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PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS

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INTRODUCTION

Benzodiazepines are usually regarded as rather selective in their actions upon the central nervous system. They are effective anticonvulsants, antianxiety drugs, and hypnotics. Peripheral actions of these drugs in patients, such as alterations in gonadal and adrenal hormone secretion, have been regarded as secondary to influences on the central nervous system. For instance, limbic and hypothalamic effects altering pituitary secretion might indirectly influence endocrine glands.

The first studies of benzodiazepine receptors used [^{3}H]diazepam (1). Binding sites with nanomolar affinity were identified in central nervous tissue membranes. The relative affinities of benzodiazepines for these sites paralleled their antianxiety and anticonvulsant actions. This "central" benzodiazepine receptor has been purified, its cDNA cloned, and functional receptors have been expressed in frog oocytes (2). The central benzodiazepine receptor is a macromolecular complex that includes a binding site for the inhibitory neurotransmitter GABA, fitting with evidence that benzodiazepines exert their CNS effects by facilitating the synaptic effects of GABA. The macromolecular GABA-benzodiazepine receptor complex also includes recognition sites for barbiturates and possibly even ethanol.

In initial studies of ^{3}H-diazepam binding, peripheral tissues were examined as controls, since one would not anticipate antianxiety receptors in peripheral tissues. Surprisingly, high densities of ^{3}H-diazepam binding sites were observed in a variety of peripheral tissues with similar nanomolar affinity for diazepam as the central receptors (1). However, an exploration of numerous

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benzodiazepines revealed pronounced differences between the central and peripheral sites. For instance, clonazepam, one of the most potent benzodiazepines pharmacologically, which is used clinically as an anticonvulsant, has a Ki value for binding sites in the rat brain in the low nanomolar range, but is several thousand-fold weaker at peripheral sites. By contrast RO5-4864, a benzodiazepine differing in structure from diazepam only in the deletion of a chlorine substituent on one of the rings (Figure 1), is devoid of antianxiety effects in animal models and in humans, and is extremely weak at central benzodiazepine binding sites. However, RO5-4864 is active in the nanomolar range at peripheral sites. The isoquinoline carboxamide derivative PK-11195 also interacts selectively and potently only with the peripheral receptors.

Based on these differences in drug specificity, peripheral-type benzodiazepine receptors (PBR) have been regarded as distinct entities from the central sites (3). They can be labeled selectively with ligands such as [^{3}H]RO5-4864 and [^{3}H]PK11195, while central sites are labeled selectively with agents such as [^{3}H]clonazepam or [^{3}H]RO15-1788. PBR have been less extensively studied than the central sites. Several investigators have referred to them as "acceptor" sites rather than receptors, implying that they possess no physiological role and so are not "functional receptors." Recently unique localizations in various organs and organelles have been described. Apparent endogenous ligands have been isolated, and the effects of peripherally selective benzodiazepines on numerous biological processes have been characterized. Progress has been made toward isolating the peripheral receptor protein. Taken together, these studies strongly imply a prominent physiological role for PBR (3).

LOCALIZATION AND REGULATION IN PERIPHERAL ORGANS AND THE BRAIN

Biochemical analyses of rat tissue homogenates reveal the greatest densities of PBR in the adrenal gland with high levels in a variety of glandular tissues, including the salivary gland, the testis, and the ovary. However, some glandular tissues, such as the pancreas and the pituitary, have relatively low densities of receptors. At the same time, tissues not conventionally regarded as glandular, such as the nasal epithelium, lung, and kidney, have high levels of receptors. Whole rat brain homogenates have relatively sparse levels of receptors, only about 2% of the adrenal gland values.

