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Peripheral benzodiazepine receptor modulation with phagocyte differentiation

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Abstract—Peripheral benzodiazepine receptor (PBR) was found to be less expressed in the immature phagocytic HL-60 and U-937 cell lines than in the more mature monocytic THP-1 cell line. Cell differentiation by several agents induced a strong enhancement of PBR density on these three phagocytic cell lines but not on the lymphocytic CEM line line. Detailed analysis of phorbol 12-myristate 13-acetate-treated THP-1 cells showed an increased PBR expression and the rise came along with an increase of CD11a and CD11b antigens and a secretion of macrophagic cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-8. Quantitation of mRNA using polymerase chain reaction (PCR)-based technique showed that overexpression of PBR did not parallel mRNA expression, indicating a gene-independent regulation. These results suggest that PBR predominance on phagocytic cells could be related to maturation process.

Benzodiazepines are drugs with a wide therapeutic use as minor tranquilizers, anticonvulsants, muscle relaxants and hypnotics. These major pharmacologic effects are mediated through the binding to the central receptor linked to the γ-aminobutyric acid (GABA*) anion channel receptor complex [1, 2]. Peripheral benzodiazepine receptors (PBRs) are distinct from the central receptors in their tissue distribution, pharmacological and protein characteristics [3–6]. The precise physiological function of PBR is still unclear despite many detailed biological effects.

Previous studies showed the presence of PBR with the typical pharmacologic profile in the various types of human blood cells. On the other hand the number of binding sites per cell was strongly different among the different cell subpopulations [7]. The rank order of PBR cell expression was: monocytes = polymorphonuclear neutrophils (PMN) > B, NK, T4 and T8-cells ≫ platelets > erythrocytes. Comparison between leukemic cell lines from T and B origin, and freshly isolated T and B lymphocytes showed a similarity of PBR density. By contrast, immature phagocytic cell lines as U-937 promonocytes expressed a much lower density than their mature counterpart suggesting a possible relationship between cell differentiation and PBR expression. This point was examined in this study.

HL-60, a human neutrophil promyelocytic cell line [8], U-937, a human promonocytic cell line [9] and THP-1, a human monocytic cell line [10] which are phagocytic cells differently committed in maturation process were used to study the modulation of PBR under the influence of various known differentiating agents such as dimethylsulfoxide (DMSO), γ-interferon (IFN-γ), phorbol 12-myristate 13-acetate (PMA), retinoic acid and vitamin D3. Maturation characteristics were monitored by analysing the expression of specific surface glycoproteins and the secretion of macrophagic cytokines together with quantitation of mRNA specific for PBR and cytokines.

Materials and Methods

Cell culture. Cell lines were grown in RPMI-1640 medium (Flow Laboratories, Irvine, U.K.) supplemented with 10% heat-inactivated fetal calf-serum (Seromed, Berlin, Germany). Based on published experiments for assessing the optimal concentrations, cells were stimulated for different periods of time either by PMA at 15 nM (Pharmacia, Uppsala, Sweden), DMSO at 1.5%, retinoic acid (RA) and 1.25-dihydroxy-vitamin D3 (VD3) at 100 nM (Sigma Chemical Co., St Quentin, France), IFN-γ at 500 U/mL (Genzyme, Cambridge, MA, U.S.A.). After each incubation period, cells were washed and viability was assessed by Trypan blue exclusion.

Cell purification. Monocytes and PMNs were purified from human venous blood as described previously [7].

Radioligand binding assays. Binding sites for [^{3}H]PK 11195 were characterized as described previously [7].

Cell surface antigens analysis. CD11a (LFA-1, αLchain, CD11b (MAC-1, αMchain), HLA-DR (MHC class II), CD64 (FcγRI), CD71 (transferrin receptor), CD23 (FcεRII), CD4 (T4) and CD14 (LPS receptor) antigens were labeled with monoclonal antibodies linked to fluorescein-isothiocyanate (Immunotech, Marseille, France). The cells were stained for 30 min at 4°, washed and fixed in a 1% paraformaldehyde solution, before analysis by flow cytometry.

Cytokine determination. Detection of interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α in cell culture supernatants was performed by ELISA (BIOTRAK-ELISA Systems, Amersham, U.K.). The lower limits of sensitivity of the different assays were 0.3, 0.35, 4.7 and 4.8 pg/mL, respectively.

mRNA quantitation. Quantitative measurement of levels of specific mRNA was performed by polymerase chain reaction (PCR) as described previously [7] using appropriate primers and oligonucleotide probes for PBR, IL-1β, IL-6, IL-8 and TNF-α.

