. Some of the potent biological activities ascribed to IL-1 appear to be mediated via the central nervous system. These include the induction of fever, slow-wave sleep and sickness syndrome [5]. Moreover, IL-1 has been shown to stimulate astrocytic growth and is detected in the brain upon cerebral trauma suggestive of a role in brain cell repair and viability [6]. Possible clues as to the basis of the effects of IL-1 in the central nervous system have come from a recent report suggesting an association between IL-1 and the peripheral-type benzodiazepine receptor in brain [7].

The peripheral-type benzodiazepine receptor differs from the central-type benzodiazepine receptor (associated with the neuronal $GABA_A$ receptor) in its pharmacological specificity and its anatomical and subcellular distribution [8]. The peripheral receptor is predominantly localized in the mitochondrial outer membrane [9, 10] and is present in both peripheral tissues and in glia in the central nervous system [11, 12]. It can be specifically labelled with ligands such as [^{3}H]Ro 5-4864 and [^{3}H]PK 11195, which have very low affinity for the central receptor [13, 14]. The peripheral receptor and its ligands have been proposed to play roles in a kaleidoscope of biological phenomena most notably stimulation of steroidogenesis [15, 16]. However, a complete mechanistic understanding of the involvement of the peripheral receptor in such a process remains to be resolved.

A recent report has suggested that IL-1 can increase the expression of the peripheral-type benzodiazepine receptor in brain [7] which may be an important response of brain tissue to injury. The mechanism of this modulation and its importance for the more general effects of IL-1 in brain have not been deduced. In order to extend these studies this work assessed the ability of IL-1 to regulate directly the expression of the peripheral receptor in two cell types: the murine thymoma line EL4. NOB-1, which has previously been used in IL-1 receptor and signalling studies [17, 18] and the rat glioma line C6, commonly used in the study of glial cell function and previously shown to be rich in the peripheral-type benzodiazepine receptor [19]. IL-1 was found to down-regulate expression in both cell types, an observation which is likely to be a first step in probing the possible role of the peripheral-type benzodiazepine receptor in IL-1-mediated effects in different cell types.

MATERIALS AND METHODS

Materials. The murine thymoma EL4.NOB-1 and rat glioma C6 lines were obtained from the European Collection of Animal Cell Cultures (Salisbury, U.K.). RPMI 1640, Dulbecco's modified Eagle's medium (DMEM) and foetal calf serum (FCS) were from Seromed (Frickenhausen, Germany). Trypsin was from GIBCO (Paisley, U.K.). Recombinant human IL-1α and β were gifts from Dr Jeremy Saklatvala (Strangeways Research Laboratory, Cambridge, U.K.). Recombinant human IL-1

* Corresponding author. Tel. (353) 1-7021802; FAX (353) 1-772400.

† Abbreviations: FCS, foetal calf serum; IL-1, interleukin-1; IL-1RA, IL-1 receptor antagonist; PMA, phorbol myristate acetate; DMEM, Dulbecco's modified Eagle's medium.

