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Enigma of the Peripheral Benzodiazepine Receptor

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I. Introduction

Benzodiazepines (BZs)[2] are used clinically as muscle relaxants, anticonvulsants, anxiolytics, and sedative-hypnotics. These effects are mediated primarily via the central BZ receptors (CBRs) located in the central nervous system (CNS; Braestrup and Squires, 1977; Möhler and Okada, 1977; Tallman et al., 1980). CBRs are part of a macromolecular complex that also contains a γ-aminobutyric acid (GABA) receptor site and a chloride ion channel (DeLorey and Olsen, 1992). This complex has been purified, its cDNA has been cloned, and its functional receptors have been expressed in *Xenopus* oocytes (Schofield et al., 1987). The GABA/BZ receptor complex is a hetero-oligomer composed of five subunits: α-, β-, γ-, δ-, and ρ-polypeptides. BZs bind to the α-subunit and facilitate the inhibitory effect obtained by GABA (DeLorey and Olsen, 1992).

BZs also bind to other receptors, located mainly in peripheral tissues and glial cells in the brain (Fig. 1), called peripheral BZ receptors (PBRs; Verma and Snyder, 1989; Gavish et al., 1992). In rats, PBRs differ from CBRs in their drug specificity: for example, the BZ clonazepam binds to CBRs with high affinity, whereas the BZ Ro 5-4864 (4′-chlorodiazepam) as well as the non-BZ ligand PK 11195 (an isoquinoline carboxamide derivative) bind to CBRs with negligible affinity. The reverse is true with regard to PBRs (Benavides et al., 1983a,b). Imidazopyridines such as alpidem bind with high affinity to both CBRs and PBRs (Langer and Arbilla, 1988). FGIN-1 (2-aryl-3-indoleacetamide) binds with high affinity to PBRs but not to CBRs (Romeo et al., 1992). The focus of the current review is the PBR.

The binding of PK 11195 and Ro 5-4864 has been studied in various species (Anholt et al., 1985b; Basile et al., 1986; Cymerman et al., 1986; Awad and Gavish, 1987, 1991; Parola et al., 1991). The binding of Ro 5-4864 has been found to differ among species (Awad and Gavish, 1987; Table 1). The order of potency of Ro 5-4864 in

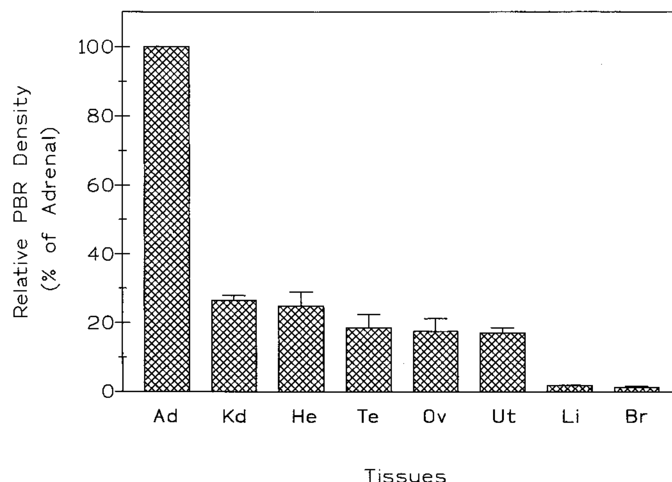

FIG 1. Relative tissue distribution of membranal [^{3}H]PK 11195 maximal binding capacity in the adult rat. Eight adult rat tissues were compared and ranked against the adrenal gland PBR density. Average values per tissue were each calculated from at least three published values, and variability was calculated as the S.E.M. (Awad and Gavish, 1987; Gavish et al., 1987; Amiri et al., 1991; Mercer et al., 1992; Weizman et al., 1992, 1997a,b; Burgin et al., 1996; Kurumaji and Toru, 1996). Ad, adrenal; Kd, kidney; He, heart; Te, testis; Ov, Ovary; Ut, uterus; Li, liver; Br, brain.

displacement of PK 11195 binding is rat = guinea pig > cat = dog > rabbit > calf. In calf, Ro 5-4864 is three orders of magnitude less potent than PK 11195 in binding to membranal PBRs (Basile et al., 1986; Awad and Gavish, 1987; Parola et al., 1991). In humans, it was found that PK 11195 is about two orders of magnitude more potent than Ro 5-4864 in binding to PBRs (Awad and Gavish, 1991). PBR species differences obtained in the membrane-bound state are retained in the soluble state and after purification and probably are attributable to variations in the molecular structure of PBRs rather than to differences in the membrane environment (Awad and Gavish, 1989b; Sprengel et al., 1989; Parola et al., 1991; Riond et al., 1991).

Protoporphyrin IX labels PBRs with nanomolar affinity and has been suggested to be an endogenous ligand for PBRs (Snyder et al., 1987). Subcellular studies have indicated that PBRs are mitochondrial in the rat brain (Basile and Skolnick, 1986), in the rat adrenal (Anholt et al., 1986b), and in the rat testis, lung, kidney, heart, skeletal muscle, and liver (Antkiewicz-Michaluk et al., 1988a). The use of confocal microscopy further confirmed the abundant mitochondrial localization of PBRs (Garnier et al., 1993). Other groups detected PBR binding in nuclei (O'Beirne et al., 1990; Hardwick et al., 1999), Golgi apparatus, lysosomes, peroxisomes (O'Beirne et al., 1990), and plasma membrane (Oke et al., 1992). It has also been reported that

[2] Abbreviations: BZ, benzodiazepine; ACTH, corticotropin; CBR, central benzodiazepine receptor; CNS, central nervous system; CRH, corticotropin-releasing hormone; DBI, diazepam-binding inhibitor; DES, diethylstilbestrol; GABA, γ-aminobutyric acid; GAD, generalized anxiety disorder; GSP, generalized social phobia; HPA, hypothalamic-pituitary-adrenal; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; NMDA, *N*-methyl-D-aspartate; OCD; obsessive-compulsive disorder; ODN, octadecaneuropeptide; P-450$_{scc}$, side-chain cleavage cytochrome P-450; PBR, peripheral benzodiazepine receptor; PD, panic disorder; PMSG, pregnant mare serum gonadotropin; PTSD, post-traumatic stress disorder; StAR, steroidogenic acute regulatory protein; TNF, tumor necrosis factor; VDAC, voltage-dependent anion channel.

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TABLE 1
Percentage of homology in 18-kDa PBR subunit gene and affinity of PK 11195 and Ro 5-4864 in various species

	% Homology to Human PBR	Reference	K_i PK 11195	K_i Ro 5-4864	Reference
			nM	*nM*	
Human kidney	100	Riond et al., 1991	4	400	Awad and Gavish, 1991
Bovine kidney	85	Parola et al., 1991	2	4,800	Awad and Gavish, 1987
Mouse kidney	82	Garnier et al., 1994b	1[a]	1[a]	Park et al., 1997
Rat kidney	77	Sprengel et al., 1989	2	12	Awad and Gavish, 1987
E. coli	55	Weisinger et al., unpublished data	>10,000	ND	Weisinger et al., unpublished data

ND, not determined.
[a] K_d values.

PBRs are present in mature human red blood cells, which lack mitochondria (Olson et al., 1988a). Titration of isolated rat adrenal mitochondria with digitonin demonstrated that PBRs are typically located on the outer membrane of the mitochondria (Anholt et al., 1986b). Another study showed that PBRs are also located on the inner membrane of the rat lung mitochondria (Mukherjee and Das, 1989). Hence, although PBRs are located mainly on the outer membrane of the mitochondria, other localizations are also possible.

Purification of PBRs contributed to an understanding of their function. The first step for purification requires a suitable detergent that will not destroy the binding activity. PBRs have been solubilized, using various detergents, without major impairment of binding activity (Martini et al., 1983; Benavides et al., 1985; Doble et al., 1985; Gavish and Fares, 1985; Anholt et al., 1986a; Awad and Gavish, 1989a). Antkiewicz-Michaluk et al. (1988b) purified one subunit (of 18-kDa molecular mass) of the PBR from the rat adrenal through utilization of photoaffinity labeling of this protein subunit with the isoquinoline carboxamide derivative [^{3}H]PK 14105 and subsequent solubilization of the membranes with the detergent digitonin, followed by purification with ion-exchange chromatography and reverse-phase HPLC.

PBRs are composed of at least three subunits: the binding site for isoquinolines, with a molecular mass of 18 kDa; the voltage-dependent anion channel (VDAC), with a molecular mass of 32 kDa, which binds BZs; and the adenine nucleotide carrier, with a molecular mass of 30 kDa, which also binds BZs (McEnery et al., 1992). Although isoquinolines can bind to the 18-kDa subunit alone, PBR-specific BZs require the interaction of all three subunits for binding (Garnier et al., 1994b). This complex is located on the outer and inner mitochondrial membrane contact sites (Papadopoulos et al., 1994).

The full-length cDNA for the 18-kDa subunit has been cloned from rat (Sprengel et al., 1989), humans (Riond et al., 1991), bovine (Parola et al., 1991), and mouse (Garnier et al., 1994b). Many functions have been attributed to PBRs, including a role in cell proliferation (Wang et al., 1984; Carmel et al., 1999), steroidogenesis (Papadopoulos et al., 1990; Papadopoulos, 1993; Kelly-Hershkovitz et al., 1998), calcium flow (Cantor et al., 1984; Python et al., 1993), cellular respiration (Hirsch et al., 1989), cellular immunity (Lenfant et al., 1986), and malignancy (Starosta-Rubinstein et al., 1987; Katz et al., 1990b,c; Alenfall and Batra, 1995).

PBRs are found in many tissues. In the current review, we survey the available data on the location, molecular characteristics, functions, and clinical implications of PBRs in these various tissues.

II. The Peripheral Benzodiazepine Receptor: Molecular Identity

The PBRs were first described (more than 20 years ago) as BZ binding sites in non-neuronal tissue (Braestrup and Squires, 1977). Nevertheless, PBRs are found not only in peripheral tissue but also in non-neuronal brain tissue (Fig. 1; Verma and Snyder, 1989; Gavish et al., 1992). Interestingly, PBR densities are high in steroidogenic tissues, in particular in the adrenal gland (Fig. 1). PBR densities in tissues such as the kidney, heart, testis, ovary, and uterus are approximately five times as low as that in the adrenal but are still one order of magnitude higher than in other tissues such as the brain (Fig. 1).

