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COMMENTARY

Multiple Forms and Locations for the Peripheral-Type Benzodiazepine Receptor

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ABSTRACT. The pharmacological effects of benzodiazepines are mediated through a class of recognition sites associated with the neuronal γ-aminobutyric acid$_A$ ($GABA_A$) receptor. A second class of benzodiazepine binding sites is found in virtually all mammalian peripheral tissues, in blood cells, and in glial cells in the brain, but its functions remain unclear. Although these peripheral-type benzodiazepine binding sites (PBBS) have been localized to the mitochondrial outer membrane in many tissues, a growing body of evidence suggests that they may also exist on the plasma membrane. Plasma membrane PBBS have been described in heart, liver, adrenal, and testis and on hemopoietic cells. In rat liver, the two subcellular forms of PBBS are found separately in two different subpopulations of cells. The discovery of a plasma membrane fraction of PBBS clearly has implications for some of its putative functions, including steroidogenesis, mitochondrial respiration, heme metabolism, calcium channel modulation, cell growth, and immunomodulation. This commentary reviews the evidence for two locations for the PBBS and discusses the relevance of mitochondrial and plasma membrane forms with regard to structure, molecular biology, and proposed roles. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;12:1805–1814, 1996.

KEY WORDS. peripheral-type benzodiazepine receptor; mitochondrial outer membrane; plasma membrane; DBI; subcellular location; multiple forms

Benzodiazepines are a class of widely prescribed anxiolytic, anticonvulsant, and sedative drugs whose pharmacological effects are mediated through high-affinity binding sites on neuronal post-synaptic plasma membrane $GABA_A$† receptors, which are GABA-gated chloride channels [1, 2]. During the discovery of these "central"-type sites in the brain, specific binding of radiolabelled diazepam to binding sites in mitochondrial fractions from peripheral tissues in the rat was also observed [3], and this second class of sites was termed the PBBS or PBR. The two classes of benzodiazepine recognition sites differ in their ligand specificity. Whereas diazepam binds to both types of site with high affinity, clonazepam and 4′-chlorodiazepam (Ro5 4864) are high affinity benzodiazepine ligands whose binding is exclusively to the central-type or the peripheral-type site, respectively [3, 4]. A chemically unrelated class of compounds, the isoquinoline carboxamides, bind with high affinity to the PBBS only [5]. The central- and peripheral-type benzodiazepine binding sites also differ in their cellular location and structure (Table 1), and whereas the association of the central-type sites with the $GABA_A$ receptor is well-established [1], no single physiological role has been assigned to the PBBS although considerable evidence has been presented for a function in steroidogenesis [28]. Putative endogenous ligands for the PBBS include porphyrins and a 10 kDa cytosolic protein, DBI [9, 11]. A recent review describes in detail the properties of the peripheral-type receptor [29].

The term "peripheral-type" receptor or binding site, although widely used throughout the literature, has been shown to be something of a misnomer for the non-neuronal class of benzodiazepine receptors since the discovery of the "peripheral" receptor in glial cells in the CNS [12]. An alternative name, "mitochondrial-type" benzodiazepine receptor, has been suggested due to the mitochondrial membrane location of this site in many tissues [13–16], but this terminology is also inaccurate since non-mitochondrial fractions of PBBS have been described in heart [17], adrenal [18], testis [19], liver [14], and human hemopoietic cells [30]. In liver, the mitochondrial and non-mitochondrial forms of the PBBS are discretely located in two different cell types: in hepatocytes and in non-parenchymal/biliary epithelial cells, respectively [31].

This article describes the evidence for two subcellular locations of the PBBS, and discusses the implications of a second location in terms of its proposed structure and diverse putative functions.

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† *Abbreviations:* $GABA_A$, γ-aminobutyric acid$_A$; PBBS, peripheral-type benzodiazepine binding site(s); PBR, peripheral-type benzodiazepine receptor; Ro5 4864, 7-chloro-1,3-dihydro-methyl-5-(*p*-chlorophenyl)-2*H*-1,4-benzodiazepine-2-one hydrochloride; PK 11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide; PK 14105, 1-(2-fluoro-5-nitrophenyl)-3-isoquinoline carboxylic acid; DBI, diazepam binding inhibitor; ANC, adenine nucleotide carrier; VDAC, voltage-dependent anion channel; and ACBP, acyl coenzyme A binding protein.

TABLE 1. Comparison of some of the properties of the two classes of benzodiazepine receptor

	"Peripheral"	Central	References
Synthetic ligands	Benzodiazepines, e.g. Ro5 4864 > diazepam	Benzodiazepines, e.g. clonazepam > diazepam	4
	Imidazopyridines	Imidazopyridines	
	Isoquinoline carboxamides, e.g. PK 11195		5
	Quinoline propanamides		6
	Benzothiazepines		7
Endogenous ligands	DBI	DBI/Endozepine	8–10
	Porphyrins		11
Cellular location	Peripheral tissues, blood cells	CNS-neuronal cells	3
	Glial cells		12
Subcellular location	Mitochondrial outer membrane	Plasma membrane	13–19, 20
	Plasma membrane		14,17–19
Structure	18 kDa, 32 kDa protein complex (±30 kDa protein)	~55 kDa α,β,γ subunits	21,20
Proposed function	Steroidogenesis	GABA-gated Cl^- uptake	22,20
	Heme biosynthesis		23
	Cell proliferation		24
	Immunomodulation		25
	Ca^{2+} channel modulation		26
	Mitochondrial respiration		27

CELLULAR LOCATION

PBBS have been found in virtually all mammalian tissues [32] as well as in the brain [12]. The density of the PBBS is highest in endocrine tissue such as adrenal gland, testis, ovary, uterus, and placenta [33–35]. It is abundant in kidney, heart, and platelets [36, 37] and is also present in erythrocytes [38]. A differential distribution has been described in various organs. Light-microscopic autoradiographic techniques using ligands specific for the PBBS showed its presence in rat adrenal cortex, testis interstitial tissue, the distal convoluted tubule and the ascending loop of Henlé in kidney, and in the choroid plexus and the ependymal lining of cerebral ventricles in the brain [34, 39]. Immunocytochemical staining using an antiserum raised against the 18 kDa-protein component of the PBBS has confirmed these staining patterns and has also shown a high density in biliary epithelial cells of the liver [40], a tissue in which there is an overall low level of the receptor [34].

MITOCHONDRIAL LOCATION

Indirect evidence for an association of the PBBS with mitochondria-rich tissues first arose from autoradiographic investigations with [^{3}H]Ro5 4864 in whole-body sections of rats [32]. Subsequently, the subcellular distribution pattern of specific binding sites for PK 11195 in rat adrenal, testis, lung, kidney, heart, skeletal muscle, liver, and brain was shown to correspond closely to that of the mitochondrial marker succinate dehydrogenase, and was unrelated to marker enzymes for plasma membranes, lysosomes, or endoplasmic reticulum [16].