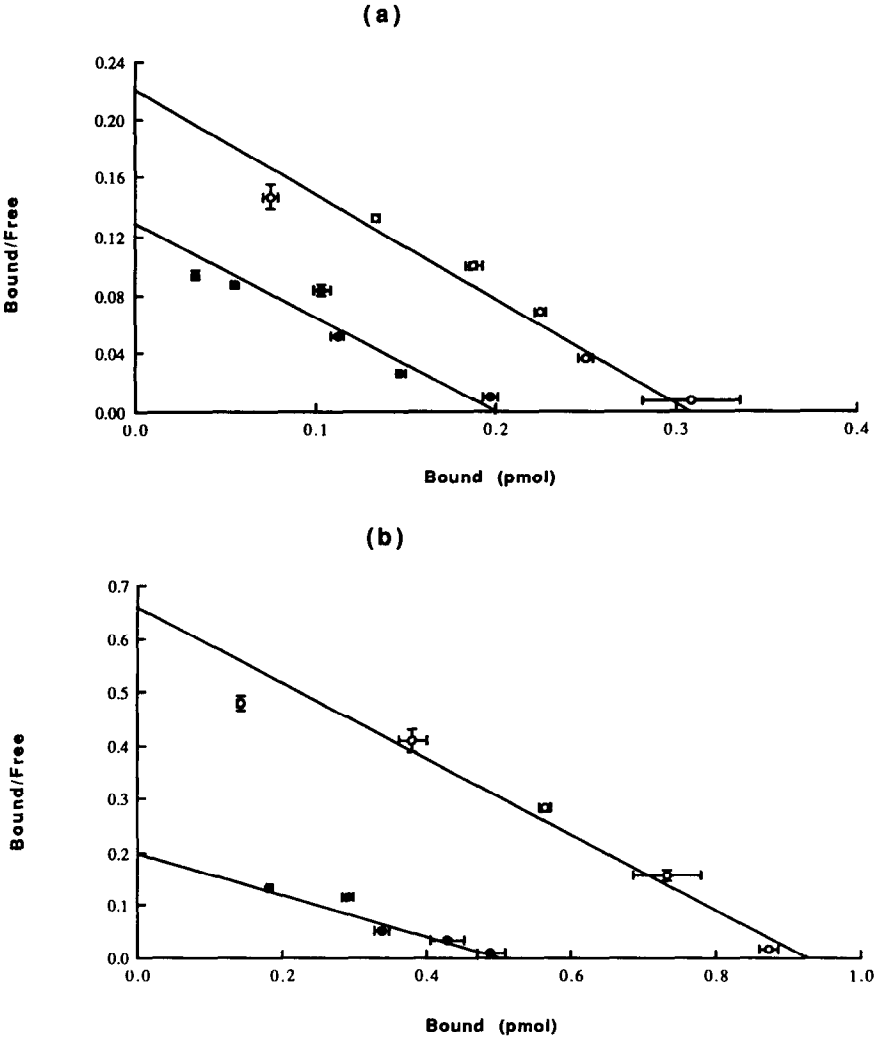

Fig. 1. Binding of [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 to EL4.NOB-1 and C6 homogenates. Homogenates of (a) EL4.NOB-1 (120 μg protein) and (b) C6 cells (40 μg protein) were assayed in triplicate for binding of 0–40 nM [^{3}H]PK 11195 (8 Ci/mmol) (○) or 0–20 nM [^{3}H]Ro 5-4864 (10 Ci/mmol) (●) as described in Materials and Methods. The lines represent the best fits to rectangular hyperbolae each of which describes the binding of ligand to a single class of binding sites [22]. The data are shown in the form of Scatchard plots for clarity.

receptor antagonist (IL-1RA) was given by Dr Robert Thompson (Synergen Inc., Boulder, CO, U.S.A.). Phorbol myristate acetate (PMA) was from the Sigma Chemical Co. (Poole, U.K.). [^{3}H]PK 11195 (86.9 Ci/mmol) and [^{3}H]Ro 5-4864 (90 Ci/mmol) were from NEN (Dreieich, Germany). PK 11195 was a gift from Dr Gerard Le Fur (Pharmuka Laboratories, Gennevilliers, France). Ro 5-4864 was from Fluka Chemicals (Glossop, U.K.).

Cell culture. The murine thymoma EL4.NOB-1 cell line was cultured in RPMI 1640 containing 10% FCS and the rat glioma C6 cell line in DMEM containing 10% FCS. The cells were maintained at 37° in a humidified atmosphere of 5% CO_2. C6 glioma were passaged using 0.25% trypsin in DMEM. Both cell types were stimulated in serum-containing medium at 37° for all experiments.

Preparation of cell homogenates. EL4.NOB-1 and C6 were stimulated with various concentrations of IL-1α, IL-1β or PMA for different time periods. In some experiments, cells were pre-treated with IL-RA for 1 hr prior to IL-1/PMA treatment. Stimulation was terminated by washing the cultures twice with ice-cold 50 mM Tris–HCl buffer, pH 7.4. Each wash in the case of EL4.NOB-1 consisted of gentle trituration of pelleted cells in the above buffer followed by centrifugation at 1500 g for 5 min. EL4.NOB-1 were thus obtained in pellet form whilst C6 were harvested, subsequent to washing, by scraping into the above buffer and centrifuging at 1500 g for 5 min. Cell pellets were resuspended in this buffer using an Ultra-Turrax homogenizer to give a protein concentration of 0.5–1 mg/mL as determined by the method of Markwell *et al.* [20].