The PBR appears to be a heteromeric complex of at least three different subunits, including an isoquinoline binding subunit (18 kDa), a VDAC (32 kDa), and an adenine nucleotide carrier (30 kDa; Snyder et al., 1990; McEnery et al., 1992; Garnier et al., 1994b). Papadopoulos et al. (1997a) reported that isoquinolines that bind specifically to PBRs interact specifically with the 18-kDa subunit, whereas PBR-specific BZ ligands bind to a site consisting of both VDAC and the 18-kDa PBR subunits (Snyder et al., 1990). In studies on the topography of PBRs in the MA-10 Leydig cell mitochondrial membrane, Papadopoulos et al. (1994, 1997a) also showed that the 18-kDa PBR protein is organized in clusters of four to six molecules associated with one VDAC molecule in such a way as to favor the formation of what they called "single pores".

The cDNA for the 850-nucleotide PBR mRNA has been cloned for a number of species, including humans (Riond et al., 1991), rat (Sprengel et al., 1989; Krueger et al., 1990), mouse (Garnier et al., 1994b), and cows (Parola et al., 1991). More recently, the genes for two of these species [humans (Lin et al., 1993) and rat

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(Casalotti et al., 1992)] have also been partially cloned and characterized. This approximately 13-kbp gene (Lin et al., 1993) was found as a single copy in the human genome and located on chromosome 22 in the 22q13.31 band (Riond et al., 1991; Chang et al., 1992). Figure 2 shows that this gene is composed of four exons, with the first exon and half of the fourth exon being untranslated. This gene has one transcription initiation site (Casalotti et al., 1992; Lin et al., 1993). Lin et al. (1993) also reported on an alternatively spliced PBR mRNA found in human tissue. In that case, compared with the full-size form, about 10-fold more of the smaller PBR mRNA form, in which its exon 2 sequences had been spliced out, was present in a congenital lipoid adrenal hyperplasia patient. Because this shorter mRNA is unable to translate PBR protein and because it is present in humans at greater levels than the full-length PBR mRNA species in some human conditions, it will be important to establish any novel function arising from any of its resultant translated products. It is of interest that the PBR promoter in both rat and humans does not contain a TATAA box but does contain multiple Sp1 boxes (Casalotti et al., 1992; Lin et al., 1993). This is also an indication that the product of this gene has a "housekeeping gene" function. To date, no additional transcription factor regulatory sites have been identified in this promoter.

The protein domain of exon 2 has been linked to the isoquinoline binding site and part of the PBR-specific BZ binding site (Farges et al., 1994; Papadopoulos et al., 1997a). Furthermore, a cholesterol recognition/interaction site has been characterized at the carboxyl end of the protein (Li and Papadopoulos, 1998). The 18-kDa

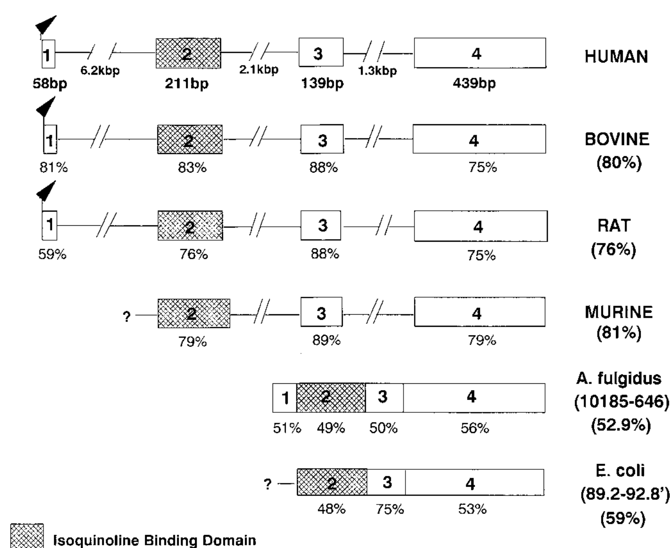

FIG. 2. PBR conservation throughout evolution. A number of different species were compared with the published human gene sequence. The human gene sequence was also compared with the *Escherichia coli* and *Archeoglobus fulgidus* genomes, which were available through GenBank. A significant homology was noted between all the sequences shown above, including a bacterial (*E. coli*) and fungal (*A. fulgidus*) sequence. Percentages reported below exon boxes are with respect to the published human sequence (Riond et al., 1991).

PBR subunit is highly conserved between the four species cloned (Fig. 2; Table 1). When querying GenBank for other homologies throughout evolution, we also found that this gene is quite well conserved (59% over exons 2–4), even in bacteria (Fig. 2). Lummis et al. (1991) reported the presence of BZ binding sites in the bacterium *Escherichia coli*. On an initial pharmacological characterization of these bacterial receptors, they claimed that these BZ receptors are distinct from both mammalian PBRs and CBRs and appear to be more closely related to those of insects. It may be that the gene sequence we found at GenBank is related to the receptor reported by Lummis and colleagues. If this is the case, its nucleotide sequence may be more related to PBR than its pharmacology. Furthermore, the fact that BZ receptors are so well conserved throughout evolution may imply that the functions of this gene must be fundamental for the cell.

Although numerous molecular studies have characterized the PBR proteins, much still needs to be undertaken to determine their basic (cellular) functions. A detailed discussion of their putative functions follows. Here, we mention a few molecular approaches that have been attempted. Efforts to generate a PBR-negative knockout mouse did not work out in that the animals died at an early embryonic stage (Papadopoulos et al., 1997b). Unfortunately, the specific reason for the early death of these fetal mice was not resolved. Nevertheless, this result does reinforce the notion that PBRs are involved in a basic cellular function that is necessary during murine fetal development. Two studies examined the effects of knockout of the 18-kDa PBR subunit gene (Papadopoulos et al., 1997b) or of 18-kDa PBR mRNA (Kelly-Hershkovitz et al., 1998) in cultured Leydig cells. Although both studies suggested the direct involvement of PBRs in Leydig cell steroidogenesis, the latter study ruled out its involvement in Leydig cell proliferation. At the level of steroidogenesis, Papadopoulos et al. (1997b) suggested that their study indicated PBR as the rate-limiting step in cholesterol transport into the mitochondria. This was not found in the antisense knockout study, which suggested that the involvement of PBR in steroidogenesis occurs later in the pathway. The reasons for the differences between the studies may reflect the different cell lines used (MA-10 versus R2C) or the different knockout approaches applied. Specifically, although *PBR* gene knockout totally abrogated PBR 18-kDa expression (Papadopoulos et al., 1997b), antisense RNA knockout in cultured Leydig cells resulted in only an approximately 50% reduction in basal PBR ligand binding (Kelly-Hershkovitz et al., 1998). Hence, observations generated from the antisense knockout approach, although suggestive, are not yet conclusive. Ultimately, the resolution of these apparently conflicting data and how this relates to the fundamental function of this gene product that could result in the abrogation of

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early fetal mouse development remains an open question.

III. Endogenous Ligands for Peripheral Benzodiazepine Receptors

The PBR is designated a receptor because the binding of specific ligands to it has been implicated in several physiological functions, including steroidogenesis (Papadopoulos et al., 1990), cell growth and differentiation (Wang et al., 1984), chemotaxis (Ruff et al., 1985), and mitochondrial respiratory control (Hirsch et al., 1989). However, the quest for an endogenous ligand for this receptor is still under way. When Beaumont et al. (1983) assayed ultrafiltrates of serum and urine collected from uremic patients, as well as from normal plasma, they isolated BZ-like molecules that inhibited [^{3}H]Ro 5-4864 binding to PBR. The affinity of these molecules for PBR was 125-fold greater than that for the CBR. The molecular mass ranged between 0.5 and 1.0 kDa, but the exact identity of the inhibitor was not specified.

Mantione et al. (1988) reported the presence of both high (molecular mass $>$ 10 kDa) and low (molecular mass $<$ 2 kDa) materials, isolated in crude form from rat antral stomach, that inhibited the specific binding of [^{3}H]Ro 5-4864 to PBRs. The high-molecular mass material was further purified to yield a 16 kDa protein called anthralin. This protein also inhibited the binding of [^{3}H]nitrendipine to the dihydropyridine Ca^{2+} channel. The protein was heat and pronase sensitive and partially sensitive to trypsin, whereas its activity was enhanced by Ca^{2+} ions in a concentration-dependent fashion. It was also found that the protein had enzymatic properties similar to the phospholipase A_2 isoenzyme. These authors proposed that anthralin may be an endogenous ligand of combined interaction, with both PBR and dihydropyridine binding sites (Mantione et al., 1988). This conclusion is consistent with pharmacological and electrophysiological observations of functional coupling between the two receptor systems (Mestre et al., 1984).

Corda et al. (1984) reported the isolation and purification to homogeneity of a 104-amino-acid-residue neuropeptide that inhibited diazepam binding to the BZ binding site. Tryptic digestion of this peptide [termed diazepam-binding inhibitor (DBI)] yielded an octadecaneuropeptide (ODN) that could compete for [^{3}H]diazepam binding, elicit proconflict responses in rodents, and antagonize the anticonflict actions of BZs (Alho et al., 1985). The proconflict effect of ODN was inhibited by Ro 15-1788 (flumazenil; ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazole-(1,5-*a*)-(1,4)benzodiazepine-3-carboxylate), a CBR-specific ligand (Ferrero et al., 1986b). DBI has been detected in human brain, and DBI-like immunoreactivity has been found in the cerebrospinal fluid of human volunteers (Ferrero et al., 1986a). This immunoreactivity is increased in dementia with normal-pressure hydrocephalus but not in Alzheimer's disease, multi-infarct dementia, or dementia with Parkinson's disease (Ferrero et al., 1988). DBI has also been located in peripheral tissues rich in PBRs, such as adrenal gland, testis, and kidney (Gray et al., 1986).

Papadopoulos and Brown (1995) have shown that the PBR is the key element in the regulation of cholesterol transport from intracellular stores to the inner mitochondrial membrane and that the presence of DBI is vital for steroidogenesis and stimulated cholesterol transport (Boujrad et al., 1993). In addition, DBI directly promotes loading of cholesterol to the inner mitochondrial membrane side chain cleavage cytochrome P-450 (P-450_{scc}) enzyme and thus starts the metabolism of cholesterol to pregnenolone (the initial step in steroidogenesis). Increased expression of DBI has been found in brain tumors (astrocytoma, glioblastoma), with the highest levels in the most neoplastic tumors (Alho et al., 1995). A close association between DBI and PBRs in ovary further emphasizes their important role in the regulation of steroid production (Alho et al., 1994). Chemical cross-linking studies of purified metabolically radiolabeled DBI to mitochondria of R2C rat Leydig tumor cells provided direct evidence that DBI specifically binds to the 18-kDa PBR protein and plays a key role in maintaining R2C constitutive steroidogenesis (Garnier et al., 1994a). Furthermore, the close localization of PBRs and DBI at the outer mitochondrial membrane has also been demonstrated by immunoelectron microscopy (Schultz et al., 1992). It can be concluded that DBI is an important candidate for an endogenous ligand to PBRs.