Using autoradiography with [^{3}H]PK 11195 and also subcellular fractionation, a mitochondrial outer membrane location for the receptor was first established in the rat adrenal gland [15]. Subcellular fractionation, density gradient centrifugation, density perturbation, and transmission electron microscopy confirmed this location for the PBBS in rat kidney and liver, although a non-mitochondrial fraction of PBBS was also observed in liver [13, 14]. Immunogold labelling, using antibodies to the 18 kDa-protein component of the PBBS, has demonstrated the PBBS on the mitochondrial outer membrane of Leydig cells [41].

A reported mitochondrial inner membrane location for the binding site in guinea pig lung [42] may reflect the location of the receptor at contact sites where the two mitochondrial membranes are in close association. This will be discussed below.

NON-MITOCHONDRIAL LOCATION

Blood Cells

Sites that bind specific ligands with the rank order of affinities expected for the PBBS have been described in human erythrocytes [38]. A later study examined ligand binding and mRNA expression of the PBBS in human blood cell subpopulations and found the highest density in monocytes and neutrophils, with an intermediate density in lymphocytes and only low levels in platelets and erythrocytes [30]. Both of these reports concluded that the PBBS must be located on the plasma membrane: in erythrocytes because the mitochondria are extruded before leaving the bone marrow, and in neutrophils because mitochondrial content decreases along the granulocyte differentiation pathway. The authors suggested that, upon extrusion of the mitochondria, part of the mitochondrial outer membrane may fuse with the plasma membrane. However, an absence

of monoamine oxidase activity indicated that the peripheral receptors may not be remnants of mitochondrial exclusion [38]. A plasma membrane location for the PBBS in neutrophils would also explain the observation that a monoclonal antibody which recognized the 18 kDa subunit of the receptor stimulated the oxidative burst in intact human polymorphonuclear lymphocytes [43].

The first report to suggest two locations for the PBBS in blood cells demonstrated high affinity binding of PBBS-specific ligands to intact lymphocytes and to cell-free preparations [44]. Immunogold labelling with anti-PBBS antibodies confirmed a cell surface site. Although the authors concluded that binding in the cell-free preparations was to the mitochondrial form of the PBBS, plasma membranes would also have been present in their assays. However, the different ability of endogenous ligands to displace binding in the two preparations suggested separate fractions of the PBBS [44]. In another study, autoradiography following photoaffinity labelling of human leucocytes with an isoquinoline carboxamide has provided direct evidence for a mitochondrial location of the PBBS in blood cells [45].

Peripheral Tissues

An investigation of the subcellular distribution of the PBBS in rat and canine cardiac membranes demonstrated high levels of high affinity binding of PBBS-specific ligands in both sarcolemmal and sarcoplasmic reticular fractions [17]. Neither of these fractions had high levels of the mitochondrial marker, succinate dehydrogenase, demonstrating a lack of mitochondrial contamination. Considerably lower levels of binding were observed in mitochondrial preparations from the same tissues [17]. A cell surface location in rat fibroblasts has been suggested to explain the observation that high affinity binding of specific PBBS ligands to both intact fibroblasts and crude membrane preparations was inhibited to the same extent by a quaternary amine derivative of PK 11195 that does not cross the plasma membrane [46].

Antibodies to the 18 kDa component of the PBBS have been used in an investigation of its cellular and subcellular distribution in mouse adrenal tissue [18]. Immunofluorescence and immunocytochemical and confocal microscopy techniques confirmed an absence of the PBBS from adrenal medulla [34], whereas both cytoplasmic and cell surface locations were observed in both the zona glomerulosa and the zona fasciculata of the cortex [18]. In both cortical zones, the diffuse cytoplasmic staining was coincident with the immunostaining pattern of $P\text{-}450_{SCC}$, a marker for the mitochondrial membranes. Confocal microscopy also demonstrated a pronounced plasma membrane location in the zona fasciculata [18].

In an examination of the distribution of the PBBS in rat testis, using biotin-streptavidin immunoperoxidase immunocytochemistry with anti-human-PBBS antiserum, immunostaining was observed only in Leydig cells [19]. Subsequent immunostaining and confocal microscopy provided evidence for both a mitochondrial and a cell surface localization of PBBS in MA-10 Leydig cells [19].

Isopycnic density gradient centrifugation of crude mitochondrial fractions from rat liver resulted in the separation of two populations of PBBS. Whereas the higher density population was mitochondrial in origin, a lower density population of PBBS did not coincide with markers for Golgi, lysosomes, peroxisomes, microsomes, or nuclei, and thus a possible plasma-membrane location was suggested [14]. Immunocytochemistry showed high levels of the 18 kDa component of the PBBS in biliary epithelial cells [40], although a definite subcellular location could not be inferred. However, binding assays and photoaffinity labelling in isolated hepatocyte and non-parenchymal/biliary epithelial cell populations showed the presence of the PBBS in both types of cell; the hepatocyte fraction was mitochondrial, whereas the non-parenchymal/biliary epithelial cell fraction was non-mitochondrial as shown by density gradient centrifugation [31].

STRUCTURE OF THE PBBS

Does the discovery of a plasma membrane form of the PBBS have implications for its proposed structure?

Photoaffinity labeling with an isoquinoline carboxamide, PK 14105, first identified an 18 kDa membrane protein as a component of the PBBS [47], whereas the benzodiazepines AHN 086 and flunitrazepam photoaffinity-labelled proteins of 30 kDa and both 30 and 35 kDa, respectively, albeit non-specifically [48, 49]. These results may be interpreted as implicating separate polypeptides within an oligomeric complex as the binding sites of isoquinoline carboxamides or benzodiazepines, even though their binding is mutually exclusive at nanomolar concentrations. However, superimposition of PK 11195 and Ro5 4864 in their supposed receptor-bound conformations indicates that these ligands may bind to a single binding site by sharing the same type of fundamental interactions [7].

The 18 kDa protein has been isolated and the cDNA has been cloned from rat, human, bovine, and murine tissues [50–53], and early expression studies indicated that this protein contains the binding domains for both isoquinoline- and benzodiazepine-type ligands [50, 52, 54]. However, purification of the PBBS in a form that retained reversible ligand binding activity seemed to indicate a close association of this 18 kDa protein with the VDAC (32 kDa) and the ANC (30 kDa) proteins of the mitochondrial outer and inner membranes, respectively, although no evidence of a functional association was provided [21]. It was suggested that the co-purification of these three proteins was indicative of the presence of the PBBS at areas of intimate association between the mitochondrial inner and outer membranes, contact sites [21]. This would account for the observed mitochondrial location for the PBBS which has been described previously, but such a structure would appear to be incompatible with a plasma membrane location.