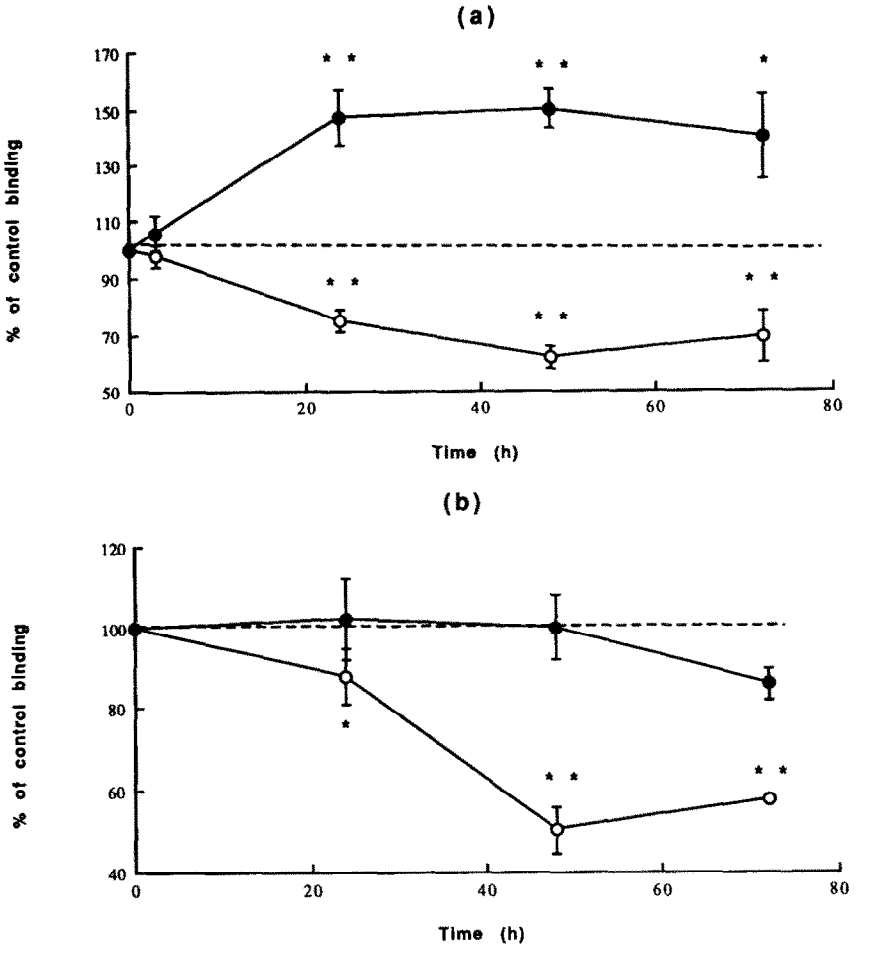

Fig. 2. Time course of effect of IL-1 or PMA on [^{3}H]PK 11195 binding to EL4.NOB-1 and C6 cells. Cultures of EL4.NOB-1 (a) and C6 (b) cells were treated with 10 ng/mL of IL-1α (○) and IL-1β (○), respectively, or PMA (●) for various time periods. Samples were then assayed for specific binding of 1 nM [^{3}H]PK 11195 (86.9 Ci/mmol) as described in Materials and Methods. Each point is the mean ± SEM of triplicate determinations from three different experiments. Results are expressed as a percentage of values obtained in untreated control cultures of cells (dashed line). Specific binding of [^{3}H]PK 11195 to control cultures of EL4.NOB-1 and C6 cells was 1.2 ± 0.1 and 6.2 ± 0.6 pmol/mg protein, respectively. Significantly different (*$P < 0.05$, **$P < 0.01$) when compared to control values.

Binding assay. Homogenates (40–120 μg protein) were incubated in triplicate for 90 min with 0–40 nM [^{3}H]PK 11195 (8–86.9 Ci/mmol) or 0–20 nM [^{3}H]Ro 5-4864 (10–90 Ci/mmol) in 50 mM Tris–HCl buffer, pH 7.4, in a total volume of 0.5 mL at 4°. Total and non-specific/non-saturable binding in each case was determined in the absence and presence of 10 μM unlabelled ligand, respectively. Specific/saturable binding was calculated from the difference between total and non-saturable binding. The incubation mixtures were filtered and counted as described previously [21]. Equilibrium binding parameters were determined by fitting data to an equation describing a rectangular hyperbola by weighted non-linear regression [22].

Statistical analysis. Significance was evaluated by Student's *t*-test for unpaired data.

RESULTS

EL4.NOB-1 thymoma and C6 glioma homogenates both displayed saturable high affinity binding of [^{3}H]PK 11195 and [^{3}H]Ro 5-4864, selective ligands for the peripheral-type benzodiazepine receptor (Fig. 1). Direct fitting of the data to a rectangular hyperbola by non-linear regression using the method of Wilkinson [22] indicated in all cases ligand binding to a single class of site. Homogenates of EL4.NOB-1 displayed K_d values of 1.5 ± 0.3 and 1.4 ± 0.2 nM and B_{max} values of 2.6 ± 0.2 and 1.8 ± 0.1 pmol/mg protein for [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 binding, respectively, where each value represents the mean ± residual for two different preparations. Similarly, homogenates of C6 demonstrated K_d values of 1.4 ± 0.1 and 2.4 ± 0.4 nM and B_{max} values