Other candidates for endogenous ligands to PBRs are porphyrins. Porphyrins are known to modulate enzymatic activity of several enzymes, including tryptophan pyrrolase, guanylate cyclase, and glutathione-5-transferase, and are involved with several mitochondrial proteins (Verma and Snyder, 1988). The major physiological porphyrins, protoporphyrin IX and heme, display inhibition constant (K_i) values of 20 to 50 nM for the PBR and 1000 times less at the CBR. This receptor specificity implies a physiological role for the PBR. Iron substitution of hemin with zinc results in an 8- to 10-fold reduction in the affinity for PBRs. Additionally, tin and cobalt replacement produces derivatives that are 1000 times less affinitive than those with iron. Cytochromes, which contain porphyrins, are selectively associated with the inner mitochondrial membrane and are involved in steroidogenesis. The striking association between steroid-forming tissues such as the adrenal gland and testis, which exhibit high PBR and porphyrin levels, also suggests a physiological role for the interaction of porphyrins with PBRs (Verma et al., 1987).

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IV. Role of Peripheral Benzodiazepine Receptors in Cellular Respiration

The PBRs are found primarily on the outer mitochondrial membrane (O'Beirne and Williams, 1988; Anholt et al., 1986b). Adrenal mitochondria possess the highest density of PBR sites, kidney mitochondria have an intermediate number, and liver has very low levels (Le Fur et al., 1983; De Souza et al., 1985). The parallels between the differential binding affinities of various PBR-specific versus CBR-specific ligands in these three tissues concerning their effect on mitochondrial respiration in the same tissues have led a few investigators to propose a role for PBRs in the regulation of the mitochondrial respiratory chain (Hirsch et al., 1989; Moreno-Sánchez et al., 1991). Hirsch et al. (1989) interpreted their data to suggest that the PBR ligands they used increased state IV and decreased state III respiration rates, which resulted in a significant decrease in the respiratory control ratio. Another group suggested that part of this inhibition of respiration was a specific effect, whereas part was nonspecific (Moreno-Sánchez et al., 1991). In particular, these authors claimed that the inhibition of reduced nicotinamide-adenine dinucleotide oxidase and the hydrolysis of ATP by PBR ligands was not specific, whereas oxidizable substrate transport was specifically inhibited by the PBR ligand AHN 086 (Moreno-Sánchez et al., 1991). In contrast, Zisterer et al. (1992) suggested that the results of those previous studies were due to nonspecific effects of PBR ligands and were not mediated through PBRs. It seems that at the present, no study definitively allows us to conclude how PBRs are actually involved in cellular respiration. If it should turn out that the PBR-specific ligands do affect mitochondrial respiration independent of PBRs, it would be essential to determine how they do this. It may be that some "alternate" peripheral-type BZ binding sites contribute to other functions or actions previously attributed to PBRs.

V. Peripheral Benzodiazepine Receptors in Endocrine System and Steroid Regulation

The biosynthesis of steroids in all steroidogenic tissues begins with the enzymatic conversion of the precursor cholesterol to form pregnenolone. This reaction is catalyzed by the enzyme P-450_{scc}, which is located on the matrix side of the inner mitochondrial membrane. The rate-limiting step in this process is the transport of cholesterol from the cellular stores across the aqueous intermembrane space of the mitochondria to the inner mitochondrial membrane and the P-450_{scc}. PBRs have been suggested to play a major role in this mitochondrial cholesterol transport (Krueger and Papadopoulos, 1990).

It has been reported that the steroidogenic acute regulatory protein (StAR) is involved in the acute trophic hormone regulation of steroid synthesis (Miller, 1995; Stocco and Clark, 1996a,b); however, the question as to how the transfer of cholesterol occurs remains open, and it is very likely that other proteins are involved in this process. In fact, a recent report suggests that PBR and StAR work together in cholesterol transport into the mitochondria (Sridaran et al., 1999). Two important observations suggest that PBRs are likely to play a role in steroidogenesis. First, PBRs are found primarily on the outer mitochondrial membrane (Anholt et al., 1986b; Mukherjee and Das, 1989; O'Beirne et al., 1990); second, PBRs are extremely abundant in steroidogenic endocrine tissues (Benavides et al., 1983a; De Souza et al., 1985).

A. Regulation of Steroidogenesis by Peripheral Benzodiazepine Receptors

It has been demonstrated that PBR-specific ligands indeed regulate mitochondrial steroidogenesis. DBI, a potential endogenous ligand for PBRs with a molecular mass of 10 kDa regulates steroidogenesis activated by corticotropin (ACTH) and luteinizing hormone via binding to PBRs and thus control mitochondrial cholesterol transport (Hall, 1991; Papadopoulos et al., 1991a,b; Brown et al., 1992). The addition of DBI to a cholesterol side chain cleavage reconstituted enzyme system was able to stimulate the conversion of cholesterol into pregnenolone (Brown and Hall, 1991), whereas the depletion of DBI from Leydig tumor cells resulted in a loss of trophic hormone-stimulated steroid production in these cells (Boujrad et al., 1993). Flunitrazepam, a partial BZ agonist for PBRs, inhibits hormone-stimulated steroid biosynthesis in Y-1 adrenocortical and MA-10 Leydig cells, via its binding to PBRs (Papadopoulos et al., 1991c). Ligands for the PBR enhance steroid production in Y-1 adrenocortical and MA-10 Leydig cell lines. These effects are attributable to their binding to PBRs (Mukhin et al., 1989; Papadopoulos et al., 1990). Ligands with high affinity to PBRs stimulate pregnenolone synthesis in brain mitochondrial preparation (Guarneri et al., 1992; Papadopoulos et al., 1992; McCauley et al., 1995). It has been suggested that PBRs play a role in the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in steroidogenesis (Krueger and Papadopoulos, 1990; Papadopoulos et al., 1990). The above findings demonstrate that PBRs play a significant role in steroid biosynthesis in various tissues.

B. Peripheral Benzodiazepine Receptors in Female Genital Tract

PBRs have been characterized in the genital tract of mature female rats (Fares et al., 1987).

1. Ovary. By means of immunocytochemical, in situ hybridization, and autoradiographic methods, it has been demonstrated that the PBR and its putative endogenous ligand (DBI) are present in all ovarian steroid-secreting cells (Toranzo et al., 1994). In the immature rat ovary, PBR density increases with age (Fares et al.,

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1987), but hypophysectomy reduced this PBR density by 68% (Bar-Ami et al., 1989; Table 2). This effect was abolished either by 2 days' treatment with pregnant mare serum gonadotropin (PMSG) or 4 days' treatment with diethylstilbestrol (DES), a synthetic derivative of estradiol (Bar-Ami et al., 1989; Table 2). [^{3}H]PK 11195 binding in the ovary of hypophysectomized rats was increased by 3.4- and 4-fold after PMSG or DES treatment, respectively (Bar-Ami et al., 1989; Table 2). In the intact immature rat, PMSG or DES also significantly increases PBR density (Fares et al., 1987; Table 2). PMSG, which contains both follicle-stimulating and luteinizing hormone activity (Moore and Ward, 1980), acts directly on the ovary to induce multiple follicular growth and development in the immature rat and elicits a significant increase in ovarian estradiol biosynthesis (Braw and Tsafriri, 1980).

Short-term (4-day) treatment of immature rats with testosterone, progesterone, and DES increased ovarian PBR density by 1.5- to 1.6-fold for each hormone (Bar-Ami et al., 1994). Long-term (10-day) treatment with testosterone and progesterone significantly reduced PBR density (40 and 14%, respectively), whereas a 2-fold increase in PBR binding capacity was obtained after long-term treatment with DES (Bar-Ami et al., 1994). These results indicate that PBR density in the ovary is altered by exogenously administrated steroids that are usually biosynthesized in the ovary and that PBRs may play an important role in the differentiation and development of the ovary.

In the adult rat, ovarian PBR density increases to a maximum on the day of proestrus (Fares et al., 1988). An increase in rat ovarian PBR density has been observed during pregnancy (Weizman et al., 1997a; Sridaran et al., 1999). Thus, it seems that the hormonal changes occurring during the estrous cycle and pregnancy play a role in the regulation of ovarian PBR.

2. Uterus and Oviduct. Autoradiographic studies have shown high PBR density in the epithelium and glands of the uterus and lower PBR density in uterine smooth muscle (Verma and Snyder, 1989). In the immature rat, PBR density in the oviduct and uterus increases with age (Fares et al., 1987). In a cycling adult rat, PBR density in both organs increases to a maximal value on the day of proestrus and has been found to correlate with the increase in serum estradiol levels throughout the 4-day cycle (Fares et al., 1988). These data suggest that PBR density in the uterus and oviduct is under hormonal regulation.

Hypophysectomy of immature rats results in a significant decrease (49%) in PBR density in uterus, whereas the administration of DES or PMSG abolishes this effect (Bar-Ami et al., 1989; Table 2). [^{3}H]PK 11195 binding increased 3.6- and 2.9-fold in DES- and PMSG-treated uterus, respectively, compared with untreated hypophysectomized rats (Bar-Ami et al., 1989; Table 2). DES or PMSG treatment of intact immature rats increased uterine PBR density by approximately 2-fold but did not affect renal PBR density (Fares et al., 1987; Table 2).

Short-term (4-day) treatment with testosterone, but not with progesterone, increased PBR density in the oviduct and uterus of immature rats by 2.0- and 1.4-fold, respectively, whereas long-term (10-day) treatment with testosterone or progesterone resulted in a significant decrease (>35%) in PBR density in the oviduct. Uterine PBR densities were significantly reduced (25%) after long-term treatment with testosterone but not with progesterone. Short-term but not long-term administration of testosterone was also associated with significant increases in estradiol levels (Bar-Ami et al., 1994). Thus, we suggest that the increase in PBR density in DES and in short-term testosterone treatment could be at least partially mediated by estrogenic action on the uterus and oviduct. Renal PBR binding was not affected by these endocrinological manipulations, perhaps due to the fact that the kidney, in contrast to the ovary and uterus, is not a target for gonadotropins and estradiol. These latter results suggest that PBRs may play an important role in the differentiation and development of the uterus and oviduct.

3. Adrenal. The highest concentration of PBRs in peripheral tissues is found in the adrenal tissue (De Souza et al., 1985; Fig. 1). Autoradiographic studies have shown PBRs to be present in the adrenal cortex and absent from the adrenal medulla (Benavides et al., 1983a; De Souza et al., 1985). Immunostaining and confocal microscopy on adrenal PBR by Oke et al. (1992) confirmed these findings. The adrenal medulla,

TABLE 2
Effect of hormonal changes on PBR density in the ovary, uterus, adrenal, and kidney of female rats

Treatment	Ovary	Uterus	Adrenal	Kidney	Reference
Hypophysectomy	↓	↓	↓	—	Bar-Ami et al., 1989 Fares et al., 1989
Hypophysectomy + DES	—	↑	↓	—	Bar-Ami et al., 1989 Fares et al., 1989
Hypophysectomy + PMSG	↑	↑	↓	—	Bar-Ami et al., 1989 Fares et al., 1989
Hypophysectomy + ACTH	ND	ND	↑	↑	Bar-Ami et al., 1989 Fares et al., 1989
Intact + DES	↑	↑	ND	—	Fares et al., 1987
Intact + PMSG	↑	↑	ND	—	Fares et al., 1987

—, unchanged; ↑, increased; ↓, decreased; ND, not determined.