More recently, cDNA corresponding to the 18 kDa pro-

tein isolated from murine Leydig tumor cells has been expressed both in mouse fibroblasts, where the density of both PK 11195 and Ro5 4864 binding sites increased, and, as a fusion protein, in *Escherichia coli* where only PK 11195 binding was observed [53]. The addition of purified VDAC to liposomes containing the fusion protein resulted in the binding of both ligands in *E. coli*, leading the authors of this comprehensive study to conclude that the isoquinoline binding site is on the 18 kDa protein subunit of the PBBS and that both the 18 kDa protein and VDAC are required for the binding of benzodiazepines [53]. The observation of benzodiazepine ligand binding in other expression studies may be explained by the fact that the mammalian cells used for transfection all contain VDAC [50, 52, 53], and, although *Saccharomyces cerevisiae* is devoid of PBBS, the presence of a VDAC (with homology to mammalian VDAC [55]) may explain the expression of binding sites for both types of ligand following transfection of yeast with human PBBS cDNA [54]. Therefore, there seems to be considerable evidence for a functional association of the 18 kDa protein and VDAC in the PBBS, but although ANC may copurify with these proteins [21], it does not appear to play a role in ligand binding. An association between the PBBS and anion channels in rat kidney has been reported following the observation of a strong correlation between the permeability of anions, relative to Cl^-, and their efficacies to inhibit [^{3}H]Ro5 4864 binding [56]. Also [^{3}H]Ro5 4864 but not [^{3}H]PK 11195 binding was reversibly inhibited by the ion transport blocker 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid [56].

VDAC or porin is a major integral protein of the mitochondrial outer membrane where it forms a channel for the non-specific passage of low molecular weight solutes [57, 58]. A portion of VDAC is located at mitochondrial contact sites [59]. It has also been demonstrated in the plasma membrane of mammalian cells including lymphocytes, epithelial cells, muscle and rat liver parenchymal cells (see Ref. 60 for a recent review) and associated with $GABA_A$ receptors in mammalian brain [61].

Consequently, the reported functional requirement for an association of the 18 kDa protein of the PBBS with VDAC is entirely consistent with either a mitochondrial or a plasma membrane location for these sites.

MOLECULAR BIOLOGY

The cDNA sequence of the 18 kDa protein specifies an open reading frame of 169 amino acids with high sequence similarity (~80%) between species, and indicates that the PBBS is not encoded in the mitochondrial genome [50–53]. Hydropathy analysis suggests that there are five potential transmembrane domains and two major hydrophilic regions, which is consistent with a membrane association of this protein [49]. The 18 kDa PBBS protein is rich in tryptophan residues, and has significant sequence similarity with the 17 kDa outer membrane protein product of the tryptophan-rich sensory protein gene (*tspO*, formerly *crtK*) of *Rhodobacter* [62, 63]. A role in photosynthetic membrane formation or in carotenoid biosynthesis has been suggested for the *Rhodobacter* TspO. Northern blot analysis of PBBS expression in nine rat and four bovine tissues revealed a single mRNA in all cases corresponding to the size of the 18 kDa component of the PBBS [50, 52]. A single species of mRNA for the PBBS in rat heart and adrenal tissue may suggest that there is a single or nearly identical amino acid sequence for the 18 kDa protein at the two locations in which it is found in these tissues. In liver, photoaffinity labelling and equilibrium binding studies on the mitochondrial and plasma membrane forms of the PBBS suggest very similar, if not identical, 18 kDa proteins [31], and an antiserum raised against the adrenal mitochondrial 18 kDa protein cross-reacted with the biliary epithelial membrane form of the PBBS, also indicating a strong similarity in proteins from the two locations [40].

The gene encoding the PBBS has been characterized from rat and human tissue [64, 65]. The gene comprises four exons spanning about 10 or 13 kb, respectively, interrupted by three introns, one of which is 6–8 kb long and is located within the 5′ non-coding region [64, 65]. Reverse transcriptase-polymerase chain reaction of human total cellular RNA from several tissues specifically amplified two cDNA products; the entire predicted PBBS sequence was contained in one product, but the second fragment carried a 211-bp internal deletion, lacking all of exon 2 [65]. This smaller, alternately spliced form of PBBS mRNA results in an altered reading frame and does not encode a protein with the binding properties of PBBS so it cannot code for a second form of the PBBS. The smaller mRNA is expressed at ten times the level of PBBS mRNA, but no function has been proposed for this species. Its 102-amino acid open reading frame does not bear any significant resemblance to any protein in GenBank, and it is not known whether the protein product is produced [65]. Such alternate splicing has not been described for the rat gene [64], but if exon 2 were deleted, a peptide product of only 62 amino acids would result, and would be clearly unrelated to the PBBS 18 kDa protein [65].

In most cases, the information for targeting of proteins to the mitochondrion is located in one or more cleavable peptide sequences at the *N*-terminal end, but cleavage does not seem to occur on proteins destined for the outer membrane [66]. One well-studied mitochondrial outer membrane protein, a major 70 kDa yeast protein, contains a short matrix-targeting sequence followed by a long stretch of hydrophobic amino acids. Any deletion within the 41 *N*-terminal amino acids interferes with transport of this protein to the outer membrane [66]. However, the deduced amino acid sequence of PBBS does not contain a typical mitochondrial matrix-targeting sequence although the first 30 amino acids comprise a hydrophobic domain [50].

Three possible differences could lead to plasma membrane- rather than mitochondrial-targeting. The amino

acid sequence could be modified to insert a glycosylation site, an *N*-terminal hydrophobic presequence could be added and/or replaced, or codons could be replaced in one or more positions to either insert a plasma-membrane targeting motif or delete a mitochondrial targeting motif. The lack of multiple-labelled bands from either photolabelling or immunoblotting from PBBS-rich tissues would appear to rule out glycosylation as a targeting motif in this case. It remains to be discovered what causes the different targeting of the two forms of the PBBS.

In the case of rat liver, the non-mitochondrial fraction of the PBBS has been found in biliary epithelial cells [31, 40]. The polarized cell surface location of plasma membrane proteins in epithelial cells is governed by a range of targeting determinants [67]. A heterogeneous class of cytoplasmic domain sequences specify basolateral targeting, and in the absence of these signals membrane proteins are transported to the apical surface [67]. During subcellular fractionation of biliary epithelial cell membranes, PBBS ligand binding activity did not correlate with that of the apical plasma membrane marker, γ-glutamyl transferase [31]. The identification of a discrete structural motif for basolateral targeting, and its presence or absence in the PBBS 18 kDa protein, would aid identification of a specific subcellular location in biliary epithelial cells.

FUNCTIONS

Benzodiazepines, including diazepam, are extensively used therapeutic drugs that alleviate anxiety, convulsions, and insomnia due to their interaction with the central-type benzodiazepine binding site associated with the $GABA_A$ receptor. However, although diazepam also binds potently to the PBBS, a clear pharmacological role has not been established for this non-neuronal site. The presence of the PBBS in both mitochondria and plasma membrane locations has implications for some of the putative roles.

Mitochondrial Steroidogenesis

The finding that the PBBS is most abundant in endocrine tissues, coupled with its mitochondrial outer membrane location first suggested a steroidogenic function. A range of ligands with binding affinity for the PBBS differing by four orders of magnitude modulated steroid biosynthesis with potencies that correlated with their affinities but at concentrations much higher than those necessary to saturate the PBBS [28]. Increased rates of steroid synthesis in isolated mitochondria as well as in steroidogenic cells has been shown to be due to an increase in the rate-limiting transfer of cholesterol from the outer to the inner mitochondrial membrane (see Ref. 28 for a review). Endogenous ligands such as DBI and, to a lesser extent, porphyrins have also been found to stimulate steroid production, suggesting an interaction with the mitochondrial PBBS [28, 68].