Results and Discussion

First, we examined the presence of high affinity binding sites for [^{3}H]PK 11195 on studied cells by Scatchard analysis (Table 1). The number of receptors per cell was similar on immature phagocytic cells HL-60, U-937 compared to CEM T-cells, whereas on the more mature monocytic THP-1 cells the density was about four times higher and was very close to the density level found on monocytes and PMNs. The apparent difference of K_d values in human monocytes and PMNs reflected PBR variability in human blood cell subpopulations, as reported previously [7]. On all these cell lines, the binding sites presented the pharmacological characteristics of the PBR: the rank order of binding potency was PK 11195 > Ro 5-4864 > diazepam = flunitrazepam ≫ Clonazepam (data not shown).

Several agents are known to induce cell activation and differentiation leading to functional changes depending on both differentiating agent and cell origin [11, 12]. We showed here for the first time, that some of these

* Abbreviations: PBR, peripheral benzodiazepine receptor; GABA, γ-aminobutyric acid; PCR, polymerase chain reaction; PMN, polymorphonuclear neutrophils; DMSO, dimethyl sulfoxide; PMA, phorbol 12-myristate 13-acetate; RA, retinoic acid; VD3, 1.25-dihydroxy-vitamin D3; IFN-γ, γ-interferon; TNF-α, tumor necrosis factor-α; IL, interleukin.

Table 1. Characteristics of the cellular PBR

	Dissociation constant K_d (nM)	Maximal binding (sites/cell)	Hill coefficient
CEM cell line	5.7 ± 3.5	211,973 ± 111,580	0.98 ± 0.05
HL-60 cell line	3.3 ± 0.4	275,415 ± 18,300	0.94 ± 0.12
U-937 cell line	9.5 ± 5.8	203,239 ± 33,826	0.91 ± 0.08
THP-1 cell line	2.4 ± 0.7	842,509 ± 92,440	1.04 ± 0.12
Human monocytes	17.6 ± 2.3	755,932 ± 54,598	0.99 ± 0.02
Human PMNs	2.8 ± 1.5	710,956 ± 97,837	1.10 ± 0.02

Values represent the means ± SEM of three to six separate experiments run in triplicate.

Table 2. PBR modulation on stimulated cell lines

	Cell treatment					
	DMSO	IFN-γ		PMA		RA + VD3
	1.5%	50 U/mL	500 U/mL	1.5 nM	15 nM	100 nM
CEM	108 ± 10		109 ± 11		102 ± 11	72 ± 30
HL-60	212 ± 14		110 ± 5		148 ± 7	89 ± 8
U-937	86 ± 8	110 ± 2	115 ± 11	143 ± 25	145 ± 19	88 ± 11
THP-1	75 ± 5	92 ± 4	193 ± 13	165 ± 12	256 ± 35	86 ± 5

Each value represents the amount of PBR per cell obtained by Scatchard analysis and expressed as the percentage of the control.

Control experiments were carried out using the solvent alone and the corresponding values were not significantly different from those given in Table 1. The incubation time was 48 hr except for DMSO which was 168 hr.

compounds induced PBR modulation: DMSO treatment induced a two-fold increase of PBR density on HL-60 cells; IFN-γ at 500 U/mL enhanced PBR expression on THP-1 cells; PMA treatment modulated PBR on HL-60, U-937 and THP-1 cells. PMA at a dose as low as 1.5 nM was sufficient to increase strongly the receptor expression. Interestingly, PBR increase was higher on THP-1 (2.6-fold) than on U-937 and HL-60 (1.5-fold). In sharp contrast, RA and VD3 which differentiated U-937 [13] were ineffective at modulating PBR on any studied cell lines (Table 2). It should be noted that these four treatments did not affect the PBR affinity. On the other hand, on CEM T-cells the PBR expression failed to enhance (Table 2); a similar result was obtained on Raji-B-cells (data not shown) suggesting that the PBR modulation is associated with phagocytic cells only.

We further explored the PBR modulation on PMA-treated THP-1 cells since they showed the much pronounced effect and also because little is known about PMA activity on THP-1 cell differentiation. We examined the time course of surface antigen expression, cytokine production and mRNA expression along with PBR modulation on PMA-treated THP-1 cells. These kinetic studies are shown in Fig. 1. The time course for the change in PBR produced in response to stimulation by PMA, revealed an enhancement after 6–48 hr which then was sustained up to 72 hr in contrast to PMA-treated CEM cells and solvent-treated THP-1 cells (Fig. 1A). Concomitantly with this PBR up-regulation, only PMA-treated THP-1 cells acquired adherence and ceased to proliferate as assessed by cell numeration (data not shown). This reflected the transformation of malignant cells into differentiating cells. PMA stimulation markedly enhanced expression of CD11b and to a lesser extent CD11a antigen, which are known for mediating cell adhesion and cellular interactions (Fig. 1B). However, PMA failed to increase the expressions of CD14, CD64, CD71, CD23, CD4 and HLA-DR antigens expressed on activated cells on monocyte and macrophage sub-populations, suggesting an incomplete maturation process. A sequential induction of TNF-α, IL-1β and IL-8, respectively, could be observed upon PMA activation whereas IL-6 remained undetectable (Fig. 1C). The failure of PMA to generate a detectable amount of IL-6 was also reported with U-937 cells [14]. In contrast, IL-6 production has been described when THP-1 cells were treated by IFN-γ or VD3 [12]. For IL-1β and IL-6, the mRNA levels paralleled with the production of the respective proteins whereas for TNF-α, an already high level of transcripts was observed which decreased by 50% upon differentiation with PMA. In a previous study, we showed a correlation between mRNA levels and PBR densities on various human leukocyte sub-populations [7]. Similarly high PBR density on undifferentiated THP-1 cells was related to high mRNA expression when compared to U-937 (data not shown) strongly suggesting that PBR is regulated at the mRNA level. Unexpectedly, the steady-state level of PBR mRNA did not change in THP-1 cells treated with PMA and even slightly decreased suggesting that PMA-induced events did not affect gene transcription (Fig. 1D). For both PBR expression and TNF-α production, the PMA could act on an intermediate step between mRNA transduction and protein expression/secretion.