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however, appears to be enriched with $GABA_A$ receptors that possess PBRs (Kitayama et al., 1989, 1990). The ontogeny of both adrenal mitochondrial PBR binding density and immunoreactivity directly parallels that of the onset of steroidogenesis in the adrenal cortex (Zilz et al., 1999). This is consistent with the notion that PBRs are a prerequisite for adrenocortical steroidogenesis (Zilz et al., 1999).

Removal of the pituitary gland, which results in the elimination of ACTH secretion, causes a significant reduction in adrenal PBR density (Anholt et al., 1985a; Bar-Ami et al., 1989; Table 2). This effect is manifested mainly in the zona fasciculata and zona reticularis, whereas no effect has been noted in zona glomerulosa density (Anholt et al., 1985a). Hormone replacement by i.p. injections of ACTH resulted in a 1.7-fold increase in PBR density compared with that in intact rats, whereas hormone replacement by PMSG and DES failed to restore adrenal PBR density (Fares et al., 1989; Table 2).

In hypophysectomized rats, the amount of DBI-like immunoreactivity in adrenal glands decreased dramatically (80%), although the administration of ACTH reduced this decrease, and there was a positive correlation between the amount of adrenal DBI-like immunoreactivity and plasma corticosterone concentrations (Massotti et al., 1991). These findings suggest that de novo synthesis of DBI in the adrenal could be an important factor in the mediation of ACTH-induced steroidogenesis.

Various PBR-specific ligands may alter steroidogenic activity in the adrenal cortex. The effect of these ligands can be seen over the entire hypothalamic-pituitary-adrenal (HPA) axis. Thus, Ro 5-4864 directly stimulates the release of corticotropin-releasing hormone (CRH) but not ACTH, whereas PK 11195 directly stimulates the secretion of ACTH (Calogero et al., 1990). In various models of induced stress in rats, the increase in corticosterone and ACTH was attenuated by treatment with diazepam (Copland and Balfour, 1987). Furthermore, in human subjects with panic disorder (PD) or generalized anxiety disorder (GAD), treatment with diazepam causes a significant reduction in ACTH and cortisol (Roy-Byrne et al., 1991). Diazepam has the capacity to bind to both CBRs and PBRs (Gobbi et al., 1987). Furthermore, Holloway et al. (1989) have shown that the CBR-specific ligand midazolam and the mixed PBR/CBR ligand diazepam inhibit cortisol and aldosterone synthesis in bovine adrenal cells in vitro. Finally, alprazolam, a CBR-specific ligand, is capable of suppressing the HPA axis in primates (Kalogeras et al., 1990). Thus, it seems that PBR-specific ligands stimulate adrenocortical steroidogenic activity by acting at a number of loci in the HPA axis and that CBR-specific ligands produce the opposite effect by suppression of the hypothalamic secretion of CRH.

In adrenal glomerulosa cell culture, the addition of various PBR-specific ligands increased angiotensin II-induced aldosterone secretion. The effect of these various ligands coincides with the rank of their potential to displace [^{3}H]PK 11195 from adrenal glomerulosa cells, which suggests a receptor-mediated action (Song and Zhou, 1989).

Adrenal PBR density is down-regulated during rat pregnancy and lactation (Weizman et al., 1997a). In addition, a significant decrease (31%) in adrenal PBR density was observed after the surgical castration of male rats (Weizman et al., 1992; Table 3). Long-term administration of testosterone acetate prevented this castration-induced PBR depletion. Moreover, long-term treatment of male rats with testosterone acetate induced an increase in adrenal PBR density, whereas cyproterone acetate induced a decrease in adrenal PBR density (Amiri et al., 1991; Table 3). Cyproterone acetate possesses glucocorticoid activity, which suppresses ACTH release via negative feedback. The decrease in the trophic effect of ACTH may lead to a marked loss in adrenal weight and to depletion of adrenal PBR. Testosterone acetate, however, has a marked anabolic effect, which is reflected by the augmentation of PBR density in the adrenal glands (Amiri et al., 1991).

In vivo treatment of rats with extract of Ginkgo biloba leaves (EGb 761) and its bioactive components ginkgolide A and B specifically reduces the ligand binding capacity and protein and mRNA expression of the PBR, resulting in decreased corticosteroid synthesis (Amri et al., 1996). Furthermore, ex vivo treatment of rat adrenocortical cells with EGb 761 and ginkgolide B for 2 days inhibited the synthesis of the PBR. In addition, these treatments resulted in 50 and 80% reduction, respectively, of ACTH-stimulated corticosterone production (Amri et al., 1997). These latter data provide further evidence that PBRs are essential for steroidogenesis in the adrenal.

4. Mammary Gland. Although high PBR density is found in acinar cells in normal mammary gland and in 7,12-dimethylbenz[*a*]anthracene-induced tumors (Tong

TABLE 3
PBR density in testis, Cowper's gland, adrenal, and heart of male rats after hormonal manipulations

Treatment	Testis	Cowper's Gland	Adrenal	Heart	Reference
Intact + testosterone	↓	ND	↑	—	Amiri et al., 1991
Intact + cyproterone	↓	ND	↓	—	Amiri et al., 1991
Testis removal	ND	↓	↓	—	Weizman et al., 1992
Testis removal + testosterone	ND	—	—	—	Weizman et al., 1992

—, unchanged; ↑, increased; ↓, decreased; ND, not determined.

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et al., 1991), PBR density does not change in response to lactation. Synthesis of DBI has been shown to occur in mammary acinar cells by in situ hybridization (Tong et al., 1991). Whether PBR density in the mammary gland is under hormonal regulation and whether PBRs are involved in mammary cell functions such as casein biosynthesis have yet to be explored.

C. Peripheral Benzodiazepine Receptors in Male Genital Tract

PBRs have been identified in the testis (Anholt et al., 1985a; De Souza et al., 1985; Mercer et al., 1992), as well as in the vas deferens, prostate, seminal vesicle, and Cowper's gland (Katz et al., 1990a). The hierarchical order of PBR density in the rat genital tract is expressed as follows: testis > Cowper's gland > prostate > vas deferens > seminal vesicle (Katz et al., 1990a). PBRs have been immunolocalized in the rat testis and were found to be present exclusively in the interstitial Leydig cells (Garnier et al., 1993). A 3-fold increase in PBR density was demonstrated in rat testis during maturation (Mercer et al., 1992). These results might reflect critical interactions between PBRs and gonadal hormone activity during development, because testicular PBRs are putatively involved in testosterone production. PBR density in the rat testis is dependent on the trophic influence of pituitary hormones: although hypophysectomy induces depletion of testicular PBR (Anholt et al., 1985a), ligands specific for PBRs increase the in vitro human chorionic gonadotropin-stimulated testosterone secretion of decapsulated rat testis and interstitial cell suspension (Ritta et al., 1987; Ritta and Calandra, 1989).

Treatment with estradiol decreases rat testicular PBR density (Anholt et al., 1985a; Gavish et al., 1986a). The effect obtained with estradiol treatment might be due to its antiandrogenic impact (Gavish et al., 1986a). Cyproterone acetate induces a decrease in rat testicular PBR density (Amiri et al., 1991; Table 3). This effect might be due to its antiandrogenic activity at the target tissue level (Murad and Haynes, 1985). The reduction in rat testicular PBR density after testosterone administration might be due to the suppressive effect of the exogenous androgen on the production of endogenous male hormone via negative feedback (Amiri et al., 1991).

The increase in adrenal PBR density after testosterone administration (Amiri et al., 1991) might be due its anabolic effect, whereas the decrease in adrenal PBR density after cyproterone administration (Amiri et al., 1991) might be due to its antiandrogenic and/or glucocorticoid activity, which suppresses pituitary ACTH secretion and can lead to a loss of adrenal weight (Poyet and Labrie, 1985). The decrease in adrenal PBR density after testicular removal accords with the trend toward diminution proposed by Anholt et al. (1985a). PBR density in the heart was not affected by either cyproterone acetate or testosterone administration (Amiri et al., 1991; Table 3).

Cowper's glands are accessory sex organs that produce the coagulating components of semen and are dependent on the trophic influence of testosterone. Testosterone administration to rats induced an increase in PBR density in Cowper's gland (Amiri et al., 1991; Table 3). PBR density in Cowper's gland was decreased (71%) after testicular removal, whereas testosterone administration prevented this castration-induced PBR depletion (Weizman et al., 1992). PBR depletion in this organ after castration, although prevented by testosterone, indicates that PBRs in this organ are localized on cells whose integrity depends on the trophic influence of testosterone. No significant changes were obtained in PBR density in the heart after testicular removal (Weizman et al., 1992), suggesting that testosterone-related PBR changes may be restricted to the male genital tract.

VI. Peripheral Benzodiazepine Receptors under Pathological Conditions

A. Stress Response

1. Relevance of Peripheral Benzodiazepine Receptors to Stress. PBRs seem to be involved in the regulation of several major stress systems: 1) the HPA axis, 2) the sympathetic nervous system, 3) the renin-angiotensin axis, and 4) the neuroendocrine-immune axis. The localization of PBRs on the outer and inner mitochondrial membranes suggests that they may be involved in basic cellular metabolic processes, in addition to their role in the regulation of biosynthesis of steroids. This assumption is supported by the observation of high PBR density in tissues that display high levels of cytochrome oxidase activity and use oxidative phosphorylation for their metabolic needs (Anholt et al., 1986a,b; Hirsch et al., 1989).

In human leukocytes and erythrocytes, PBRs have been identified on the plasma membranes (Olson et al., 1988a; Cahard et al., 1994). These plasma membrane receptors might be involved in neuroendocrine-immune function, in contrast to the mitochondrial receptors, which seem to be involved in the transfer of specific molecules into the mitochondria (Berkovich et al., 1993). The involvement of PBRs in the regulation of basic biological processes that are pertinent to physiological response to stress, such as cellular metabolism, neuroendocrine activity, and immune functioning, points to their possible role in the mediation of the organism's adaptation to stress.