Glial cells synthesize neurosteroids [69], so-called because they are synthesized and are believed to act locally in the brain via an indirect action on the $GABA_A$ receptor complex [70]. Ro5 4864, a specific PBBS ligand, regulates glial cell steroidogenesis, suggesting a mechanism involving the PBBS [71, 72], and thus it appears that ligands acting at the PBBS may also ultimately affect the function of the $GABA_A$ receptor. No evidence has been presented to support an involvement of the plasma membrane form of the PBBS in steroid synthesis.

An early suggestion of a location for the mitochondrial PBBS at contact sites [73], borne out by the finding that VDAC is functionally associated with the 18 kDa protein [53], supports the proposed role for PBBS in mitochondrial steroidogenesis. Contact sites are dynamic areas of intimate contact between the two mitochondrial membranes whose formation is increased in phosphorylating (state III) mitochondria. Contact site components include a fraction of VDAC with a high affinity for hexokinase, a fraction of the ANC, and creatine kinase, and it has been proposed that such channels may play a key role in regulating energy metabolism in cells [74].

Mitochondrial Respiration

A function for the PBBS in modulating cellular energy metabolism was proposed in a report describing the inhibition of the respiratory control ratio due to the binding of specific PBBS ligands, both synthetic and endogenous [27]. It is tempting to suggest that interactions at mitochondrial contact sites could explain such an effect. However, in a more recent report it has been shown that PBBS ligands affect respiration only at very high concentrations and with no correlation to binding site density or pharmacology [75]. Furthermore, the energized state of mitochondria, and hence the degree of contact site formation, does not appear to have any effect on PBBS ligand binding [31].

It therefore appears unlikely that there is a direct function for the mitochondrial form of the PBBS in mitochondrial respiration.

Heme Metabolism

The finding that porphyrins are endogenous ligands for the PBBS [11] points towards a role for the mitochondrially located PBBS in heme biosynthesis. During heme biosynthesis, cytosolic coproporphyrinogen III traverses the mitochondrial outer membrane and is converted, via protoporphyrin IX, to heme which is subsequently exported from the mitochondria. Diazepam, albeit at a concentration vastly in excess of its K_d value for the PBBS, induced the differentiation of mouse erythroleukemia cells, and PBBS mRNA was induced in parallel with mRNA for heme biosynthetic enzymes although the levels of VDAC remained unchanged [76]. It has been reported that protoporphyrin, hemin, and coproporphyrinogen can displace the specific binding of [^{3}H]PK 11195 to the PBBS, and that benzodiazepines, by inhibiting binding to the PBBS, decrease coproporphyrinogen conversion to protoporphyrinogen [23].

Interestingly, the authors reported binding of [^{3}H]PK 11195 to two classes of PBBS, both with nanomolar K_d values, whereas heme bound only to the higher affinity site [23]. It may be speculated that these two classes of PBBS may correspond to the receptor from two different locations, because the fractions that were assayed (Cos-1-cell homogenates and mouse liver crude mitochondrial membranes) would have contained both mitochondrial and plasma membranes. However, PBBS from different locations may not necessarily show different ligand binding affinities since a single population of high affinity binding sites has been reported in crude mitochondrial membrane preparations from whole rat liver even though both mitochondrial and plasma membrane fractions of PBBS have been demonstrated in this tissue [31].

Evidence that there is an *in vivo* interaction of porphyrins with the PBBS has been provided by the observation of reduced [^{3}H]PK 11195 binding in induced hepatic porphyria in rats [77].

An involvement of PBBS in modulation of mitochondrial transport of porphyrinogens and heme may occur at contact sites [23]. In patch clamp studies on isolated rat heart mitoplasts, nanomolar concentrations of protoporphyrin IX as well as Ro5 4864 and PK 11195 influenced the activities of two ion channels of the mitochondrial inner membrane at potencies consistent with their affinities for the PBBS [78]. The central-type ligand clonazepam did not elicit any effect. Since PBBS ligands are thought to bind to an 18 kDa protein of the mitochondrial outer membrane, these results may only be explained by portions of the outer membrane remaining closely associated with the inner membrane mitoplasts, as would occur in contact sites.

Therefore, it seems likely that the mitochondrial form of the PBBS may be located in contact sites where it appears to be involved in translocation of cholesterol, porphyrinogens, and heme.

Calcium Channel Modulation

Several early studies suggested a possible interaction between the PBBS and voltage-dependent Ca^{2+} channels on plasma membranes. Decreased action potential duration and contractility in guinea pig heart muscle was observed in the presence of either Ro5 4864 or calcium channel blockers, and this effect was reversed in the presence of PK 11195 or extracellular calcium [79]. Although Ro5 4864, but not central-type ligands, appeared to competitively displace dihydropyridine calcium channel blockers from rat heart, kidney, and brain, this effect was only observed with higher concentrations of benzodiazepine than would be required to saturate the PBBS [80]. Similarly, ligands specific for PBBS decreased ileal smooth muscle contraction, but with poor correlation to their affinities for the PBBS [81]. Whereas the effects of specific dihydropyridine calcium channel blockers were abolished by PK 11195 [26], separate binding sites for PBBS-ligands and dihydropyridines, with parallel subcellular locations, have been described subsequently in rat heart, and no direct or allosteric interaction between the two types of site could be demonstrated at concentrations of ligands up to 10^{-5} M [17]. It has been postulated that a sarcolemmal subpopulation of PBBS of either high or low affinity may modulate voltage-dependent calcium channels indirectly through an interaction with dihydropyridine receptors [82]. An unrelated type of receptor, a low-affinity neuronal benzodiazepine binding site ("micromolar" receptor), has been described to mediate benzodiazepine effects on calcium channels in the brain [83], and it may be that some of the effects described above could be through this type of receptor.

A plasma membrane form of the PBBS binds specific ligands with K_d values in the nanomolar range [31]. However, because the effects of such ligands on calcium channels are elicited only by concentrations well in excess of those at which they interact with the PBBS, a functional coupling of the plasma membrane PBBS with calcium channels appears to be unlikely.

Cell Proliferation and Differentiation

The reported effects on cell growth by ligands that bind to the PBBS include differentiation of Friend erythroleukemia cells [84], induction of ornithine carboxylase and neurite outgrowth in PC12 cells [85], and superinduction of c-*fos* by nerve growth factor in PC12 cells [86]. However, there was no clear correlation between the specificity of ligands in mediating these effects and their specificity for the PBBS. Fifteen peripheral-type benzodiazepines inhibited the proliferation of mouse thymoma cells with affinities comparable to those for the PBBS, but the concentrations of ligand required were three orders of magnitude greater than necessary to saturate the PBBS [24]. The antiproliferative actions of PK 11195 and Ro5 4864 on rat C6 glioma and mouse neuro-2A neuroblastoma cells were also observed only at greater than micromolar concentrations, and it was concluded that this effect was not mediated via the PBBS [87].