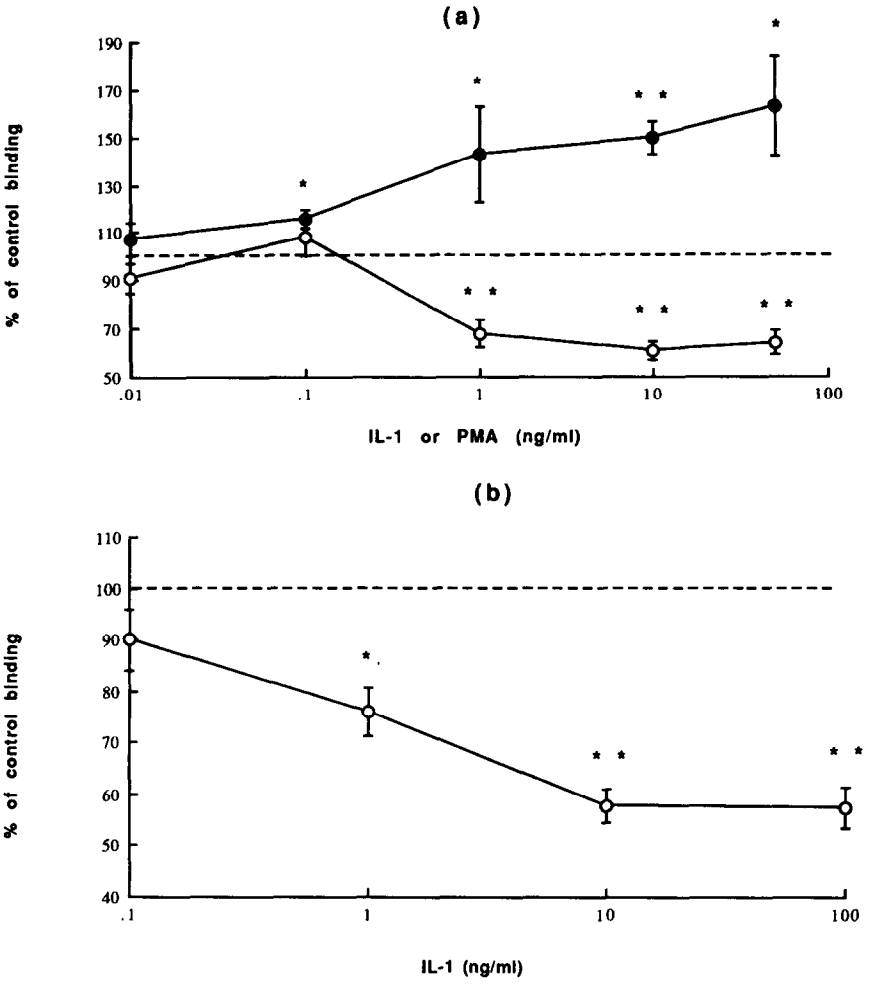

Fig. 3. Effect of various concentrations of IL-1 or PMA on [^{3}H]PK 11195 binding to EL4.NOB-1 and C6 cells. Cultures of EL4.NOB-1 (a) and C6 (b) cells were exposed for 48 hr to various concentrations (0.1–100 ng/mL) of IL-1α (○) and IL-1β (○), respectively. EL4.NOB-1 cells were also treated for 48 hr with 0.1–50 ng/mL of PMA (●). Cultures were assayed after treatment for specific binding of 1 nM [^{3}H]PK 11195 (86.9 Ci/mmol) as described in Materials and Methods. Each point represents the mean ± SEM of triplicate determinations from three separate experiments. Results are expressed as a percentage of values obtained in untreated control cultures of cells (dashed line). Specific binding of [^{3}H]PK 11195 to control cultures of EL4.NOB-1 and C6 cells was 1.2 ± 0.1 and 6.2 ± 0.6 pmol/mg protein, respectively. Significantly different ($^*P < 0.05$, $^{**}P < 0.01$) when compared to control values.

of 23.1 ± 0.7 and 12.3 ± 0.6 pmol/mg protein for [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 binding, respectively.