2. Peripheral Benzodiazepine Receptor Ligands and Behavior. Behavioral studies have demonstrated that Ro 5-4864 possesses anxiogenic and convulsant properties, whereas PK 11195 has been found to be anxiolytic and anticonvulsant (Weissman et al., 1983, 1984a,b; Bénavidès et al., 1984; Pellow and File, 1984; Mizoule et al., 1985; Massotti and Lucantoni, 1986; Hariton et al., 1988). PK 11195 reverses acute anxiety-induced (forced

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swimming stress) increase in platelet aggregation, indicating a possible anxiolytic effect of the drug (Serrano et al., 1988). Using Vogel's conflict model of anxiety (thirsty-lick test) in rats, Mizoule et al. (1985) reported that Ro 5-4864 decreased, whereas PK 11195 increased, punished drinking in a dose-dependent fashion. Furthermore, the punishment effects of Ro 5-4864 were antagonized by low doses of PK 11195. These authors showed in this model that Ro 5-4864 and PK 11195 act similarly to CBR inverse agonists and partial agonists, respectively, in a conflict situation. It is of note that neither the Ro 5-4864 nor the PK 11195 behavioral effects were blocked by the CBR antagonist Ro 15-1788, suggesting that the action of Ro 5-4864, at least in Vogel's conflict model of anxiety, is independent of the CBR. Other experimental models support the anxiogenic activity of Ro 5-4864, although some have failed to antagonize this effect with PK 11195 (Pellow and File, 1984; File and Pellow, 1985).

These neural activities indicate that PBRs and CBRs, although different structurally, pharmacologically, and physiologically, seem to share some common behavioral functions (Drugan and Holmes, 1991). Behavioral and electroencephalographic studies have shown that Ro 5-4864 induces convulsions in mice, rats, and guinea pigs and facilitates audiogenic seizures in genetically susceptible mice in a fashion similar to CBR inverse agonists and GABA-coupled chloride channel blockers (Weissman et al., 1983; Bénavidès et al., 1984; Rastogi and Ticku, 1985). The Ro 5-4864-induced convulsions can be antagonized by PK 11195, but not by Ro 15-1788 (Weissman et al., 1983; Bénavidès et al., 1984). These data support the notion that Ro 5-4864 convulsant activity is not mediated by CBRs. However, it has been shown that Ro 5-4864-induced supraspinal convulsions can be antagonized by the selective CBR antagonist Ro 15-1788 (Massotti and Lucantoni, 1986). Furthermore, it has been demonstrated that flurazepam-potentiated muscimol responses in a cuneate nucleus slice preparation can be attenuated by Ro 5-4864 (Simmonds, 1985). It has been suggested that anxiogenic and convulsant effects of high doses of Ro 5-4864 are mediated via a low-affinity binding site at the GABA-gated chloride ion channel (Gee, 1987), indicating a possible link between PBRs and CBRs. The relevance of the pharmacological data on PBR ligands to anxiety and response to stress is still unclear.

3. Involvement of Peripheral Benzodiazepine Receptors in Acute Stress.

a. Animal Studies. The sensitivity of PBRs to acute stress has been demonstrated in various animal models. Drugan et al. (1986) were the first to show the involvement of PBRs in the physiological response to stress, using inescapable tail shocks as an animal model of stress. Five shocks induced a significant increase in the density of renal but not cerebral cortex PBRs. The exposure of mice to acute maximal electroshock induced rapid up-regulation of PBR density in mouse cerebral cortex and cardiac ventricles, measured 30 min after the procedure (Basile et al., 1987).

Surgery is a traumatic procedure associated with transitory alterations in levels of stress hormones, such as cortisol, prolactin, growth hormone, and β-endorphin (Noel et al., 1972; Cohen et al., 1981). Abdominal wall surgery in rats was associated with a significant elevation in the density of cerebral and renal PBRs as well as cerebral CBRs on days 1 and 3 after the surgical procedure but not on day 7. The up-regulation of PBRs and CBRs was not accompanied by any alteration in [^{3}H]quinuclidinyl benzilate binding to cholinergic muscarinic receptors and monoamine oxidase A and B activity, indicating that BZ receptors are selectively sensitive to acute surgical stress. It is noteworthy that the elevation and later normalization of the BZ receptors correspond to the stages of repair of surgical wounds (Okun et al., 1988).

Acute single forced swimming stress in rats is associated with a significant increase in PBR density in cerebral cortex, olfactory bulb, kidney, platelets, and lymphocytes, as well as a parallel increase in cerebral CBRs (Novas et al., 1987; Rago et al., 1989a,b). Up-regulation of forebrain PBR has also been demonstrated in chicks submitted to acute swimming stress (Martijena et al., 1992; Marin et al., 1996). In addition, low subsolubilizing concentrations of Triton X-100 caused a significant increase in measurable forebrain PBR density in the unstressed chicks but not in the stressed chicks. These results indicate that both acute stress and Triton X-100 enhance [^{3}H]Ro 5-4864 accessibility to a pool of receptors not detected before stress or in the absence of the detergent. Thus, it appears that the in vitro increase caused by stress could not be enhanced further by Triton X-100. It was suggested that the acute stress-induced increase in forebrain PBR could be explained by recruitment of receptors, via translocation of receptors coming from another subcellular pool, but not by immediate increase in the receptor protein biosynthesis (Marin et al., 1996). It is of note that a similar recruitment of CBRs was demonstrated in chicks exposed to acute stress (Martijena et al., 1992).

Exposing handling-habituated rats to acute noise stress resulted in an 80% increase in PBR density in the cerebral cortex and a decrease of 30 to 40% in CBR density in the same tissue, demonstrating that the stress-induced change in PBRs is not necessarily associated with a parallel change in CBRs (Mennini et al., 1989). Noise stress in rats also induced an increase in adrenal PBR and DBI-like immunoreactivity in adrenal gland and hippocampus (but not in the cerebral cortex, striatum, hypothalamus, or cerebellum), as well as in plasma corticosterone (Ferrarese et al., 1991). It has been suggested that the stress-induced up-regulation of adrenal DBI and PBR may play a role in the activation of adrenal release of steroids and/or neurosteroids and

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could thereby be responsible for changes in brain BZ receptors and hippocampal DBI. In turn, the increase in hippocampal DBI in stressed rats could possibly lead to increased biosynthesis of neurosteroids via hippocampal glial cells, and these neurosteroids might act as GABA agonists (Majewska et al., 1986; Deutsch et al., 1992) and have a negative feedback action, inactivating the HPA axis (Mennini, 1993).

b. Human Studies. Platelet PBRs were assessed in psychiatric residents who were completing the written part of the Israeli Board-certification examination in comparison with age- and sex-matched controls. The platelet PBR density was significantly elevated immediately after the examination and showed a trend toward a decrease to normal range 10 days later. Similar changes were detected in anxiety levels, as measured by the appropriate rating scale (Karp et al., 1989). Thus, as shown in animal studies, acute stress in humans can lead to an increase in PBRs.

4. Involvement of Peripheral Benzodiazepine Receptors in Chronic Stress.

a. Animal Studies. As described above, exposure of rats to five inescapable tail shocks induced a significant increase in renal PBR density (Drugan et al., 1986). In contrast, 80 repeated tail shocks resulted in a significant decrease of PBR density in kidney, heart, pituitary gland, and cerebral cortex. This biphasic effect of inescapable shock may indicate differences in PBR response to short- versus long-term exposure to stress. This assumption is supported by the finding that repeated swimming stress (daily forced swimming for 21 days) was associated with a significant reduction in renal PBR, in contrast to the up-regulatory effect of single swimming stress on PBR density in this organ (Burgin et al., 1996). Another model demonstrating the association between down-regulation of renal PBR and long-term stress is that of Maudsley reactive rats, which have been bred for high levels of fearfulness. Maudsley reactive rats exhibited decreased renal PBR density compared with Maudsley nonreactive rats (Drugan et al., 1987).

Food deprivation has also been used as an animal model of chronic stress in rats. Five days of food deprivation induced a decrease in PBR density in the kidney (33%), heart (34%), and adrenal (35%) but not in the hypothalamus, cortex, and ovary. Refeeding for 5 days restored PBR densities to control values in the heart and kidney but not in the adrenal. Starvation also resulted in a reduction (35%) in cerebellar $GABA_A$ receptors, whereas CBRs in the cerebral cortex and hypothalamus remained unaltered (Weizman et al., 1990). Food deprivation is known to cause adrenal hyperfunction (Young et al., 1987); thus, some of the receptor alterations could be attributable to the suppressive effect of hypercortisolemia or to the metabolic changes associated with starvation.

b. Human Studies. Platelet PBRs were studied in Israeli soldiers at the beginning of a parachute training course, after 6 days of preparatory exercises, and after the fourth actual parachute jump. Reduced (26%) density of PBRs was observed immediately after the repeated actual jumps, indicating that repeated stress is associated with a down-regulation of PBR. The PBR half-life is approximately 3 days; thus, the binding measure after the fourth parachute jump (8 days after the baseline measurement) was due to platelets not present at the time of the first sampling. It is possible that the repeated stress-induced receptor changes occur at the precursor cells (megakaryocytes) and not at the peripheral blood platelets. The decrease in PBR density was accompanied by a parallel decrease in systolic blood pressure. Both effects of repeated stress may be related to an habituation process that involves an adaptive reduction in sympathetic responsivity (Dar et al., 1991).

The density of platelet PBRs was also assessed in Israeli civilians exposed to missile attacks during the Persian Gulf War. PBR density was shown to be 22 and 15% lower before and during the war, respectively, than at 4 weeks after the end of the war. Thus, it appears that relief of stress, as assessed by the Hamilton Anxiety Rating Scale, led to an increase in PBR density, which correlated with the relief of anxiety (Weizman et al., 1994). It is of note that the human studies showed the same results as the animal studies (i.e., up-regulation and down-regulation of PBRs in response to acute and chronic stress, respectively).

5. Putative Mechanisms Involved in Peripheral Benzodiazepine Receptor Response to Stress. The exact mechanisms that play a role in modulation of PBR expression in response to stress are not well established. PBRs are localized predominantly on the outer mitochondrial membrane, are very dense in peripheral organs that are highly activated during stress (heart, kidney, adrenal, and lung), and have been suggested to be involved in oxidative metabolism and steroidogenesis. Activation of the PBR during acute exposure to a stressor may provide neural and metabolic preparation for better coping with the stress. Furthermore, in some situations, the changes in PBRs are accompanied by a simultaneous increase in CBR activity, a combination that enables efficient psychological and physical fitness (Drugan and Holmes, 1991). Renal PBR activation during acute stress may be relevant to stress-induced hypertension via activation of the renin-angiotensin system (Holmes and Drugan, 1993; Drugan, 1996). In steroidogenic tissues, PBR ligands can affect the translocation of cholesterol from the outer to the inner mitochondrial membrane, although the absolute rate changes are limited (Krueger and Papadopoulos, 1990). Because stress is accompanied by an increase in glucocorticoid synthesis and release, it is possible that this receptor plays a pivotal role in the neuroendocrine response to stress.