More recently, it has been shown that nanomolar concentrations of PBBS ligands and DBI increased [^{3}H]thymidine uptake and cell numbers in MA-10 Leydig cells, whereas an inhibitory effect on cell growth was reported for micromolar concentrations of the same ligands [19]. Competition and reversibility studies indicated that these effects were mediated via the PBBS. However, whereas both PK 11195 and Ro5 4864 reversed the mitogenic effect, only PK 11195 could reverse the DBI inhibition of DNA synthesis [19], suggesting that this latter phenomenon may not be through the PBBS. Similar effects were reported for 3T3 fibroblast cell proliferation [19]. The same study, using confocal microscopy following immunostaining, identified a plasma membrane fraction of the PBBS in Leydig cells and immunolocalization and immunoprecipitation demonstrated that the cytoplasmic protein, DBI, was secreted by both Sertoli and Leydig cells [19]. The authors have postulated that interstitial fluid DBI interacts with the plasma

membrane form of the PBBS to function as a paracrine/autocrine regulator of Leydig cell proliferation [19]. DBI is reported to stimulate steroid biosynthesis in Leydig cells. If such an effect was also due to extracellular DBI binding to a cell surface PBBS, it might be expected in other cells in which a second location of the receptor has been described. However, although DBI stimulated adrenal mitochondrial steroidogenesis, it did not stimulate steroidogenesis in adrenocortical cells, and competition studies also indicated that the effect in Leydig cells is likely to be mediated via a PBBS-independent mechanism [19].

It seems that a possible function of the plasma membrane PBBS in rat testis is to interact with extracellular DBI to elicit effects on cell proliferation, but not on steroidogenesis.

Immunomodulation

The presence of PBBS on lymphoid cells and macrophages is indicative of a potential role in immune function. In addition to both potentiating and inhibitory effects on lymphoid cell growth (at nanomolar and micromolar concentrations, respectively), specific effects on immune cells have been reported [88]. Picomolar concentrations of Ro5 4864 and diazepam induced human monocyte chemotaxis [89], and nanomolar concentrations of specific PBBS ligands or a specific monoclonal antibody stimulated oxidative bursts in neutrophils or macrophages *in vitro* [43, 90]. Subsequent *in vivo* studies demonstrated a reduced oxidative response in macrophages and decreased macrophage secretion of interleukins 1 and 6 and tumor necrosis factor following injection of Ro5 4864 in mice [25]. This is another example of the biphasic effects of benzodiazepines: stimulatory at low concentrations and inhibitory at high doses.

Interestingly, the PBBS at the two different locations that have been identified in human lymphocytes has different specificities for two types of endogenous ligand [44]. The plasma membrane PBBS is recognized essentially only by DBI fragments, whereas the intracellular form of the PBBS, which has been reported to be present in only 22% of lymphocyte mitochondria, binds both DBI fragments and protoporphyrin with low affinity [44, 45]. This is consistent with the suggested role for the mitochondrial PBBS in tetrapyrrole biosynthesis [23].

An interaction of extracellular DBI with the plasma membrane form of the PBBS may be of functional significance for the immunomodulatory effects of peripheral-type ligands. Such a functional association of DBI with the plasma membrane PBBS has already been proposed to account for effects on Leydig cell growth [19].

CONCLUDING REMARKS

The liver, although low in receptor density, is the only tissue in which mitochondrial and plasma membrane forms of the PBBS have been described in separate cell populations [31]. DBI, in varying amounts, has been found in all tissues examined, and it has been reported that its distribution correlates with that of PBBS, although liver DBI content is relatively high due to its diffuse presence in every hepatocyte [84]. A rat liver ACBP, with high affinity for long chain (C_{14}–C_{22}) acyl-CoA esters, has been found to be identical to DBI [91, 92]. Its gene exhibits all the hallmarks of a typical housekeeping gene, suggesting a general role in acyl-CoA metabolism [93]. A dual function in the protection of cellular processes against inhibition by long chain acyl-CoA esters and in the maintenance of a cytosolic pool of acyl-CoA has been proposed [94].

Most cholesterol synthesis in vertebrates takes place in the liver where it is converted to bile acids or to cholesterol esters. Bile acids are ultimately secreted via bile canaliculi, ductules, and ducts. DBI/ACBP is present in biliary epithelial cells [31], and it is tempting to speculate on an interaction of this endogenous ligand with the PBBS of the biliary epithelial cell membrane in the regulation of biliary secretion. Cytosolic cholesterol storage is mainly as oleal-cholesterol esters, and it may be that ACBP donates acyl-CoA substrates for acyl-CoA cholesterol acyltransferase when cholesterol levels are high. Subsequently, the cholesterol esters are either stored in the liver or transported to other tissues where they may be hydrolysed to provide cholesterol for steroidogenesis. The ability of ACBP/DBI to interact with mitochondrial PBBS where it appears to influence steroidogenesis, coupled with its ability to bind acyl-CoA esters, may indicate a function for this protein in cholesterol metabolism. The reported presence in rat testis cells of extracellular as well as cytosolic DBI/ACBP suggests that further studies with this endogenous ligand may be of benefit in determining the roles of the plasma membrane and mitochondrial forms of the PBBS.

In conclusion, there is growing evidence that the PBBS is on plasma membranes, in addition to its well-documented mitochondrial outer membrane location. Further structural studies and analysis of the binding characteristics of both endogenous and synthetic ligands will determine the identity or lack thereof of the PBBS in each location. It may be that the two forms are present in different subcellular organelles in all/many peripheral tissues, as has already been demonstrated in liver, and that there are separate functions. The results of such studies are likely to have positive implications for the elucidation of the physiological significance of the PBBS.

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References

1. Haefely W, Kulskar A, Mohler H, Pieri L, Polc P and Schnaffer R, Possible involvement of GABA in the central actions of benzodiazepine derivatives. In: *Mechanisms of Actions of Benzodiazepines* (Eds. Costa E and Greengard P), Vol. 14, pp. 131–151. Raven Press, New York, 1975.
2. Costa E and Guidotti A, Molecular mechanisms in the receptor action of benzodiazepines. *Annu Rev Pharmacol Toxicol* **19:** 531–543, 1979.