EL4.NOB-1 and C6 cultures were treated with IL-1α and IL-1β, respectively, for various time periods in order to assess the effect of such cytokines on [^{3}H]PK 11195 binding in these cells (Fig. 2). IL-1β was chosen to stimulate C6 because of previous studies which reported human recombinant IL-1β to be more potent than its α equivalent in rat model systems [23, 24]. Exposure to the respective forms of IL-1 (each at a concentration of 10 ng/mL) for at least 24 hr resulted in significant decreases of [^{3}H]PK 11195 binding in both cell types, effects which reached their maximum at 48 hr. The latter time period was used to demonstrate the dose-dependent decrease of [^{3}H]PK 11195 binding activity by the two forms of IL-1 (Fig. 3). The maximum effect in EL4.NOB-1 cells was obtained with IL-1α at a concentration of 10 ng/mL and resulted in a decrease of [^{3}H]PK 11195 binding activity to 61% of control levels (Fig. 3a). Similarly, [^{3}H]PK 11195 binding activity in C6 cells decreased maximally to 58% of control levels in response to 10 ng/mL of IL-1β (Fig. 3b).

PMA, which usually mimics IL-1 effects in EL4.NOB-1 cells, interestingly increased the [^{3}H]PK 11195 binding activity of these cells in a time- (Fig. 2a) and dose-dependent (Fig. 3a) manner. No such effect could be demonstrated in C6 cells in response to PMA (Fig. 2b).

The effect of IL-1RA on the decrease of [^{3}H]PK

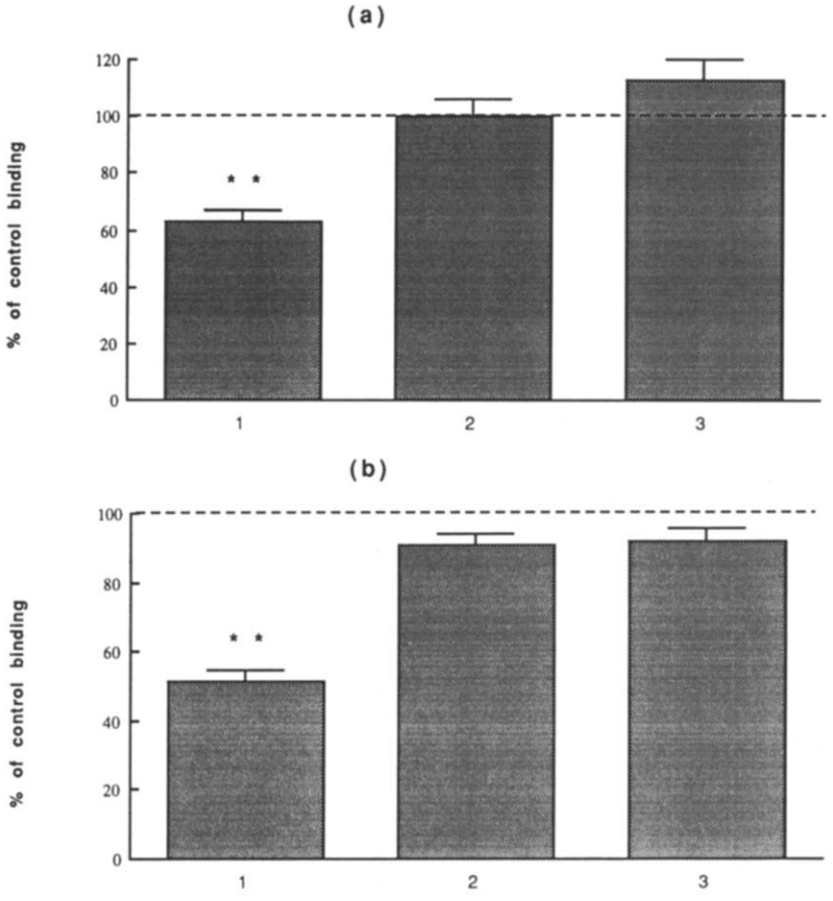

Fig. 4. Inhibition of IL-1 effects by IL-1 receptor antagonist. Cultures of EL4.NOB-1 (a) and C6 (b) cells were maintained in the presence (1 and 2) and absence (3) of IL-1α and IL-1β (10 ng/mL), respectively, for 48 hr. Samples (2) and (3) had been pre-treated with the IL-1 receptor antagonist (5 μg/mL) for 1 hr. After 48 hr, cultures were assayed for specific binding of 1 nM [^{3}H]PK 11195 (86.9 Ci/mmol) as described in Materials and Methods. Each value is the mean ± SEM of triplicate determinations from three separate experiments. Results are expressed as a percentage of values obtained in untreated control cultures of cells (dashed line). Specific binding of [^{3}H]PK 11195 to control cultures of EL4.NOB-1 and C6 cells was 1.2 ± 0.1 and 6.2 ± 0.6 pmol/mg protein, respectively. Significantly different (**$P < 0.01$) when compared to control values.