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It seems that to avoid long-term hypercortisolemia secondary to chronic stress, which may provoke damage to the CNS, metabolic changes, and impairment in the immune functioning, the maximal binding capacity of PBRs is diminished. The down-regulation of the mitochondrial PBR associated with chronic stress may reflect a neuroendocrine defense mechanism that inhibits mitochondrial cholesterol transport and overproduction of glucocorticoids. It has been shown that chronic stress, in contrast to acute stress, leads to diminished cortisol secretion (Mason et al., 1990). Furthermore, recovery from the stress is accompanied by restoration of PBR density to a normal range (Weizman et al., 1994). However, it should be noted that there are insufficient data regarding the impact of chronic stress on adrenal PBR and that regarding PBR, only a small fraction of intracellular cholesterol is available for steroid synthesis; uptake of cholesterol from plasma is also directly stimulated by ACTH (Krueger and Papadopoulos, 1990). Furthermore, it seems that there are no unequivocal data demonstrating that PBRs are a necessary and sufficient condition for steroidogenesis (Stocco and Clark, 1996a,b; Weizman et al., 1997b), but according to Zilz et al. (1999), they are an absolute prerequisite. The decrease in PBRs in steroidogenic and nonsteroidogenic peripheral organs after chronic stress may reflect a mechanism with an aim to diminish the sensitivity of these receptors to cope with increased sympathetic tone and hypercortisolemia. The suppression of renal PBRs during repeated stress may prevent overactivity of the renin-angiotensin system, which might lead to stress-induced hypertension (Holmes and Drugan, 1994; Drugan, 1996). It is noteworthy that the chronic stress-induced reduction in PBRs is more pronounced in males than in females, in both rats and humans (Drugan et al., 1991; Weizman et al., 1994); however, the cause of this gender-related difference is unclear. Thus, the present suggested mechanisms for the involvement of PBRs in stress are largely hypothetical.

B. Anxiety Disorders

1. Generalized Anxiety Disorder. GAD is characterized by excessive anxiety and worry occurring frequently for at least 6 months. The anxiety, worry, or physical symptoms cause clinically significant distress and impairment in social and occupational functioning (American Psychiatric Association, 1994). Reduced (24%) [^{3}H]PK 11195 binding to platelet membranes was observed in GAD patients compared with age- and sex-matched normal controls. Four weeks of diazepam treatment induced a reduction in the anxiety, accompanied by an elevation (69%) in PBR density. One week of diazepam withdrawal resulted in a slight decrease (16%) in PBR density compared with the level during the drug treatment (Weizman et al., 1987b). Similar results are reported in lymphocyte PBRs, and the decrease was restored to a normal value after long-term treatment with BZs (Ferrarese et al., 1990; Rocca et al., 1991). It has been suggested that endogenous neuropeptides such as DBI may be released in anxiety and down-regulate PBR expression (Ferrarese et al., 1990). It is of note that Ro 5-4864 possesses proconflict and anxiogenic effects that can be antagonized by PK 11195 (Mizoule et al., 1985). Furthermore, PK 11195 has been reported to possess anxiolytic properties in psychiatric patients (Papart et al., 1988; Ansseau et al., 1991).

An animal study demonstrated that chronic low-dose (0.5 mg/kg for 21 days) diazepam treatment up-regulated PBR density in the heart and cerebral cortex and that withdrawal of the drug for 5 days resulted in the normalization of PBR levels in these organs (Weizman and Gavish, 1989). It seems that the elevation of PBRs induced by chronic diazepam treatment, as observed in GAD patients, can also be achieved in unstressed rats. Thus, the stress-induced low PBR levels are not a prerequisite for the up-regulatory effect of diazepam. The increased PBR density in the heart after diazepam treatment may reflect a drug-induced reduction in the sympathetic tone due to the nonspecific general sedative effect of diazepam treatment (Basile and Skolnick, 1988).

2. Panic Disorder. PD is characterized by recurrent unexpected panic attacks, which lead to persistent concern and worry about having additional attacks and to impairment in daily functioning (American Psychiatric Association, 1994). Platelet PBR density was found to be reduced (29%) in PD patients compared with normal controls (Marazziti et al., 1994), although an earlier study failed to demonstrate such an alteration in lymphocyte membranes (Rocca et al., 1991).

3. Generalized Social Phobia. Patients with GSP have a persistent and excessive fear of one or more social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others. Exposure to the feared social situation provokes anxiety, which may be similar to situationally predisposed panic attack (American Psychiatric Association, 1994). An abnormally low number of platelet PBRs (36%) have been demonstrated in GSP patients (Johnson et al., 1998).

4. Post-Traumatic Stress Disorder (PTSD). PTSD is a severe anxiety disorder that may occur after the exposure to a traumatic event. The traumatic event is persistently reexperienced, and persistent avoidance of stimuli associated with the trauma, numbing of general responsiveness, and symptoms of increased arousal are present (American Psychiatric Association, 1994). Platelet PBRs were assessed in drug-free PTSD patients. All were Israeli citizens who had been exposed to repeated missile attacks during the Persian Gulf War. The PTSD symptoms persisted for 2 years. Civilians from the same geographical area who were exposed to the same attacks composed the control group. Decreased platelet PBR density (62%) was observed in the PTSD patients compared with the control group. The reduction in PBR

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density in the PTSD patients was more prominent in men than in women (Gavish et al., 1996).

5. Obsessive-Compulsive Disorder (OCD). OCD is characterized by recurrent obsessions and compulsions that cause marked distress, interfere with the patient's daily function, and are commonly associated with depression (American Psychiatric Association, 1994). Two studies have demonstrated a lack of alteration in platelet PBR in OCD patients (Weizman et al., 1993a; Marazziti et al., 1994), whereas one study has demonstrated decreased lymphocyte membrane PBR density (25%) in OCD (Rocca et al., 1991).

It appears that platelet PBRs are down-regulated in the anxiety disorders associated with chronic hyperarousal, overactivity of the sympathetic system, and anxious mood, such as GAD, PD, GSP, and PTSD. These anxiety disorders can be relieved by the administration of BZs. It may be that the marked and long-term stress associated with these disorders leads to down-regulation of PBR, as documented in animals and humans exposed to repeated stress (Gavish et al., 1992, 1993; Weizman and Gavish, 1993).

It is of note that ablation of the amygdaloid central nucleus attenuates stress-induced changes in PBR density (Holmes and Drugan, 1993), indicating that PBR down-regulation may be specifically associated with anxiety disorders characterized by overactivity of this nucleus (Johnson and Lydiard, 1995). The receptor down-regulation may reflect an adaptive response that prevents chronic overproduction of glucocorticoids in hyperarousal anxiety states. OCD, in contrast to the other anxiety disorders, is not associated with down-regulation of PBRs. OCD is not ameliorated by BZs but rather by serotonin reuptake inhibitors. Furthermore, this disorder is not associated with overactivation of the amygdala (Johnson and Lydiard, 1995). Thus, it seems that PBRs are selectively suppressed by anxiety disorders associated with long-term repeated hyperactivity of the autonomic nervous system. It is not clear whether the altered PBRs are also accompanied by functional alterations in CBR activity, yet it is possible that there is a CBR/PBR/endogenous ligand/steroid/immune network that becomes operational on the exposure of an organism to stress or anxiety (Dodd and Lenfant, 1993).

C. Mood Disorders

To evaluate the relationship between depression and PBRs, platelet PBRs were measured in untreated depressed patients in comparison with normal controls. The density and the dissociation constant of PBRs on platelets of those patients did not differ from those of controls. Furthermore, no correlation was found between PBR density and the severity of the depression in these patients (Weizman et al., 1995). It seems that depression, like OCD and in contrast to stress and some anxiety disorders, is not associated with suppression of PBR density. In accordance with this, the amygdala, which seems to play a role in stress-induced depletion of PBR, is not overactive in depression or OCD (Holmes and Drugan, 1993).

It is of note that chronic treatment with antidepressants has been demonstrated to modulate adrenal and hepatic PBRs in rats, a phenomenon that seems to be irrelevant to the antidepressive activity of the drugs but relevant to their effects on the function of these peripheral organs (Weizman et al., 1993b). Moreover, electroconvulsive therapy down-regulates platelet PBR density in medication-resistant depressed patients, a phenomenon that appears to be related to the repeated stress associated with the treatment and not to its antidepressive properties (Weizman et al., 1996).

The mood stabilizer carbamazepine has an up-regulatory effect on platelet PBRs; however, the significance of this activity with regard to the antiepileptic and/or mood-stabilizing properties of this agent is unclear (Weizman et al., 1987a).

D. Neurodegenerative Disorders

1. Parkinson's Disease. Diminished platelet PBR density has been detected in Parkinson's disease patients (Bonuccelli et al., 1991). The depletion of PBRs was not dependent on antiparkinsonian treatment, although animal studies have demonstrated a tissue-specific modulatory effect of dopamine agonists on PBR expression (Amiri et al., 1993). The decreased PBR expression in mitochondrial receptors involved in respiratory control may reflect an impairment in cellular respiratory function (Hirsch et al., 1989). It is of note that reduced PBR density on platelets has also been found in schizophrenic patients under antidopaminergic treatment; however, the relevance of this finding to extrapyramidal syndromes is unclear (Gavish et al., 1986b; Weizman et al., 1986).

2. Alzheimer's Disease. Postmortem brain studies have demonstrated increased PBR density in the temporal lobe (Owen et al., 1983; Diorio et al., 1991), Broca's area, and the precentral and postcentral gyri (McGeer et al., 1988) in Alzheimer's disease. Such alterations were not detected in multi-infarct dementia, indicating that these changes may be specific for Alzheimer's disease. Because PBRs in the brain are localized mainly in glial cells, it is possible that the increased PBR density reflects brain damage and gliosis. Ex vivo study has demonstrated increased platelet PBR density in Alzheimer's disease patients compared with multi-infarct demented patients and age- and sex-matched normal controls (Bidder et al., 1990). These results are consistent with the postmortem brain studies. Because Alzheimer's disease is associated with enhanced HPA axis activity (Christie et al., 1987), it is possible that the increased endocrine steroid activity plays a role in PBR up-regulation.

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E. Peripheral Benzodiazepine Receptors and Brain Damage

1. Peripheral Benzodiazepine Receptors and Neurotoxic Brain Damage. Chemical sympathectomy, which causes destruction of catecholaminergic neurons, results in an increase in hypothalamic, striatal, and cardiac PBRs. Other tissues (cerebral cortex, kidney, lung, and so on) remain unaffected (Basile and Skolnick, 1988). This observation suggests that sympathetic tone is important to the expression of PBRs in certain organs.