3. Braestrup C and Squires RF, Specific benzodiazepine receptors in rat brain characterized by high-affinity [^{3}H]diazepam binding. *Proc Natl Acad Sci USA* **74:** 3805–3809, 1977.
4. Bourguignon JJ, Endogenous and synthetic ligands of mitochondrial benzodiazepine receptors: Structure–affinity relationships. In: *Peripheral Benzodiazepine Receptors* (Ed. Giesen-Crouse E), pp. 59–82. Academic Press, London, 1993.
5. Le Fur G, Perrier ML, Vaucher N, Imbault F, Flamier A, Benavides J, Uzan A, Renault C, Dubroeucq MC and Guérémy C, Peripheral benzodiazepine binding sites: Effects of PK 11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide. I. *In vitro* studies. *Life Sci* **32:** 1839–1847, 1983.
6. Dubroeucq M-C, Bénavidès J, Doble A, Guilloux F, Allam D, Vaucher N, Bertrand P, Guérémy C, Renault C, Uzan A and Le Fur G, Stereoselective inhibition of the binding of [^{3}H]PK 11195 to peripheral-type benzodiazepine binding sites by a quinolinepropanamide derivative. *Eur J Pharmacol* **128:** 269–272, 1986.
7. Fiorinni I, Nacci V, Ciani SM, Garafola A, Campiani G, Savini L, Novellino E, Greco G, Bernasconi P and Mennini T, Novel ligands specific for mitochondrial benzodiazepine receptors: 6-arylpyrrolo[2,1-*d*][1,5]benzothiazepine derivatives. Synthesis, structure–activity relationships and molecular modeling studies. *J Biol Chem* **37:** 1427–1438, 1994.
8. Bovolin P, Schlichting J, Miyata M, Ferrarese C, Guidotti A and Alho H, Distribution and characterization of diazepam binding inhibitor (DBI) in peripheral tissues of rat. *Regul Pept* **29:** 267–281, 1990.
9. Guidotti A, Forchetti CM, Corda MG, Konkel D, Bennett CD and Costa E, Isolation, characterization, and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. *Proc Natl Acad Sci USA* **80:** 3531–3535, 1983.
10. Shoyab M, Gentry LE, Marquardt H and Todaro GJ, Isolation and characterization of a putative endogenous benzodiazepineoid (endozepine) from bovine and human brain. *J Biol Chem* **261:** 11968–11973, 1986.
11. Verma A, Nye JS and Snyder SH, Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor. *Proc Natl Acad Sci USA* **84:** 2256–2260, 1987.
12. Marangos PJ, Patel J, Boulenger J-P and Clark-Rosenberg R, Characterization of peripheral-type benzodiazepine binding sites in brain using [^{3}H]Ro5-4864. *Mol Pharmacol* **22:** 26–32, 1982.
13. O'Beirne GB and Williams DC, The subcellular location in rat kidney of the peripheral benzodiazepine acceptor. *Eur J Biochem* **174:** 413–421, 1988.
14. O'Beirne GB, Woods MJ and Williams DC, Two subcellular locations for peripheral-type benzodiazepine acceptors in rat liver. *Eur J Biochem* **188:** 131–138, 1990.
15. Anholt RRH, Pederson PL, De Souza EB and Snyder SH, The peripheral-type benzodiazepine receptor: Localization to the mitochondrial outer membrane. *J Biol Chem* **261:** 576–583, 1986.
16. Antkiewicz-Michaluk L, Guidotti A and Krueger KE, Molecular characterization and mitochondrial density of a recognition site for peripheral-type benzodiazepine ligands. *Mol Pharmacol* **34:** 272–278, 1988.
17. Doble A, Benavides J, Ferris O, Bertrand P, Menager J, Vaucher N, Burgevin M-C, Uzan A, Gueremy C and Le Fur G, Dihydropyridine and peripheral type benzodiazepine binding sites: Subcellular distribution and molecular size determination. *Eur J Pharmacol* **119:** 153–167, 1985.
18. Oke BO, Suarez-Quain CA, Riond J, Ferrara P and Papadopoulos V, Cell surface location of the peripheral-type benzodiazepine receptor (PBR) in adrenal cortex. *Mol Cell Endocrinol* **87:** R1–R6, 1992.
19. Garnier M, Boujrad N, Oke BO, Brown AS, Riond J, Ferrara P, Shoyab M, Suarez-Quian CA and Papadopoulos V, Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: Action via peripheral-type benzodiazepine receptor and independent mechanisms. *Endocrinology* **132:** 444–458, 1993.
20. Olsen RW and Tobin AJ, Molecular biology of $GABA_A$ receptors. *FASEB J* **14:** 1469–1481, 1990.
21. McEnery MW, Snowman AW, Trifletti RR and Snyder SH, Isolation of the mitochondrial benzodiazepine receptor: Association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci USA* **89:** 3170–3174, 1989.
22. Mukhin AG, Papadopoulos V, Costa E and Krueger KE, Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proc Natl Acad Sci USA* **86:** 9813–9816, 1989.
23. Taketani S, Kohno H, Furukawa T and Tokunga R, Involvement of peripheral-type benzodiazepine receptors in the intracellular transport of heme and porphyrins. *J Biochem (Tokyo)* **117:** 875–880, 1995.
24. Wang JKT, Morgan JI and Spector S, Benzodiazepines that bind at peripheral sites inhibit cell proliferation. *Proc Natl Acad Sci USA* **81:** 753–756, 1984.
25. Zavala F, Taupin V and Descamps-Latscha B, *In vivo* treatment with benzodiazepines inhibits murine phagocyte oxidative metabolism and production of interleukin 1, tumor necrosis factor and interleukin 6. *J Pharmacol Exp Ther* **255:** 442–450, 1990.
26. Mestre M, Carriot T, Belin C, Uzan A, Renault C, Dubroeucq MC, Guérémy C, Doble A and Le Fur G, Electrophysiological and pharmacological evidence that peripheral type benzodiazepine receptors are couled to calcium channels in the heart. *Life Sci* **36:** 391–400, 1985.
27. Hirsch JD, Beyer CF, Malkowitz L, Beer B and Blume AJ, Mitochondrial benzodiazepine receptors mediate inhibition of mitochondrial respiratory control. *Mol Pharmacol* **35:** 157–163, 1988.
28. Papadopoulos V, Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: Biological role in steroidogenic cell function. *Endocr Rev* **14:** 222–240, 1993.
29. Krueger KE, Molecular and functional properties of mitochondrial benzodiazepine receptors. *Biochim Biophys Acta* **1241:** 453–470, 1995.
30. Canat X, Carayon P, Bouaboula M, Cahard D, Shire D, Roque C, Le Fur G and Casellas P, Distribution profile and properties of peripheral-type benzodiazepine receptors on human hemopoietic cells. *Life Sci* **52:** 107–118, 1992.
31. Woods MJ, Zisterer DM and Williams DC, Two cellular and subcellular locations for the peripheral-type benzodiazepine receptor in rat liver. *Biochem Pharmacol* **51:** 1283–1292, 1996.
32. Anholt RRH, De Souza EB, Oster-Granite ML and Snyder SH, Peripheral-type benzodiazepine receptors: Autoradiographic localization in whole-body sections of neonatal rats. *J Pharmacol Exp Ther* **233:** 517–526, 1985.
33. Verma A and Snyder SH, Peripheral-type benzodiazepine receptors. *Annu Rev Pharmacol Toxicol* **29:** 307–322, 1989.
34. De Souza EB, Anholt RRH, Murphy KMM, Snyder SH and Kuhar MJ, Peripheral-type benzodiazepine receptors in endocrine organs: Autoradiographic localization in rat pituitary, adrenal, and testis. *Endocrinology* **116:** 567–573, 1985.
35. Fares F, Bar-Ami S, Brandes JM and Gavish M, Gonadotrophin- and estrogen-induced increase of peripheral-type benzodiazepine binding sites in the hypophyseal-genital axis of rats. *Eur J Pharmacol* **133:** 97–102, 1987.
36. Taniguchi T, Wang JKT and Spector S, [^{3}H]Diazepam-binding sites on rat heart and kidney. *Biochem Pharmacol* **31:** 589–590, 1982.
37. Benavides J, Quarteronet D, Plouin P-F, Imbault F, Phan T,

Uzan A, Renault C, Dubroeucq M-C, Gueremy C and Le Fur G, Characterization of peripheral type benzodiazepine binding sites in human and rat platelets by using [^{3}H]PK 11195. Studies in hypertensive patients. *Biochem Pharmacol* **33:** 2467–2472, 1984.

