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Effect of 1-(2-Chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline Carboxamide (PK11195), a Specific Ligand of the Peripheral Benzodiazepine Receptor, on the Lipid Fluidity of Mitochondria in Human Glioma Cells

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ABSTRACT. When human glioma cells were incubated for 24 hr in serum-free medium with nanomolar concentrations of 1-(2-chlorophenyl)-*N*-methyl-*N*(1-methylpropyl)-3-isoquinoline carboxamide (PK11195), a specific ligand of the peripheral benzodiazepine receptor (PBR), a significant increase in the membrane fluidity of mitochondria isolated from these cells was registered. These effects were not observed with a shorter incubation time (2 hr) of the cells with PK11195 nor in the presence of serum. Other significant associated changes were observed: a significant increase of 16 ± 4% of [^{3}H]thymidine incorporation into DNA was detected in cells in the presence of PK11195 in serum-free medium, and an increase of 33 ± 5% as compared to controls in nonyl acridine orange uptake, as indicator of mitochondrial mass, was also registered in cells treated with 10 nM PK11195. [^{3}H]PK11195 binding was decreased in cells incubated with PK11195; a 45% decrease compared to controls was obtained. In view of the effect of PBR ligands on DNA synthesis, changes in mitochondrial lipid metabolism through interaction with PBRs might lead to biogenesis of mitochondria to support the increased metabolic requirements for cell division, which is even higher in malignant cells. BIOCHEM PHARMACOL **58**;4:715–721, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. peripheral benzodiazepine receptors; human glioma cells; mitochondria; lipid fluidity; serum deprivation; cell proliferation

PBRs† are present in numerous peripheral tissues [1, 2] as well as in the CNS [3]. PBRs have been chiefly localized to the mitochondrial outer membrane [4] where they bind some benzodiazepines (diazepam, Ro5-4864 [7-choro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2-*H*-1,4-benzodiazepin-2-one]) [5], isoquinoline carboxamides (PK11195 [1-(2-chlorophenyl)-*N*-methyl-*N*(1-methylpropyl)-3-isoquinoline carboxamide]) [6], and putative endogenous ligands (porphyrins and diazepam-binding inhibitor) with nanomolar affinities [7]. However, non-mitochondrial sites have been described [8]. McEnery *et al.* [9] described the purification of PBR in close association with the ADP–ATP translocator and the voltage-dependent anion channel (VDAC) and suggested contact site location of the receptors, which may be important for their function. Hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1), which initiates glycolysis, is bound to the outer mitochondrial membrane at the level of VDAC, preferentially at these contact sites [10]. When bound to mitochondria, hexokinase plays a critical role in the regulation of energy metabolism of gliomas [11, 12].

Numerous different roles have been proposed for ligands of the PBR (for review, see [13]), including inhibition of respiration [14] and stimulation of the steroidogenesis [13]. However, the biological role of PBRs is not yet understood. Micromolar concentrations of peripheral benzodiazepines were needed to affect the proliferation rate of various cells in a dose-dependent manner [15, 16], while nanomolar concentrations of high-affinity ligands play a stimulatory role in cell proliferation [17]. Increased PBR expression in tumours has been reported [18], but their role in neoplastic cells remains unclear. Increased density of PBRs in glioblastomas was found to be correlated with malignancy and glucose utilization [19, 20]. The localization of the benzodiazepine receptor to mitochondria has led to speculation that PBRs take part in the regulation of mitochondrial

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† *Abbreviations:* NAO, 10-*N* nonyl acridine orange bromide; PBR, peripheral benzodiazepine receptor; DPH, 1,6-diphenyl-1,3,5-hexatriene; and TMA-DPH, (1-(4-trimethylammonium-phenyl)-6-phenyl~-1,3,5-hexatriene).

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function. Some reports suggest that peripheral benzodiazepines act as modulators of mitochondrial function, causing inhibition of mitochondrial respiratory control [14]. Shiraishi *et al.* [21] reported morphological changes in mitochondria from glioma cells exposed to nanomolar concentrations of PK11195 or Ro5-4864. Hence, stimulation of proliferation of mitochondria by these ligands was associated with increased γ-DNA polymerase activity, which is specific to mitochondrial DNA replication [22].