PBRs may also be used as markers of neuronal damage induced by a variety of factors. The organophosphate soman causes neuronal lesions with inflammatory reaction and subsequent glial proliferation. These changes are well correlated with alterations in PBR binding site densities. Two days after soman treatment, PBR densities were markedly increased in the hippocampus. When, before soman challenge, *N*-[1-(2-thienyl)cyclohexyl]-3,4-piperidine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, was given, neuronal damage was partially prevented but not the increase in PBR density. A non-NMDA (α-amino-3-hydro-5-methyl-4-isoxazole propionic acid) antagonist, 2,3-dioxo-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline, abolished both brain damage and PBR increase (Lallement et al., 1993).

Systemic injections of kainic acid caused hippocampal lesions (an excitotoxically induced phenomenon) that were quantified by PBR binding. The lesions showed gliosis, which is typical of excitotoxic neural death. Treatment with an adenosine analog soon after kainic acid injection prevented neurotoxicity, as demonstrated by preserved PBR binding (Miller et al., 1994).

The potential for in vitro visualization of lesioned brain areas by positron emission tomography scanning, using [^{11}C]PK 11195 as a ligand, has been shown in a local lesion model. This lesion is of a type in which the primary pathology is characterized by an acute inflammatory reaction, and not by gliosis (Myers et al., 1991). The lesion was elicited by intracortical injection of the photosensitive dye rose bengal and exposure to an intense green light shone on the brain surface.

In another study, it was shown that ischemic brain damage can be attenuated by anti-ischemic drugs belonging to several chemical classes: NMDA receptor antagonists, calcium channel blockers, platelet-activating factor antagonists, adenosine receptor antagonists, and opioid receptor antagonists (Gotti et al., 1990). Limited protection was provided by adenosine receptor antagonist, and effective protection was achieved with NMDA receptor antagonist. Lesions and sparing effects were assessed using PBR as the biomarker (Gotti et al., 1990).

Another model used as a biomarker of neurotoxicity is systemic application of the neurotoxin domoic acid (Kuhlmann and Guilarte, 1997). This agent causes amnesia, seizures, coma, and death, as well as extensive forebrain damage. PBRs have been used as an indicator of neurotoxicity and compared with functional and histological parameters. PBR expression is increased in the limbic structures, striatum, and substantia nigra 5 days after domoic acid treatment. The histological changes are accompanied by spatial learning and memory impairments. The strong correlation between receptor and behavioral changes shows the extent and effect of brain injury elicited by the acute administration of the excitotoxin (Kuhlmann and Guilarte, 1997).

In summary, PBR determination is a highly effective tool for quantification of lesions caused by excitatory amino acids and by a variety of neurotoxic agents leading to brain damage in which the principal pathology is expressed by inflammation or gliosis.

2. Peripheral Benzodiazepine Receptors and Traumatic-Ischemic Brain Damage. The PBR and its specific ligands have been found to participate in traumatic-ischemic brain damage. In the rat, a mechanical brain injury was elicited by lateral fluid percussion over the parietal cortex (Toulmond et al., 1993). The percussion was a short, constant impact pressure that resulted in accumulation of blood in the subarachnoid space and cortical edema in the hyperacute post-traumatic phase. In the acute (4- to 24-h post-traumatic) phase, phagocytes invaded the injured brain areas. Three to 7 days after injury, complete neuronal loss was observed around the impact areas. These pathophysiological changes were similar to those described clinically in cortical and subcortical injury. A spatial correlation between the histological alterations and PBR binding was shown that reached highest values at 7 days after injury, at which time the lesion was consolidated and astroglial cells replaced neuronal cells (Toulmond et al., 1993).

The fluid percussion trauma model has also been used to measure interleukin (IL)-6 and tumor necrosis factor (TNF) levels in lesioned brain areas (Taupin et al., 1993). These mediators increased in the acute post-traumatic phase. The administration of Ro 5-4864 enhanced the increase of both mediators, suggesting the ability of PBRs to modulate the inflammatory reaction at the site of brain injury (Taupin et al., 1993). A conflicting observation was reported by Zavala et al. (1990). They found that i.p. injection of Ro 5-4864 inhibited the capacity of macrophages to produce IL-1, IL-6, and TNF. The cause of dissimilar effects of Ro 5-4864 on the abilities of brain astroglia and peritoneal macrophages to produce cytokines is not known.

Encephalopathy of various causes, such as hepatic insufficiency or thiamine deficiency, leads to an increase in PBRs as well as coinciding with gliosis in the latter (Lavoie et al., 1990; Leong et al., 1994). Portacaval anastomosis, a model of hepatic encephalopathy, also produced an increase in brain PBR, suggesting a putative role for neurosteroids (not inactivated by the liver) in modulation of PBRs (Giguère et al., 1992). Further studies will show whether PBRs can be used clinically for the

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assessment of the severity of brain lesions and encephalopathy.

VII. Peripheral Benzodiazepine Receptors in Cancer and in Immune Function

Attention has focused on the involvement of the PBRs in cell proliferation and differentiation for two main reasons: 1) the growing number of neoplastic tissues reported to show altered binding characteristics of PBRs (Table 4) and 2) the effects that ligands for PBRs have on differentiation and proliferation of normal and malignant cells in vitro (Table 5). Evidence of the involvement of ligands for PBRs in the regulation of cellular processes is accumulating and suggests a few pathways, depending on the tissue concerned.

During the 1980s, data regarding the change of PBR binding characteristics in neoplastic tissues accumulated and suggested a possible involvement in cancer. Ovarian carcinoma is a malignant disease that is usually diagnosed at late stages, making overall therapy less effective. Increased PBR density in human epithelial ovarian carcinoma was noticed in comparison to benign tumors (5-fold) and normal ovaries (3-fold; Katz et al., 1990b; Table 4). In binding experiments with [^{3}H]PK 11195, Katz et al. (1990c) demonstrated the presence of PBRs in normal colonic tissue and a 3.2-fold increase in PBR density for adenocarcinoma of the colon (Table 4). It has been shown for guinea pig ileum that diazepam inhibits the longitudinal smooth muscle contraction, coinduced by intracellular release of Ca^{2+} in a concentration-dependent manner. This finding may strengthen the theory that the inhibition of smooth muscle contraction and the reduction in digestive tract motility lead to a longer time of exposure of the intestinal mucosa to carcinogenic factors, which will consequently induce a higher occurrence of bowel cancer (Sugarbaker et al., 1982).

Several studies have demonstrated increased binding site densities for BZ ligands in various brain tumors (Ferrarese et al., 1989; Black et al., 1990; Ikezaki et al., 1990; Table 4). In particular, one study of PBR density in several types of brain tumors showed marked increases in high-grade astrocytoma and glioblastoma cells in comparison with normal brain parenchyma, whereas low-grade gliomas and meningiomas exhibited much lower elevations in PBR binding site densities (Cornu et al., 1992; Table 4). Olson et al. (1988b) examined PBR ligand binding in postmortem glioma samples and found high densities of PBR-specific binding in intact glioma tumor cells compared with cells of normal cerebral cortex or necrotic areas of the tumor. The clinical relevance is obvious, because PBRs may accurately delineate glioma borders with the use of positron emission tomography in preference to other, less accurate, imaging techniques (Starosta-Rubinstein et al., 1987; Van Dort et al., 1988). The PBR itself may be not only a proliferation marker or gliosis border delineator but also the route by which cancer cells may be specifically targeted. Verma et al. (1998) showed that the high affinity of porphyrins to PBRs, and their elevated levels in cancer cells allowed selective retention and cell-specific cytotoxicity through light irradiation (photodynamic therapy).

One study demonstrated that patients expressing high levels of PBR-immunoreactive cells were correlated with shorter life expectancies (Miettinen et al., 1995). In addition, in a recent study focusing on human breast cancer, PBRs were found to be highly expressed in aggressive metastatic human breast tumor biopsy samples compared with normal breast tissues (Hardwick et al., 1999). Moreover, it was found that the more aggressive the breast cancer cell lines, the more abundant were PBR ligand binding and mRNA. The same study went on to characterize the change in cellular location of PBR protein when the more aggressive and the less aggressive breast cancer cell lines were compared. The more aggressive cell lines showed a nuclear localization for PBR, as opposed to the “normal” or the less aggressive tumor mitochondrial location (Hardwick et al., 1999).

Matthew et al. (1981) showed that tyrosinase activity and melanin synthesis (differentiation markers) were induced by diazepam in B16/C3 mouse melanoma cells (Table 5). Furthermore, BZs with higher affinities to PBR showed higher potencies in melanogenesis induction. In human prostatic nodular hyperplasia, the density of PBR in response to Ro 5-4864 did not alter, but the affinity was lowered 15-fold in comparison with normal prostate (Escubedo et al., 1993; Table 4). Black et al. (1994) demonstrated that PK 11195 and Ro 5-4864 stimulated mitochondrial proliferation (1.6-fold) and growth hormone stimulation (2.4-fold) in pituitary tumor GH_3 cells (exposure to central-type BZ ligands did not have any effect; Table 5).

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TABLE 4
Alterations in PBR binding characteristics in various hyperplastic and cancer tissues

Tissue	Proliferative Disorder	Ligand Affinity to PBR	PBR Density	Reference
Ovary	Benign	Unaltered	Unaltered	Katz et al., 1990b
	Malignant	Unaltered	3- to 5-fold increase	Katz et al., 1990b
Colon	Adenocarcinoma	Unaltered	3-fold increase	Katz et al., 1990c
Prostate	Nodular hyperplasia	15-fold less for Ro 5-4864 and unaltered for PK 11195	Unaltered	Escubedo et al., 1993
Brain	High-grade astrocytoma	Unaltered	15-fold increase	Cornu et al., 1992
	C6 glioma	Unaltered	17-fold increase	Black et al., 1990
	LK Walter 256 metastatic	Unaltered	15-fold increase	Black et al., 1990

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TABLE 5
Effect of PBR ligands on cellular activity in different cell types

Cell Type	Type of Ligand	Activity Type	Reference
Pituitary cells	Diazepam, Ro 5-4864	Stimulation of mitosis	Pawlikowski et al., 1987
	PK 11195, Ro 5-4864	1.6-fold increase in number of mitochondria	Black et al., 1994
		2.5-fold increase in mitochondrial divisions	Black et al., 1994
		1.4-fold increase in number of nucleolar organizers	Black et al., 1994
C6 glioma	PK 11195, Ro 5-4864	Inhibition of cellular proliferation	Gorman et al., 1989
Glioma cells	PK 11195, Ro 5-4864	2.5-fold increase in number of mitochondria with elongated cristae	Shiraishi et al., 1991
	PK 11195	5-fold increase in mitochondrial divisions	Shiraishi et al., 1991
Lymphocytes (mouse spleen)	Diazepam, Ro 5-4864	Concentration-dependent inhibition of DNA synthesis	Pawlikowski et al., 1988
Lymphoma cells	PK 11195, Ro 5-4864, PPIX	No change in prolactin-activated mitogenesis	Gerrish et al., 1990
Thymus cells	Diazepam	Stimulation of mitosis	Stepien et al., 1988
	Ro 5-4864	Decrease in mitosis	Stepien et al., 1988
	Ro 15-1788	Unaltered mitosis	Stepien et al., 1988
B16/C3 mouse melanoma cells	Diazepam	Increase in differentiation markers	Matthew et al., 1981
MCF-7 breast cancer	PK 11195, Ro 5-4864	Inhibition of proliferation	Carmel et al., 1999
	Clonazepam	Unaltered	Carmel et al., 1999

PPIX, protoporphyrin IX.