Table 1. Effect of IL-1 and PMA on binding parameters for the ligands, [^{3}H]PK 11195 and [^{3}H]Ro 5-4864, binding to EL4.NOB-1 and C6 cell homogenates

	[^{3}H]PK 11195			[^{3}H]Ro 5-4864		
	Control	IL-1	PMA	Control	IL-1	PMA
EL4.NOB-1:						
K_d (nM)	1.3 ± 0.3	1.6 ± 0.2	1.1 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.3 ± 0.2
B_{max} (pmol/mg protein)	2.4 ± 0.1	1.87 ± 0.07*	3.3 ± 0.2*	1.74 ± 0.09	1.34 ± 0.08*	2.25 ± 0.08*
C6:						
K_d (nM)	1.4 ± 0.1	1.5 ± 0.3	ND	2.4 ± 0.4	2.2 ± 0.2	ND
B_{max} (pmol/mg protein)	23.1 ± 0.7	18 ± 1*	ND	12.3 ± 0.6	8.8 ± 0.2*	ND

Homogenates (100 μg protein) of EL4.NOB-1 and C6 cells, previously treated with IL-1α and IL-1β (10 ng/mL), respectively, or PMA (10 ng/mL) for 48 hr, were assayed for saturable binding of [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 over a range of radioligand concentrations.

Binding parameters were obtained by computer analysis of the binding data using the method of Wilkinson [22].

Each value represents the mean ± SEM from triplicate determinations.

Significantly different (*$P < 0.01$) when compared to control levels.

ND, not determined.

11195 binding activity by IL-1α and β was examined. IL-1RA was preincubated for 1 hr with EL4.NOB-1 or C6 cells prior to stimulating them with IL-1α and β, respectively. IL-1RA prevented the decrease of [^{3}H]PK 11195 binding activity in both cases (Fig. 4). IL-1RA alone displayed no significant effect on [^{3}H]PK 11195 binding in either cell type.

Equilibrium binding parameters were determined by saturable binding studies in an attempt to elucidate whether changes of binding activity with respect to ligands selective for the peripheral-type benzodiazepine receptor reflected alterations in expression of new binding sites or modulation of affinity of existing binding sites. Treatment of EL4-NOB-1 and C6 cells with IL-1α and IL-1β, respectively, caused a decrease in the binding capacities of the cells for both [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 without altering the affinities for the ligands (Table 1). In contrast, PMA increased the capacity of EL4.NOB-1 cells to bind both ligands without affecting affinities.

DISCUSSION

It has been demonstrated in these studies that the murine thymoma EL4.NOB-1 and the rat glioma C6 lines contain significant levels of the peripheral-type benzodiazepine receptor as determined by high affinity, saturable binding of both [^{3}H]PK 11195 and [^{3}H]Ro 5-4864. The binding parameters determined for C6 agree with values reported previously [19]. However the present study demonstrates more reproducible B_{max} values for different batches of cells. This greater consistency is not understood as Gorman *et al.* [19] did not study the nature of their variability.

EL4.NOB-1 and C6 cells were thus used as models to examine the direct effect of IL-1 on the peripheral-type benzodiazepine receptor in cells of both immune and central nervous system lineage. IL-1 was found to down-regulate the receptor in these cells possibly via inhibition of its expression as suggested by IL-1 causing a decrease of B_{max} values for [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 binding. This is in apparent contrast to a previous study which reported that IL-1 could actually increase the expression of the receptor in brain [7]. However, the latter was a systemic response and may well be explained by the indirect effect of gliosis which results from the mitogenic activity of IL-1 [6].

The signalling mechanism(s) employed by IL-1α and β to decrease the binding capacity of EL4.NOB-1 and C6 cells for ligands, selective for the peripheral-type benzodiazepine receptor, is not fully understood. The type I IL-1 receptor is probably involved based on the inhibitory action of IL-1RA on the IL-1 effects and a recent study reporting that the human IL-1RA binds strongly to the murine type I IL-1 receptor, but only weakly, if at all, to the type II receptor [25]. Past studies have shown that many of the effects of IL-1 are mimicked by PMA [26, 27], suggestive of a common signalling pathway possibly involving protein kinase C. However, other lines of evidence now point to a lack of involvement of protein kinase C in IL-1 signalling [28, 29]. The present study adds further evidence to the latter argument, in that PMA failed to mimic IL-1 action in C6 cells and indeed showed opposing effects in EL4.NOB-1 cells thus implying a protein kinase C-independent transducing pathway with respect to IL-1 modulation of the peripheral-type benzodiazepine receptor. It also points to the complexity of cellular responses to IL-1 and PMA whereby the signalling pathways for both agents may converge on one response [26, 27] but diverge on another.