Biomembranes are dynamic structures, mainly consisting of lipids and proteins, which are constantly in motion and form the basis of membrane fluidity. Lipid organization is involved in membrane functions, signal transduction, and cellular processes which can be altered by fluidity changes. In view of the effect of PBR ligands on cell proliferation [17], these ligands might increase the number of mitochondria so as to support the increased requirements for cell division in highly proliferating cells such as malignant cells. The stimulation by PBR ligands of phospholipid metabolism [23] also raises the possibility that PBRs might influence lipid metabolism [24], but the effects of these ligands on mitochondrial membrane fluidity have received little attention until now. Steady-state fluorescence anisotropy has been used here to monitor the motion of fluorophores in membranes, allowing the evaluation of the structural changes in plasma and mitochondrial membranes. The fluorescence polarization of DPH and TMA-DPH, which probe different regions of the phospholipid bilayer, was used to investigate lipid fluidity. The function of PBRs and the effects of PBR ligands on plasma and mitochondrial membrane fluidity were investigated in relation to [^{3}H]PK11195 binding, DNA synthesis, cell cycle, and mitochondrial mass in human glioma cells.

MATERIALS AND METHODS

Chemicals

Ro5-4864 and PK11195 were obtained from RBI; diazepam from Roche; [^{3}H]PK11195 (85 Ci/mmol) and [^{3}H]thymidine (20 Ci/mmol) from Dupont/NEN; NAO and TMA-DPH from Molecular Probes; DPH from Aldrich; clonazepam, Dulbecco's modified Eagle's medium, L-glutamine, PBS (150 mM NaCl, 150 mM sodium phosphate, pH 7.4), trypsin–EDTA, bromodeoxyuridine (BrdU), propidium iodide, and penicillin–streptomycin from Sigma; fetal bovine serum from Dutscher; rat anti-BrdU antibody from Seralab (Sigma); fluorescein isothiocyanate-conjugated goat anti-rat antibody from Cliniscience.

Cell Culture and Treatment

The cell culture conditions of the human glioblastoma cell line SNB-19 were as described previously [12]. The medium of attached cells was replaced by fresh serum-free medium with or without PK11195, diazepam, clonazepam, or vehicles for 24 hr.

[^{3}H]Thymidine Uptake Assay

[^{3}H]thymidine uptake assay was performed as described [25].

Cell Cycle Determination

The assessment of the cell cycle was performed as described previously [25]. Data were analyzed automatically by a LYSIS II program on 10^4 cells. The percentage of cells present in areas corresponding to each phase of the cell cycle was evaluated.

Mitochondrial Membrane Extraction

Attached cells ($40–50 \times 10^6$) were washed twice with PBS, trypsinized, and homogenized in 20 vol. of ice-cold buffer (320 mM sucrose, 1 mM K^+EDTA, 10 mM Tris–HCl, pH 7.4) by seventy strokes of a tight-fitting glass pestle in a Potter Elvehjem glass homogenizer. The resulting homogenate was centrifuged at 1500 *g* for 5 min. The pellet containing cell debris and nuclei was discarded and the supernatant was centrifuged at 12,000 *g* for 10 min. The pellet was resuspended in 20 vol. of buffer, and the washing was repeated once more. The resulting pellet was centrifuged at 30,000 *g* for 20 min. Protein concentration was estimated by the method of Lowry *et al.* [26] using BSA as standard.