While biochemical measurements of receptor numbers indicate the ubiquity of PBR, they do not shed much insight into potential functions. Autoradiographic localizations of these receptors in various organs have been particularly illuminating. For instance, while whole rat pituitary homogenates have relatively low levels of receptors, autoradiographic analysis reveals high

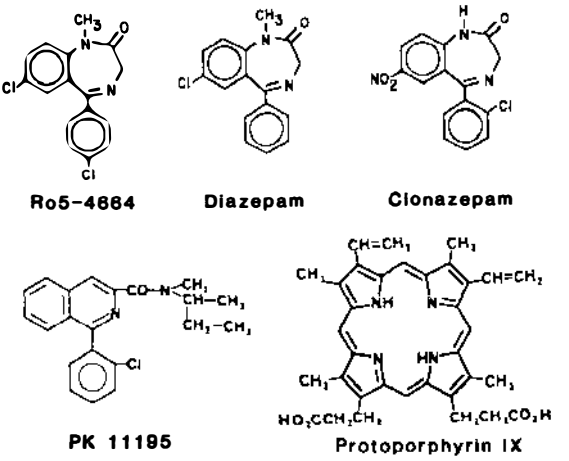

Figure 1 Structures of compounds interacting at benzodiazepine recognition sites.

levels of receptors concentrated in the posterior and intermediate lobes, which by weight are minor components of the pituitary (4). Within the adrenal gland localizations are quite discrete. The adrenal medulla is devoid of receptors, yet in the adrenal cortex substantial numbers of receptors are apparent in all layers, with highest densities in the outer zona glomerulosa, which is principally concerned with the elaboration of the salt-controlling hormone aldosterone (4).

Since diazepam has nanomolar affinity for peripheral benzodiazepine receptors and micromolar concentrations of diazepam circulate following treatment with therapeutic doses of the drug, it should influence these receptors in clinical settings. Benzodiazepines alter both the release of growth hormone, ACTH, prolactin, and luteinizing hormone from anterior pituitary gland (5), and plasma levels of adrenocortical and testicular hormones (6–8). Stress-induced elevations in plasma levels of glucocorticoids are attenuated by benzodiazepines (9), Diazepam inhibits aldosterone production in bovine adrenal glomerulosa cells (10), indicating a direct action of benzodiazepines on adrenal function mediated by PBR.

Within the testes receptors are highly localized to the interstitial tissue where Leydig cells produce testosterone (4). Much lower densities of receptors occur in the seminiferous tubules where spermatogonia and Sertoli cells predominate. Changes in plasma testosterone levels occur after administration of diazepam (7). A direct role of these receptors in testicular function is evident from the enhancement of testosterone production from rat testes in vitro elicited by RO5-4864 (11). PBR in the testes are regulated by the pituitary gland and are markedly depleted following hypophysectomy (12). Interestingly, within the adrenal cortex zonal differences exist in the influence

of the pituitary, as hypophysectomy depletes receptors from the glucocorticoid-producing zona fasciculata and zona reticularis while not influencing receptors in the aldosterone-producing zona glomerulosa (12). Similar effects of trophic hormones on peripheral benzodiazepine receptors are seen in the female reproductive structures of the rat. Treatment of prepubertal female rats with either pregnant mare's serum, gonadotropins or β-estradiol enhances PBR levels (13). Autoradiography using [^{3}H]PK11195 has localized PBR to the steroidogenic interstitial and thecal layers. While granulosa cells appear to lack benzodiazepine receptors, there is a dramatic expression of these sites upon luteinization of these cells. PBR are also enriched in the oviduct epithelium and the epithelium and glands of the uterus, with somewhat lower levels seen in the smooth muscle of these structures (14). These localizations and regulation imply a role for these receptors in modulating endocrine function.

In the kidney PBR have been localized both by microdissection and by autoradiographic analysis. Receptors are primarily concentrated in the thick ascending portion of the loop of Henle, the distal convoluted tubules, and the collecting ducts, with much lower levels in the proximal nephron (15, 16). These distal components of the nephron are the primary sites of action of mineralocorticoids and vasopressin that derive from the adrenal zona glomerulosa and posterior pituitary respectively, tissues with high levels of receptors themselves. These localizations suggest a role for the receptors in water and electrolyte disposition. No clinical studies have reported major effects of benzodiazepines upon kidney function.