The end result of IL-1 and PMA actions on the cells is apparently due to regulation of the expression of peripheral-type benzodiazepine receptor rather than direct modification of existing receptor as judged by changes in receptor density without altering receptor affinity. This was reflected in changes of binding site density for both [^{3}H]PK 11195 and [^{3}H]Ro 5-4864. The binding of both radioligands was similarly affected in all cases. Previous reports have failed to demonstrate unequivocally whether the binding sites for these ligands are located on the same or different polypeptides [30, 31]. The similar regulation of the binding sites in the present study proposes that the former scenario is more probable but the alternative which would necessitate closely coordinated regulation of the expression of different polypeptides cannot be excluded.

The functional significance of this novel finding that IL-1 down-regulates the expression of the peripheral-type benzodiazepine receptor in cells representative of the immune and central nervous systems is unclear. However, IL-1 has been demonstrated to be a very potent inhibitor of steroidogenesis in primary culture [32] and whilst this may be due in part to inhibition of expression of cholesterol side-chain cleavage cytochrome P450 [33], it is highly plausible that down-regulation of the peripheral-type benzodiazepine receptor may play a key role in such an IL-1 mediated process. The latter argument arises from the many recent reports which indicate that the receptor regulates the rate-limiting step in steroidogenesis [15, 16, 34]. As certain steroids display immunosuppressive properties [35], regulation of their production by IL-1 may be of physiological significance with respect to the immunomodulatory activity of IL-1. In addition, neurosteroids play crucial roles in the brain, including control of convulsive and anaesthetic states [36–38] and thus modulation of their production by IL-1 may constitute an important mechanism for regulating neuronal excitability. The present study may, therefore, provide a basis to explain some of the effects of IL-1 in brain as well as intimating a further aspect of IL-1 action in the immune system.

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REFERENCES

1. Lindemann A, Riedel D, Oster W, Meuer SC, Blohm D, Mertelsmann RH and Herrmann F, Granulocyte/macrophage colony-stimulating factor induces interleukin-1 production by human polymorphonuclear neutrophils. *J Immunol* **140**: 837–839, 1988.