A strong and positive correlation has been shown between the affinity of PBR ligands and the antiproliferative activity of mouse thymoma cells (Wang et al., 1984). Such a correlation was not found for CBR ligands. This finding reinforces the notion of PBR involvement in growth control and cellular proliferation. The effects that BZs have on different cells are not always similar, as they have been reported to inhibit mitogenesis in Swiss 3T3 cells while inducing mitogenesis in Friend erythroleukemia cells (Clarke and Ryan, 1980). Pawlikowski et al. (1988) noted concentration-dependent inhibition of cellular proliferation in mouse spleen lymphocytes by diazepam and Ro 5-4864 (Table 5). Increased mitochondrial proliferation and morphological changes in glioma cells were shown to take place after the application of PK 11195 and Ro 5-4864 (Shiraishi et al., 1991; Table 5). Carmel et al. (1999) found that PK 11195 and Ro 5-4864 inhibited MCF-7 breast carcinoma cell line proliferation at concentrations of 10^{-5} to 10^{-4} M, whereas clonazepam (CBR-specific ligand) had no effect (Table 5). Ikezaki and Black (1990) showed that for C6 glioma cells, growth rate and thymidine incorporation increased 20 to 30% after PK 11195 stimulation in the nanomolar range. They found a correlation between the affinity of peripheral BZs for PBRs in the nanomolar range and their potency for inhibiting proliferation (in the micromolar range), although it is difficult to explain inhibition through high-affinity receptors for BZs. This three-orders-of-magnitude discrepancy does not permit unequivocal proof of antiproliferative action through PBR. Furthermore, Gorman et al. (1989) showed that rat NCTC epithelial cells and mouse Sp2/0-Ag14 hybridoma cells, which do not possess any detectable levels of PBR, were inhibited by PK 11195 and Ro 5-4864 (Table 5). This result suggests the possibility of another pathway for the inhibition of cellular growth.

The association between PBRs and Ca^{2+} channels (see above) suggests a role in PBR-related proliferation and differentiation. Increased cytoplasmic levels of Ca^{2+} are a prerequisite for mitosis in eukaryotic cells. Studies on the effect of PBR ligands on Ca^{2+} channels showed that at high concentrations (micromolar range), these ligands inhibited Ca^{2+} flow through plasma membranes by modulating voltage-sensitive Ca^{2+} channels (Cantor et al., 1984). In addition, the same group found that a Ca^{2+} ion channel blocker was displaced by the PBR ligand Ro 5-4864 in cardiac, renal, and cerebral membranes. Similar results were obtained by Python et al. (1993), who reported that PBR ligands may block Ca^{2+} ion flow through voltage-activated channels in glomerulosa cells of the adrenal gland.

The evidence of PBR involvement in the regulation of cellular proliferation suggests a few pathways, one of which might be modulation of the capability of the immune system in elimination of neoplasms. Stepien et al. (1988) demonstrated that different BZs modulate thymus cell proliferation, thus regulating immune system function (Table 5). Numerous observations indicate that macrophages play a significant role in the immune response to tumors. For example, macrophages are often observed to cluster around tumors, and their presence is often correlated with tumor regression. BZs have been found to bind to specific receptors on macrophages and to modulate in vitro their metabolic oxidative responsiveness (Lenfant et al., 1985). Zavala et al. (1990) showed that the capacity of macrophages to produce IL-1, TNF, and IL-6 was inhibited by i.p. injection of Ro 5-4864 (with no effect by the central-type BZ clonazepam). This result demonstrated in vivo immunosuppressive properties of PBRs and mixed ligands, but not of CBR ligands, affecting characteristic phagocyte functions involved in host-defense mechanisms.

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The antitumor activity of macrophages is probably mediated by several macrophage products. Activation of macrophages with interferon (IFN)-γ and macrophage-activating factor increases not only their secretion of various products but also their cytotoxicity to tumor cells. Peripheral BZ ligands have been found to potentiate the antiproliferative activity of recombinant human IFNs (Solowey et al., 1990). Activated macrophages secrete large amounts of lytic enzymes, which can reach high levels around a tumor, especially if antitumor antibodies bind to Fc receptors on macrophages and serve to bridge the macrophages to the tumor. Diazepam (both a CBR and a PBR ligand) and Ro 5-4864 (a PBR ligand) have been found to inhibit neutrophil chemotaxis and superoxide production in a stimulus-dependent way, thus possibly affecting antitumor activity (Finnerty et al., 1991). Macrophages also secrete a cytokine called TNF-α that has potent antitumor properties. When cloned, TNF-α was injected into tumor-bearing animals; it induced hemorrhage and necrosis of the tumor. Interestingly, in response to TNF-α and IL-2β, PBR density increased in both a dose- and time-dependent manner in cultured polygonal astrocytes (Oh et al., 1992). Supporting results were obtained by Matsumoto et al. (1994), who showed that PBR modulates lipopolysaccharide-induced TNF activity in mouse macrophages.

Prenatal exposure to low doses of BZs can result in long-lasting alterations of the cytokine network, as indicated by the reduced release of TNF-α, IL-1, IL-6, IL-2, and IFN-γ (Schlumpf et al., 1993). The concomitant reduction of PBR on macrophages has been suggested as a possible link between prenatal treatment and postnatal disturbed immune function.

Rosenberg (1984) injected FBL-3 lymphoma into the footpad of syngeneic mice and found that in the presence of high concentrations of cloned IL-2, large numbers of activated lymphoid cells were generated that could kill fresh tumor cells but not normal cells. Rosenberg called these cells lymphokine-activated killer cells. These cells appear to be a heterogeneous population of lymphoid cells that include natural killer (NK) cells and natural cytotoxic cells. Bessler et al. (1997) demonstrated that Ro 5-4864 and PK 11195 significantly and specifically suppressed NK cell activity; this result was completely reversed by the addition of human recombinant IL-2 or human leukocyte IFN. This suppression of NK cells may connect the involvement of PBRs in reducing the immunological capacity for eliminating malignant growths. On the other hand, Solowey et al. (1990) demonstrated that peripheral-acting BZs were found to potentiate the antiproliferative action of recombinant human IFNs, which have been found to inhibit cell division of both normal and malignantly transformed cells in vitro, perhaps through modulation of major histocompatibility complex (MHC) expression, whereas IFN-γ has been shown to increase the expression of class II MHC on macrophages.

These findings are significant if a better understanding of the specific modes of PBR involvement in the tumoricidal activity of the immune system, which might be relevant to the control of cell growth and tumorigenesis, is to be reached.

VIII. Summary and Future Prospects

The combined facts that the *PBR* gene is conserved throughout evolution from bacteria to humans and that this gene appears to have the hallmarks of a typical housekeeping gene suggest that this gene's product has a basic cellular function. Even though many functions have been attributed to this gene product, its primary roles remain an open question. Hence, a resolution of the primary functions of the *PBR* gene products must be a central theme for future work with PBRs.

One of the often-suggested putative roles attributed to PBRs is as the rate-limiting step in steroidogenesis. The relevance of PBRs for steroidogenesis is reflected by their abundance in the adrenal gland and in male and female gonadal tissues. It is explained that this role is mediated through regulation of cholesterol transport from the cytoplasm to the mitochondrial matrix. Because another protein, StAR, has the same attribute, either a biochemical redundancy is apparent or some interaction between these proteins may mediate mitochondrial cholesterol transport.

Another putative function for PBRs involves a regulatory role in cell proliferation. This role may be closely associated with some type of regulatory function in cellular respiration, which also is one of the many assigned roles for the *PBR* gene product. Hence, it has been argued that the dysregulation of PBRs in some tissues may lead to diseases of cellular proliferation, including cancer. A number of reports have, in fact, found PBR overexpression in particular tumor types and some transformed cell lines. In all of these cases, a causative pathology for PBR overexpression has never been shown, hence not ruling out a passive coincident dysregulation of the expression of *PBR* genes in these tumors. In support of this, we recently reported that on antisense knockout of PBRs in a mouse Leydig tumor cell line, no apparent changes in the cell proliferation or cell cycle were measurable (Kelly-Hershkovitz et al., 1998). We acknowledge that this may be unique to these specific cells, and hence a more extensive study must still be undertaken. Furthermore, other as-yet-undefined effects of PBR overexpression may be important in malignancy. In this respect, the modulatory role of PBRs on immune system function should also be taken into consideration.

In addition, at behavioral levels, PBRs appear to be involved in the biological coping with stress and anxiety disorders. It has been suggested that PBRs play a role in the regulation of several stress systems such as the HPA axis, the sympathetic nervous system, the renin-angio-

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tensin axis, and the neuroendocrine-immune axis. In these systems, acute stress typically leads to increases in PBR density, whereas chronic stress typically leads to decreases in PBR density. Furthermore, in GAD, PD, GSP, and PTSD, PBR density is typically decreased in platelets. In the brain, where PBRs are associated with glial cells, PBRs are increased in specific brain areas in neurodegenerative disorders and also after neurotoxic and traumatic-ischemic brain damage. These accumulating data indicate a possible role for PBR in adaptation of the organism to stress and brain damage.

Because PBRs appear to be involved in a large variety of physical diseases, mental disorders, and responses to stress, clinical benefit may be attainable by the increasing pharmacological knowledge surrounding these receptors. Nevertheless, there still is much to be learned about the structure of PBRs as well as their cellular location, regulation of gene expression of the PBR subunits, and the interaction between PBR subunits. As mentioned above, other proteins like StAR may also be involved in this complex. A molecular understanding of the protein component subunits, as well as how they fit together and function as a whole, still requires much work. The 18-kDa isoquinoline-binding PBR subunit is not only found on the mitochondrial membrane; it is also found to a lesser extent on the plasma membrane of the cell, as well as on the membrane of other cellular organelles. Its role, structure, and other interacting subunits in these other cellular locations also must be addressed.

Much has been learned in the decade since the 18-kDa PBR subunit mRNA was cloned and still much must be learned from and about the genes encoding PBR subunits. This will involve many levels of study. It may be that once we understand why evolution so carefully preserves the sequence of the 18-kDa PBR subunit gene, we will be able to uncover new biochemical pathways that will link the various putative PBR functions now being discussed. Only the future can tell.

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