Labeling with Fluorescent Probes and Fluorescence Instrumentation

Fluorescence anisotropy (⟨r⟩), involving measurement of the polarized emissions of a fluorophore in the membrane, can provide a detailed description of the rotational motion of a fluorophore and, thus be used to characterize the microviscosity of the environment surrounding the fluorophore [27]. DPH provides information on the hydrophobic areas, enters the cells and labels both the plasma membranes and the intracytoplasmic compartments [28], and constitutes a probe of the membrane core [29]. The positive charge of TMA-DPH provides a plasma membrane surface anchor, thereby improving its specific localization in the phospholipid headgroup region. For plasma membrane fluidity determination, intact SNB-19 cells (3×10^6) were incubated with DPH and TMA-DPH at a final concentration of 1 μM. For mitochondrial membrane fluidity determination, mitochondrial proteins (300 μg) were labeled with DPH and TMA-DPH at a final concentration of 0.75 μM. DPH- and TMA-DPH-labeled suspensions were incubated in the dark at 25° for 30 and 10 min, respectively. Steady-state fluorescence anisotropy (⟨r⟩) and fluorescence intensity were measured as described [28, 30]. Membrane fluidity was expressed as ⟨r⟩, which is inversely related to fluidity. The steady-state estimations provide a weighted average of membrane microenvironments. Fluorescence

TABLE 1. Fluorescence anisotropy ⟨r⟩ of SNB-19 intact cells and of mitochondria isolated from cells incubated with PBR ligands

	Fluorescence anisotrophy ⟨r⟩			
	Mitochondria		Intact cells	
Ligands (nM)	DPH	TMA-DPH	DPH	TMA-DPH
Control	0.212 ± 0.0023	0.261 ± 0.0020	0.212 ± 0.0022	0.278 ± 0.0018
PK11195 (10)	0.201 ± 0.0018*	0.247 ± 0.0013*	0.207 ± 0.0010	0.277 ± 0.0021
PK11195 (50)	0.196 ± 0.0015*	0.244 ± 0.0016*	ND	ND
Diazepam (10)	0.208 ± 0.0025	0.255 ± 0.0021	0.209 ± 0.0019	0.279 ± 0.0019
Diazepam (50)	0.209 ± 0.0022	0.253 ± 0.0024	ND	ND
Clonazepam (10)	0.214 ± 0.0018	0.259 ± 0.0014	0.212 ± 0.0017	0.277 ± 0.0014
Clonazepam (50)	0.217 ± 0.0020	0.258 ± 0.0018	ND	ND

Cells in logarithmic phase of growth were continuously incubated with 10 or 50 nM PK11195, diazepam, or clonazepam for 24 hr in serum-free medium. Mitochondria were extracted by differential centrifugation from 40–50 × 10^6 cells. Mitochondria (300 μg protein) and cells (3 × 10^6 cells) were then stained with DPH or TMA-DPH and assayed for fluorescence polarization as described in Materials and Methods. A decrease in ⟨r⟩ reflects an increase in membrane fluidity. Data are means ± SEM values of 4 (for mitochondria) and 3 experiments (for intact cells) performed in triplicate.

*$P < 0.01$ compared to control with the use of Student's *t*-test; ND, not determined.

lifetimes were determined with an Aminco-SLM 48000-S apparatus (SLM Instruments) as described [30].

Binding of [^{3}H]PK11195

Binding assays were performed on attached cell cultures as previously described [31]. Binding studies were carried out using 1 nM [^{3}H]PK11195 in the presence or absence of unlabeled PK11195 (10 μM). Saturation experiments were carried out using 0.5 to 20 nM [^{3}H]PK11195.

Subcellular Distribution of Hexokinase

Determination of the subcellular distribution of hexokinase activity was performed in the same mitochondrial and cytosolic fractions previously isolated as described [11, 32].

Mitochondrial Mass Determination

Cells were trypsinized, fixed with 4% paraformaldehyde, and 5 × 10^5 cells were labeled in 1 mL PBS with 100 nM NAO for 15 min at room temperature in the dark. The medium was discarded and the washed cells were suspended in 500 μL of PBS. Dye uptake was measured by flow cytometry [33]. Data were analyzed by a LYSIS II program on 10^4 cells. The percentage of positive cells in PK11195-treated cells compared to control (increased NAO fluorescence intensity) was evaluated using the Kolmogorov–Smirnov statistics.