2. Luger TA, Stadler BM, Luger BM, Mathieson BJ, Mage M, Schmidt JA and Oppenheim JJ, Murine epidermal cell-derived thymocyte-activating factor resembles murine interleukin-1. *J Immunol* **128**: 2147–2152, 1982.
3. Fontana A, Kristensen F, Dubs R, Gemsa D and Weber E, Production of prostaglandin E and an interleukin-1 like factor by cultured astrocytes and C6 glioma cells. *J Immunol* **129**: 2413–2419, 1982.
4. Mizel SB, Interleukin-1 and T cell activation. *Immunol Rev* **63**: 51–72, 1982.
5. Kent S, Bluthe R, Kelley KW and Dantzer R, Sickness behavior as a new target for drug development. *Trends Pharmacol Sci* **13**: 24–28, 1992.
6. Giuliam D and Lachman LB, Interleukin-1 stimulation of astroglial proliferation. *Science* **228**: 497–499, 1985.
7. Bourdiol F, Toulmond S, Serrano A, Benavides J and Scatton B, Increase in ω (peripheral type benzodiazepine) binding sites in the rat cortex and striatum after local injection of interleukin-1, tumour necrosis factor-α and lipopolysaccharide. *Brain Res* **543**: 194–200, 1991.
8. Krueger KE, Peripheral-type benzodiazepine receptors: a second site of action for benzodiazepines. *Neuropsychopharmacology* **4**: 237–244, 1992.
9. Anholt RRH, Pederson PL, De Souza EB and Snyder SH, The peripheral-type benzodiazepine receptor: localization to the mitochondrial outer membrane. *J Biol Chem* **261**: 576–583, 1986.
10. O'Beirne GB and Williams DC, The subcellular location in rat kidney of the peripheral benzodiazepine acceptor. *Eur J Biochem* **175**: 413–421, 1988.
11. Braestrup C and Squires RF, Specific benzodiazepine receptors in rat brain characterized by high-affinity [^{3}H]diazepam binding. *Proc Natl Acad Sci USA* **74**: 3805–3809, 1977.
12. Schoemaker H, Morelli M, Deshmukh P and Yamamura HI, [^{3}H]Ro 5-4864 benzodiazepine binding in the kainate lesioned striatum and Huntington's diseased basal ganglia. *Brain Res* **248**: 396–401, 1982.
13. Schoemaker H, Boles RG, Horst WD and Yamamura HI, Specific high-affinity binding sites for [^{3}H]Ro 5-4864 in rat brain and kidney. *J Pharmacol Exp Ther* **225**: 61–69, 1983.
14. Le Fur G, Perrier ML, Vaucher N, Imbault F, Flamier H, Benavides J, Uzan A, Renault C, Dubroeucq MC and Gueremy C, Peripheral benzodiazepine binding sites: effect of PK 11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide. *Life Sci* **32**: 1839–1847, 1983.
15. Muhkin AG, Papadopoulos V, Costa E and Krueger KE, Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proc Natl Acad Sci USA* **86**: 9813–9816, 1989.
16. Papadopoulos V, Mukhin AG, Costa E and Krueger KE, The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis. *J Biol Chem* **265**: 3772–3779, 1990.
17. Bird TA, Gearing AJH and Saklatvala J, Murine interleukin 1 receptor. *J Biol Chem* **263**: 12063–12069, 1988.
18. O'Neill LAJ, Ikebe T, Sarsfield SJ and Saklatvala J, The binding subunit of pertussis toxin inhibits IL-1 induction of IL-2 and prostaglandin production. *J Immunol* **148**: 474–479, 1992.
19. Gorman AMC, O'Beirne GB, Regan CM and Williams DC, Antiproliferative action of benzodiazepines in cultured brain cells is not mediated through the peripheral-type benzodiazepine acceptor. *J Neurochem* **53**: 849–855, 1989.
20. Markwell M, Haas SM, Bieker L and Tolbert NA, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87**: 206–210, 1978.
21. Moynagh PN, Bailey CJ, Boyce SJ and Williams DC, Immunological studies on the rat peripheral-type benzodiazepine acceptor. *Biochem J* **275**: 419–425, 1991.
22. Wilkinson GN, Statistical estimations in enzyme kinetics. *Biochem J* **80**: 324–332, 1961.
23. Xiao D and Levine L, Stimulation of arachidonic acid metabolism: differences in potencies of recombinant human interleukin-1α and interleukin-1β on two cell types. *Prostaglandins* **32**: 709–718, 1986.
24. Ferreira SH, Lorenzetti BB, Bristow AF and Poole S, Interleukin-1β as a potent hyperalgesic agent antagonised by a tripeptide analogue. *Nature* **334**: 698–700, 1988.
25. Hannum CH, Wilcox CJ, Arend WP, Joolin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP and Thompson RC, Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* **343**: 336–340, 1990.
26. Taylor DJ, Evanson JM and Woolley DE, Comparative effects of interleukin-1 and a phorbol ester on rheumatoid synovial cell fructose 2,6-bisphosphate content and prostaglandin E production. *Biochem Biophys Res Commun* **150**: 349–354, 1988.
27. Chedid M, Yoza BK, Brooks JW and Mizel SB, Activation of AP-1 by IL-1 and phorbol esters in T cells. *J Immunol* **147**: 867–873, 1991.
28. Bird TA and Saklatvala J, IL-1 and TNF transmodulate epidermal growth factor receptors by a protein kinase C-independent mechanism. *J Immunol* **142**: 126–133, 1989.
29. Stylianou E, O'Neill LAJ, Rawlinson L, Edbrooke MR, Woo P and Saklatvala J, Interleukin 1 induces NF-κB through its Type I but not its Type II receptor in lymphocytes. *J Biol Chem* **267**: 15836–15841, 1992.
30. Sprengel R, Werner P, Seeburg PH, Mukhin AG, Santi MR, Grayson DR, Guidotti A and Krueger KE, Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *J Biol Chem* **264**: 20415–20421, 1989.
31. McEnery MW, Snowman AM, Trifiletti RR and Snyder SH, Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci USA* **89**: 3170–3174, 1992.
32. Calkins JH, Sigel MM, Nankin HR and Lin R, Interleukin-1 inhibits Leydig cell steroidogenesis in primary culture. *Endocrinology* **123**: 1605–1610, 1988.
33. Lin T, Wang D, Nagpal ML, Calkins JH, Chang W and Chi R, Interleukin-1 inhibits cholesterol side-chain cleavage cytochrome P450 expression in primary cultures of Leydig cells. *Endocrinology* **129**: 1305–1311, 1991.
34. Krueger KE and Papadopoulos V, Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. *J Biol Chem* **265**: 15015–15022, 1990.
35. Cupps TR and Fauci AS, Glucocorticoid-mediated immunoregulation in man. *Immunol Rev* **65**: 133–155, 1982.
36. Majewska MD, Harrison NL, Schwartz RD, Barker JL and Paul SM, Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **232**: 1004–1007, 1986.
37. Majewska MD and Schwartz RD, Pregnenolone-sulfate: an endogenous antagonist of the γ-aminobutyric acid receptor complex in brain? *Brain Res* **404**: 355–360, 1987.
38. Baulieu E and Robel P, Neurosteroids: a new brain function. *J Steroid Biochem Mol Biol* **37**: 395–403, 1990.