38. Olson JM, Ciliax BJ, Macini WR and Young AB, Presence of peripheral-type benzodiazepine binding sites on human erythrocyte membranes. *Eur J Pharmacol* **152:** 47–53, 1988.
39. Gehlert DR, Yamamura HI and Wamsley JK, Autoradiographic localization of 'peripheral' benzodiazepine binding sites in the rat brain and kidney using [^{3}H]Ro5-4864. *Eur J Pharmacol* **95:** 329–330, 1983.
40. Moynagh PN, Bailey CJ, Boyce SJ and Williams DC, Immunological studies on the rat peripheral-type benzodiazepine acceptor. *Biochem J* **275:** 419–425, 1991.
41. Papadopoulos V, Boujard N, Ikonomovic MB, Ferrara P and Vidic B, Topography of the Leydig cell mitochondrial peripheral-type benzodiazepine receptor. *Mol Cell Endocrinol* **104:** R5–R9, 1994.
42. Mukherjee S and Das SK, Subcellular distribution of "peripheral type" binding sites for [^{3}H]Ro5-4864 in guinea pig lung: Localization to the mitochondrial inner membrane. *J Biol Chem* **264:** 16713–16718, 1989.
43. Zavala F, Masson A, Brys L, de Baetselier P and Descamps-Latsha B, A monoclonal antibody against peripheral benzodiazepine receptor activates the human neutrophil NADPH-oxidase. *Biochem Biophys Res Commun* **176:** 1577–1583, 1991.
44. Berkovich A, Ferrarase C, Cavaletti G, Alho H, Marzorati C, Bianchi G, Guidotti A and Costa E, Topology of two DBI receptors in human lymphocytes. *Life Sci* **52:** 1265–1277, 1993.
45. Cahard D, Canat X, Carayon P, Roque C, Casellas P and Le Fur G, Subcellular localization of peripheral benzodiazepine receptors on human leukocytes. *Lab Invest* **70:** 23–28, 1994.
46. Weissman BA, Elson HF, Kaplan D and Newman AH, Presence of peripheral benzodiazepine binding sites on primary rat skeletal fibroblasts. *Eur J Pharmacol* **187:** 369–375, 1990.
47. Doble A, Ferris O, Burgevin MC, Menager J, Uzan A, Dubroeucq MC, Renault C, Gueremy C and Le Fur G, Photoaffinity labelling of peripheral-type benzodiazepine binding sites. *Mol Pharmacol* **31:** 42–49, 1984.
48. McCabe RT, Schoenheimer JA, Skolnick P, Newman AH, Rice KC, Reig J-A and Klein DC, [^{3}H]AHN 086 acylates peripheral benzodiazepine receptors in the rat pineal gland. *FEBS Lett* **244:** 263–267, 1989.
49. Snyder SH, Verma A and Trifiletti RR, The peripheral-type benzodiazepine receptor: A protein of mitochondrial outer membranes utilizing porphyrins as endogenous ligands. *FASEB J* **1:** 282–288, 1987.
50. Sprengel R, Werner P, Seeburg PH, Mukhin AG, Santi MR, Grayson DR, Guidotti A and Krueger KE, Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *J Biol Chem* **264:** 20415–20421, 1989.
51. Riond J, Mattei MG, Kaghad M, Dumont X, Guillemot JC, Le Fur G, Caput D and Ferrara P, Molecular cloning and chromosomal location of a human peripheral-type benzodiazepine receptor. *Eur J Biochem* **195:** 305–311, 1991.
52. Parola AL, Stump DG, Pepperl DJ, Krueger KE, Regan JW and Laird HE II, Cloning and expression of a pharmacologically unique bovine peripheral-type benzodiazepine receptor isoquinoline binding protein. *J Biol Chem* **266:** 14082–14087, 1991.
53. Garnier M, Dimchev AB, Boujrad N, Price JM, Musto NA and Papadopoulos V, *In vitro* reconstitution of a functional peripheral-type benzodiazepine receptor from mouse Leydig tumor cells. *Mol Pharmacol* **45:** 201–211, 1993.
54. Riond J, Leplatois P, Laurent P, Le Fur G, Caput D, Loison G and Ferrara P, Expression and pharmacological characterization of the human peripheral-type benzodiazepine receptor in yeast. *Eur J Pharmacol* **208:** 307–312, 1991.
55. Blachly-Dyson E, Zambronicz EB, Yu WH, Adams V, McCabe ERB, Adelman J, Colombini M and Forte M, Cloning and functional expression in yeast of two human isoforms of the outer mitochondrial membrane channel, the voltage-dependent anion channel. *J Biol Chem* **268:** 1835–1841, 1993.
56. Luedeens HWM and Skolnick P, 'Peripheral-type' benzodiazepine receptors in the kidney: Regulation of radioligand binding by anions and DIDS. *Eur J Pharmacol* **133:** 205–214, 1987.
57. Colombini M, A candidate for the permeability pathway of the outer mitochondrial membrane. *Nature* **279:** 643–645, 1979.
58. Zalman LS, Nikaido H and Kagawa Y, Mitochondrial outer membrane contains a protein producing nonspecific diffusion channels. *J Biol Chem* **255:** 1771–1774, 1980.
59. Ohlendieck K, Riesinger I, Adams V, Krause J and Brdiczka D, Enrichment and biochemical characterization of boundary membrane contact sites from rat-liver mitochondria. *Biochim Biophys Acta* **860:** 672–689, 1986.
60. Reymann S, Flörke H, Heiden M, Jakob C, Stadtmüller U, Steinacker P, Lalk VE, Pardowitz I and Thinnes FP, Further evidence for multitopological localization of mammalian porin (VDAC) in the plasmalemma forming part of a chloride channel complex affected in cystic fibrosis and encephalomyopathy. *Biochem Mol Med* **54:** 75–87, 1995.
61. Bureau MH, Khrestchatisky M, Heeren MA, Zambrowicz EB, Kim H, Grisar TM, Colombini M, Tobin AJ and Olsen RW, Isolation and cloning of a voltage-dependent anion channel-like M_r 36,000 polypeptide from mammalian brain. *J Biol Chem* **267:** 8679–8684, 1992.
62. Baker ME and Fanestil DD, Mammalian peripheral-type benzodiazepine receptor is homologous to CrtK protein of *Rhodobacter capsulatus*, a photosynthetic bacterium. *Cell* **65:** 721–722, 1991.
63. Yeliseev AA and Kaplan S, A sensory transducer homologous to the mammalian peripheral-type benzodiazepine receptor regulates photosynthetic membrane complex formation in *Rhodobacter sphaeroides* 2.4.1. *J Biol Chem* **270:** 21167–21175, 1995.
64. Casalotti SO, Pelaia G, Yakovlov AG, Csikós T, Grayson D and Krueger KE, Structure of the rat gene encoding the mitochondrial benzodiazepine receptor. *Gene* **121:** 377–382, 1992.
65. Lin D, Chang YJ, Strauss JF and Miller WL, The human peripheral benzodiazepine receptor gene: Cloning and characterization of alternative splicing in normal tissues and in a patient with congenital lipoid adrenal hyperplasia. *Genomics* **18:** 643–650, 1993.
66. Schatz G, Signals guiding proteins to their correct locations in mitochondria. *Eur J Biochem* **165:** 1–6, 1987.
67. Matter K and Mellman I, Mechanisms of cell polarity: Sorting and transport in epithelial cells. *Curr Opin Cell Biol* **6:** 545–554, 1994.
68. Tsankova V, Magistrelli A, Cantoni L and Tacconi MT, Peripheral benzodiazepine ligands in rat liver mitochondria: Effect on cholesterol metabolism. *Eur J Pharmacol* **294:** 601–607, 1995.
69. Jung-Testas I, Hu ZY, Baulieu EE and Robel P, Neurosteroids: Biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* **125:** 2083–2091, 1989.
70. Costa E, Auta J, Guidotti A, Korneyev A and Romeo E, The pharmacology of neurosteroidogenesis. *J Steroid Biochem Mol Biol* **49:** 385–389, 1994.
71. Guarneri P, Papadopoulos V, Pan B and Costa E, Regulation