Statistical Analysis

Values are the means ± SD or SEM. Student's *t*-test was used to compare the results of fluidity and DNA synthesis in the controls and in the presence of PBR ligands.

RESULTS

⟨r⟩ decreased significantly compared to control values ($P < 0.01$) in both DPH- and TMA-DPH-labeled mitochondria extracted from PK11195-treated cells (Table 1); at 10 nM PK11195, decreases in ⟨r⟩ compared to controls of 0.011 and 0.014 were recorded in DPH- and in TMA-DPH-labeled mitochondria, respectively, indicating a more fluid microenvironment in the regions surrounding the fluorescent probes. When cells were treated with 50 nM PK11195, a slight increase in fluidity compared to the 10 nM concentration was recorded. In biological membranes, the range of ⟨r⟩ values is limited and is generally 0.2–0.4. Diazepam, a benzodiazepine ligand with high affinity for both central and peripheral benzodiazepine receptors, was also used to treat cells; a decrease in ⟨r⟩ compared to control values (≤ 0.06; not significant) was also observed, but to a lesser extent than with PK11195 ligand. Clonazepam, a central benzodiazepine ligand, had no effect on ⟨r⟩. These changes, observed after 24 hr of treatment, were neither found after 2 hr of cell treatment with PK11195 or diazepam nor in the presence of 10% fetal bovine serum (data not shown). Fluorescence lifetimes of the excited state of DPH and TMA-DPH in mitochondria extracted from treated cells were unaffected (data not shown), thus excluding the possibility that alterations in fluidity may have been due to altered excited state lifetime. As a control experiment, no change in ⟨r⟩ was noted in intact human glioma cells exposed for 24 hr to different ligands in serum-free medium (Table 1).

The specific binding of [^{3}H]PK11195 was saturable, whereas non-specific binding increased linearly with increasing concentrations of the labeled ligand (10–20% of the total binding). In a typical experiment performed in the presence of serum-containing, serum-free, and PK11195-containing serum-free medium (Fig. 1), Scatchard analysis of the specific binding showed a single class of sites with a K_d of 10.7, 11.8, and 12.5 nM, respectively, and a B_{max} (maximal amount of ligand bound) of 150, 425, and 225 fmol/10^6 cells, respectively, which corresponds to 1.2, 3.4, and 1.8 pmol/mg protein. The K_d remained unchanged in the presence or absence of serum or after treatment. The [^{3}H]PK11195 binding was performed at 4° or 37° and similar results were obtained. Cells treated for 24 hr with 10

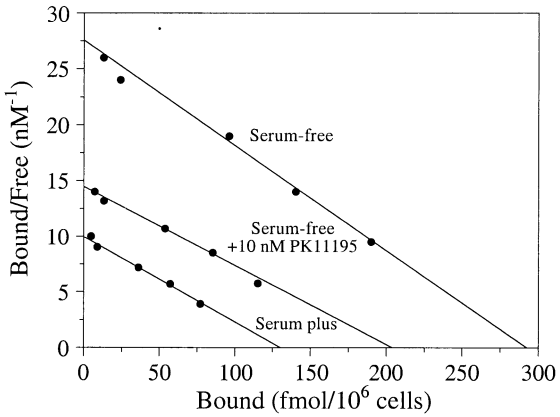

FIG. 1. Scatchard plots and effect of PK11195 and serum deprivation on [^{3}H]PK11195 binding to intact SNB-19 glioma cells. Binding assays were performed as described under Materials and Methods. A typical experiment performed in serum-containing, serum-free, and PK11195-containing serum-free medium is shown. Scatchard plot of the specific binding data was determined using a least-square fit to determine the regression line.