We showed here for the first time that an up-regulation of PBR occurred on phagocytic cells during treatment with

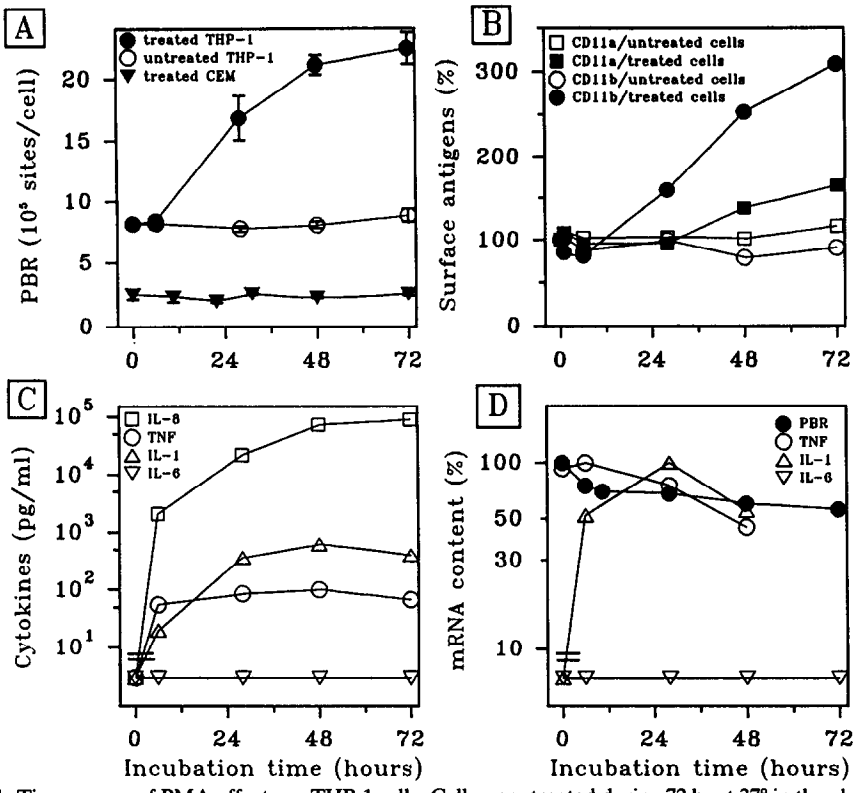

Fig. 1. Time course of PMA effects on THP-1 cells. Cells were treated during 72 hr at 37° in the absence or presence of 15 nM PMA and: (A) PBR number per cell was determined by Scatchard analysis. (B) THP-1 surface antigens CD11a, CD11b, HLA-DR, CD64, CD71, CD23, CD4 and CD14 were analysed by flow cytometry. Only CD11a and CD11b showed a significant increase. Their values are expressed as the percentage of fluorescence units at time 0 of the incubation. (C) Cytokine production was analysed. Values for untreated THP-1 cells (not shown) and values for IL-6 were under the detection limit. (D) Quantitation of TNF-α, IL-1β, IL-6 and PBR specific mRNA was realized from PMA-treated THP-1 cells. IL-8 mRNA values were similar to the IL-1β values (not shown) and IL-6 values were under the detection limit. Values are expressed as the percentage of the maximal mRNA content.

differentiating agents. The range of PBR modulations was shown to be dependent on both stimulating agents and phagocytic cell type, probably reflecting that very complex mechanisms are involved in the regulation of the differentiation pathways. However, using the PMA-treated THP-1 model we clearly demonstrated that overexpression of PBR was associated with an increase of other markers of differentiation such as the adhesion molecule CD11 and the production of macrophagic cytokines. This result confirms that the phagocytic lineage could be a key component in the benzodiazepine-mediated immunomodulatory effects involving leukocyte proliferation, cytokine production [15], monocyte chemotaxis [16] and phagocyte-dependent host defense mechanisms [17].

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