of pregnenolone synthesis in C6-2B glioma cells by 4'-chlorodiazepam. *Proc Natl Acad Sci USA* **89:** 5118–5122, 1992.
72. Papadopoulos V, Guarneri P, Krueger KE, Guidotti A and Costa E, Pregnenolone biosynthesis in C6-2B glioma cell mitochondria: Regulation by a mitochondrial diazepam binding inhibitor receptor. *Proc Natl Acad Sci USA* **89:** 5113–5117, 1992.
73. Anholt RRH, Mitochondrial benzodiazepine receptors as potential modulators of intermediary metabolism. *Trends Pharmacol Sci* **7:** 506–511, 1986.
74. Moynagh PN, Contact sites and transport in mitochondria. *Essays Biochem* **30:** 1–14, 1995.
75. Zisterer DM, Gorman AMC, Williams DC and Murphy MP, The effects of peripheral-type benzodiazepine acceptor ligands, Ro5 4864 and PK 11195, on mitochondrial respiration, *Methods Find Exp Clin Pharmacol* **14:** 85–90, 1992.
76. Taketani S, Kohno H, Okuda M, Furukawa T and Tokunaga R, Induction of peripheral-type benzodiazepine receptors during differentiation of mouse erythroleukemia cells. *J Biol Chem* **269:** 7527–7531, 1994.
77. Cantoni L, Rizzardini M, Skorupska M, Cagnotto A, Codegoni A, Pecora N, Frigo L, Ferrarase C and Mennini T, Hepatic protoporphyria is associated with a decrease in ligand binding for the mitochondrial benzodiazepine receptors in the liver. *Biochem Pharmacol* **44:** 1159–1164, 1992.
78. Kinnally KW, Zorov DB, Antonenko YN, Snyder SH, McEnery MW and Tedeschi H, Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligand. *Proc Natl Acad Sci USA* **90:** 1374–1378, 1993.
79. Mestre M, Carriot A, Belin C, Uzan A, Renault C, Dubroeucq MC, Gueremy C and Le Fur G, Electrophysiological and pharmacological characterization of peripheral benzodiazepine receptors in a guinea pig heart preparation. *Life Sci* **35:** 953–962, 1984.
80. Cantor EH, Kenessy A, Semenuk G and Spector S, Interaction of calcium channel blockers with non-neuronal benzodiazepine binding sites. *Proc Natl Acad Sci USA* **81:** 1549–1552, 1984.
81. Hullihan JP, Spector S, Taniguchi T and Wang JK, The binding of [^{3}H]diazepam to guinea pig ileal longitudinal muscle and the *in vitro* inhibition of contraction by benzodiazepines. *Br J Pharmacol* **78:** 321–327, 1983.
82. Bolger GT, Cardiovascular actions of peripheral benzodiazepines. In: *Peripheral Benzodiazepine Receptors* (Ed. Giesen-Crouse E), pp. 153–167. Academic Press, London, 1993.
83. Taft WC and De Lorenzo RJ, Micromolar-affinity benzodiazepine receptors regulate voltage-sensitive calcium channels in nerve terminal preparations. *Proc Natl Acad Sci USA* **81:** 3118–3122, 1984.
84. Wang JKT, Morgan JI and Spector S, Differentiation of Friend erythroleukemia cells induced by benzodiazepines. *Proc Natl Acad Sci USA* **81:** 3770–3772, 1984.
85. Morgan JI, Johnson MD, Wang JKT, Sonnenfeld KH and Spector S, Peripheral-type benzodiazepines influence ornithine decarboxylase levels and neurite outgrowth in PC12 cells. *Proc Natl Acad Sci USA* **82:** 5223–5226, 1985.
86. Curran T and Morgan JI, Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* **229:** 1265–1268, 1985.
87. Gorman AMC, O'Beirne GB, Regan CM and Williams DC, Antiproliferative action of benzodiazepines in cultured brain cells is not mediated through the peripheral-type benzodiazepine acceptor. *J Neurochem* **53:** 849–855, 1989.
88. Pawlikowski M, Immunomodulating effects of peripherally acting benzodiazepines. In: *Peripheral Benzodiazepine Receptors* (Ed. Giesen-Crouse E), pp. 125–135. Academic Press, London, 1993.
89. Ruff MR, Pert CB, Weber RJ, Wahl LM, Wahl SM and Paul SM, Benzodiazepine receptor-mediated chemotaxis of human monocytes. *Science* **229:** 1281–1283, 1985.
90. Zavala F and Lenfant M, Peripheral benzodiazepines enhance the respiratory burst of macrophage-like P388 D_1 cells stimulated by arachidonic acid. *Int J Immunopharmacol* **9:** 269–274, 1987.
91. Rosendal J, Ertbjerg P and Knudsen J, Characterization of ligand binding to acyl-CoA-binding protein. *Biochem J* **290:** 321–326, 1993.
92. Knudsen J, Højrup P, Hansen HO, Hansen HF and Roepstorff P, Acyl-CoA-binding protein in the rat. *Biochem J* **262:** 513–519, 1989.
93. Mandrup S, Hummel R, Ravn S, Jensen G, Andreasen PH, Gregersen N, Knudsen J and Kristiansen K, Acyl-CoA-binding protein/diazepam binding inhibitor gene and pseudogenes. *J Mol Biol* **228:** 1011–1022, 1992.
94. Rasmussen JT, Rosendal J and Knudsen J, Interaction of acyl-CoA binding protein (ACBP) on processes for which acyl-CoA is a substrate, product or inhibitor. *Biochem J* **292:** 907–913, 1993.