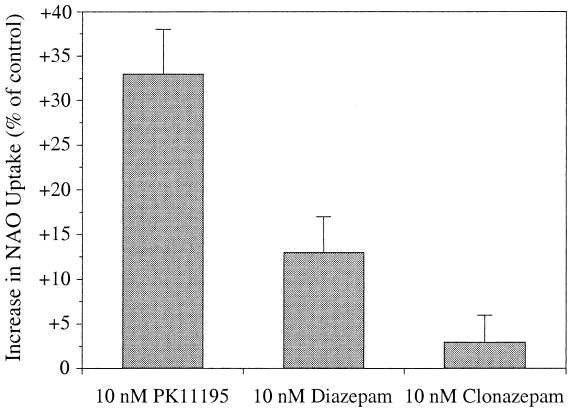

FIG. 2. Effect on NAO uptake of SNB-19 glioma cells exposed to benzodiazepine ligands. Glioma cells in the logarithmic phase of growth were incubated with 10 nM PK11195, diazepam, or clonazepam for 24 hr without serum. After the incubation period, glioma cells were fixed and 5 × 10^5 cells were stained with 100 nM NAO for 15 min and subjected to flow cytometry. The results are expressed as the percentage increase in NAO uptake as compared to control cells (the control being 0%) and data are means ± SD (bars) values (N = 6) of 2 experiments.

and 50 nM PK11195 in serum-free medium showed a 1.8- and 2-fold decrease, respectively, in [^{3}H]PK11195 binding compared to untreated cells in serum-free medium, while a 2.8-fold decrease in [^{3}H]PK11195 binding was obtained in cells cultured in serum-containing medium compared to cells cultured without serum (Fig. 1). Under conditions with serum, PK11195 treatment did not affect [^{3}H]PK11195 binding (data not shown).

The fluorescent dye NAO binds selectively to cardiolipin, a molecule that is selectively localized in the inner mitochondrial membrane in an energy-independent manner. NAO uptake correlates with both the number and size of mitochondria [34]. An increase in NAO uptake of 33 ± 5% (corresponding to 46% of positive cells) and 13 ± 4% compared to controls was registered in cells treated for 24 hr with PK11195 and diazepam, respectively, while clonazepam had no significant effect (Fig. 2). The potential effect of 10 and 50 nM PBR ligands on the activity and distribution of hexokinase was investigated in the same samples. The total specific activity of hexokinase and its distribution between cytosol and mitochondria were unaffected (data not shown).

As shown in Fig. 3, an increase of 16 ± 4% (21,500 ± 2,300 cpm in control and 25,900 ± 3,200 cpm in PK11195-treated cells, N = 20; $P < 0.02$) and 7 ± 3% of [^{3}H]thymidine incorporation into DNA was found in cells treated for 24 hr in serum-free medium by PK11195 and diazepam, respectively. A 60% decrease in [^{3}H]thymidine uptake was achieved in cells cultured in serum-free medium compared to 10% serum-containing medium (data not shown). An accumulation of cells in G_0-G_1 phase (89% vs 65% for control cells) with a decrease of 18% of the cells in S phase and a stable G_2 + M phase (10–14%) was observed in serum-free medium compared to cells in serum-containing medium. The cycle of the cells incubated with 10 nM PK11195 for 24 hr in serum-free medium remained unchanged compared to serum-free medium.

DISCUSSION

Relationships between the composition of biological membranes, their structural state (fluidity) and their function are well established. Several important functional properties, such as the activity of membrane proteins (enzymes, transport proteins or receptors), membrane fusion, and

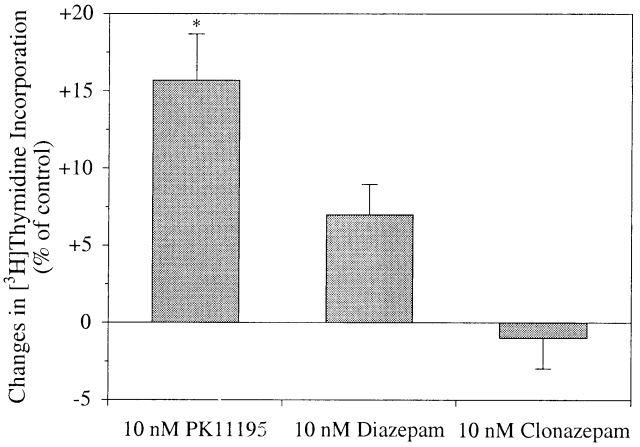

FIG. 3. Effect on [^{3}H]thymidine uptake into SNB-19 glioma cells by serum deprivation and PBR ligands. Glioma cells in the logarithmic phase of growth were incubated with 10 nM PK11195, diazepam, or clonazepam for 24 hr without serum. [^{3}H]thymidine uptake was performed for 4 hr as described in Materials and Methods. The results are expressed as the percentage increase in [^{3}H]thymidine uptake as compared to control wells (the control being 0%) and are means ± SD values (N = 20) of 3 experiments. Significance was determined to be *$P < 0.02$ compared to control with the Student's *t*-test.

changes in shape depend on membrane fluidity. Variations in membrane fluidity may induce changes in the structure of some membrane proteins that could lead to alteration of their function [35]. The early finding that Ro5-4864 stimulates phospholipid methylation in rat C6 glioma cells [23] suggests that PBRs may be functionally related to mitochondrial lipid metabolism. Recently, it has been shown that the cell cholesterol/lipid distribution was altered in Leydig's cells treated with the human choriogonadotropin. A fraction of the membrane lipids released was transported to the inner mitochondrial inner membrane probably through the PBRs [36]. These observations prompted us to investigate changes in mitochondrial lipid metabolism by PBR ligands in human glioma cells. For this purpose, SNB-19 glioma cells were grown in serum-free medium to minimize the interference in lipid metabolism and cell proliferation by the serum lipoproteins, steroids, and growth factors. Taken together, the data presented here suggest a close relationship between PBR ligands through interaction with their receptor and mitochondrial lipid metabolism via fluidity changes. An increase in mitochondrial membrane fluidity in the presence of nanomolar concentrations of PK11195 was observed both in the polar and hydrophobic areas (probed by TMA-DPH and DPH, respectively), indicating a general fluidization of the mitochondrial membranes. This mitochondrial fluidization seemed to be accomplished through a slow and durable regulation (24 hr), since it was not detected after a 2-hr treatment with 10 or 50 nM PK11195 (data not shown).

Benzodiazepines (diazepam and Ro5-4864) and isoquinoline carboxamides (PK11195) have different binding properties. PK11195 completely displaces benzodiazepines and vice versa, and consistently shows high affinities for PBRs across many species, whereas the affinity for Ro5-4864 varies widely. In human, PBRs have a greater affinity for PK11195 than for benzodiazepines [13]. When binding experiments were performed with tritiated Ro5-4864 in the SNB-19 glioblastoma cell line, which is of human origin, the non-specific binding represented more than 50% of the total binding; therefore, Scatchard analysis and the effects of these drugs on lipid fluidity and in other experiments were not completed. Furthermore, the ability of PBRs to bind these two classes of ligands differs with various physico-chemical perturbations, such as an increased temperature sensitivity of benzodiazepine binding [6]. In our study, the incubation of tumour cells with PBR ligands was performed at 37°, leading to a less efficient binding to benzodiazepines. The partial binding efficacy of diazepam correlates with its lower affinity compared to PK11195 for PBRs [13], and its temperature sensitivity could be in part responsible for the reduced effects on mitochondrial membrane fluidity. Mitochondrial biogenesis takes place when cells begin to proliferate and enter the cell cycle [37]. In the absence of serum, ~ 90% of SNB-19 cells are resting (G_0–G_1 phase). A comparison can be drawn with lymphoblasts, which contain significantly more total inner mitochondrial membrane surface and a higher number of mitochondria compared to resting lymphocytes. Mitogenic stimulation of resting cells requires additional genesis of mitochondria during the transition from G_0 to G_1 [37].

Several laboratories have studied the effects of nanomolar concentrations of PBR ligands on mitochondrial physiology. PBR ligands alter mitochondrial respiration [14], increase mitochondria number, and modify their morphology and distribution [21, 22]. As observed by us, the mitochondrial mass was increased by PK11195 more efficiently than by diazepam. The [^{3}H]PK11195-binding parameters measured in intact SNB-19 cells were in accordance with other studies [38]. In contrast to other authors [17, 39], we show that [^{3}H]PK11195-binding sites in cells treated with 10 and 50 nM PK11195 for 24 hr in the absence of serum were down-regulated compared to controls, despite an increase in the mitochondrial mass. The possibility that the unlabeled PK11195 that was used to treat the cells could be responsible for the decrease in binding observed after 24 hr in serum-free medium was ruled out, since in the presence of serum no decrease in binding was observed in cells treated with unlabeled PK11195 compared to untreated cells. Furthermore, [^{3}H]PK11195 binding was increased in cells cultured without serum compared to the same cells cultivated in the presence of serum. Beaumont *et al.* [24] reported in detail on the inhibition of PBR binding by specific lipids. Inhibitory potencies of fatty acids on PBR ligand binding were strongly related to structures that tend to increase membrane fluidity. Shinitsky [40] showed that modifications of the lipid bilayer led to fluidity changes and consequently modified the accessibility of some receptors to their respective ligands. The early finding that Ro5-4864 stimulates phospholipid methylation in rat C6 glioma cells [23] suggests that PBRs may be functionally related to mitochondrial lipid metabolism. Woods and Williams [41] have proposed that either PBRs play a role in the synthesis of phospholipids in some tissues or alternatively that phospholipids regulate receptor function. The down-regulation of [^{3}H]PK11195-binding sites after binding suggests that the effects of receptor occupation are indirect and may involve as yet undefined signal transduction mechanisms.

The increase in cell growth or DNA synthesis in serum-free medium (fetal bovine serum contains a variety of growth factors) observed by us and others in various cells [17, 42, 43] in the presence of nanomolar concentrations of PBR ligands supports the hypothesis that PBRs recognize a premitogenic signal which subsequently leads to the initiation of cell growth. The increase in DNA synthesis found here in glioma cells in serum-free medium confirmed this hypothesis. Taken together, these results suggest that PK11195 could act through interaction with PBRs as a premitogenic signal to increase the proliferation of mitochondria in serum-deprived cells in the absence of growth factor stimulation. The relationship between PBR ligands, mitochondrial fluidity, and growth-associated alterations suggest that PBR ligands regulate some physiological functions of the mitochondria in response to various stimuli

(growth factors, hormones, energy status, oxygen, and ions) through changes in the structure and physical properties of the membranes. Membrane-structural alterations are involved in the mediation of the physiological effects of hormones [44], and, as stated by Halestrap *et al.* [45], changes in lipid fluidity of mitochondria may be a significant switch for long-term regulation such as hormonal regulation of oxidative metabolism. Very recently, Yeliseev *et al.* [46] found that pk 18 (an M_r 18,000 isoquinoline-binding subunit) functions as a bacterial oxygen sensor, able to complement a mutant lacking the tryptophan-rich sensory protein from the photosynthetic bacterium *Rhodobacter sphaeroides*. This finding may explain the diversity of activities attributed to the PBRs. Depending on the tissue, the PBRs would transmit a regulatory signal to different downstream responsive genes, leading to a tissue-specific response.

The slow rate in the change in mitochondrial fluidity (detected at 24 hr but not at 2 hr) could mean that the cell initiates a complex multistep process including synthesis and/or degradation of various membrane proteins or lipids, possibly involving altered gene expression, to give the membrane different physical properties as a long-term regulatory event. The fluidization effect observed in mitochondria treated with PK11195 could be an outcome of changes in phospholipid or fatty acid content. Further studies are needed to analyze the lipid composition of mitochondria and identify which membrane is preferentially affected following PBR ligand treatment. An analysis of functional consequences of the induced-lipid fluidity changes on the activity of integrated membrane proteins (transporters or enzymes) is also needed [47]. In view of the effect of PBR ligands on the initiation of DNA synthesis in resting cells, changes in the lipid metabolism through interaction with PBRs leading to mitochondrial membrane fluidization might increase the number of mitochondria in order to support the greater metabolic requirements for cell division.

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