PBR may influence epithelial regulation of electrolyte transport in other organs with high receptor concentrations, such as the choroid plexus and ependyma of the brain, the salivary gland, the skin, the ciliary body and trabecular meshwork of the eye, and epithelia of the oviduct and uterus (14, 17, 18).

While the designation of peripheral-type receptors originally resulted from their relative absence from the brain, use of the specific, high-affinity ligands [^{3}H]RO5-4864 and [^{3}H]PK11195 later demonstrated these sites in the CNS as well (19, 20). Autoradiography in rat brain showed diffuse, low-level ligand binding throughout the parenchyma of the brain, with selective localizations in the ependyma, choroid plexus, and olfactory neurons (20, 21).

Although rat and mouse brains normally display low levels of PBR, a rapid and striking increase in their density occurs following various insults to the brain. Increases of 400% or more appear after intrastriatal (22) or systemic injections (23) of neuronal excitotoxins. Chronic ethanol treatment augments PBR 40–50% in the mouse brain with no change in the central type receptors (24). This rapid and impressive induction of PBR by CNS insults may reflect an adaptive metabolic response of the brain analogous to the induction of

detoxifying enzymes in the liver. The anticonvulsant actions of carbamazepine have been attributed to these receptors (25) and conceivably may reflect such an induction (26). PBR associated with proliferating glia (27) may account for increases following neuronal destruction and associated gliosis. Very high densities of PBR occur in glial brain tumors (28).

Unlike the rat and mouse, CNS tissue of cat and human displays high levels of PBR selectively associated with grey matter (29, 30). High levels of PBR occur in the extrapyramidal motor, vestibulo-cerebellar, visual and auditory relay and blood pressure regulating structures (29, 30). The ability to image PBR in living humans by positron emission tomography (31) may clarify their response to neuronal damage.

ASSOCIATION OF RECEPTORS WITH MITOCHONDRIA

Autoradiographic studies of PBR in whole body sections of fetal rats provided the first suggestion of a relationship between these sites and mitochondria (17). High levels of receptors are closely associated with tissues that derive their metabolic energy primarily from oxidative phosphorylation, whereas only low levels were present in tissues that derive their metabolic energy largely from glycogenolysis. A link to mitochondrial energy metabolism is supported by histochemical maps of cytochrome oxidase activity that closely resemble the distribution of PBR (17).

Direct evidence for a mitochondrial localization comes from subcellular fractionation studies (32). Relative densities of peripheral receptors in various subcellular fractions of the adrenal gland (32), liver, and kidney (33) correlate closely with cytochrome oxidase activity but not with markers for nuclei, lysosomes, peroxysomes, endoplasmic reticulum, plasma membrane, or cytoplasm. Titration of isolated mitochondria with digitonin simultaneously releases PBR and monoamine oxidase, a marker for outer mitochondrial membranes, but not cytochrome oxidase, which is localized to the inner mitochondrial membranes. Thus, in a variety of tissues these receptors are associated primarily with the outer membrane of the mitochondria.

In tissues such as the adrenal gland, kidney, and liver, essentially all of the receptor content can be accounted for by the binding sites localized to the outer membrane of mitochondria. It is extraordinarily difficult to obtain completely pure subcellular fractions so that definitive conclusions cannot readily be drawn. A substantial portion of PBR could occur at sites other than the mitochondria but go undetected in our studies. The cross-contamination of subcellular fractionations is apparent in the high concentration of PBR binding sites in nuclear fractions, which appear to reflect contamination by mitochondria (19).

POTENTIAL ENDOGENOUS LIGANDS: A ROLE FOR PORPHYRINS

Insight into a possible function for the peripheral receptor would be greatly facilitated if one knew the identity of endogenous ligands for the site. Prior to the identification of GABA as an allosteric regulator of central benzodiazepine receptors, numerous laboratories examined brain extracts for materials that might compete for central receptors. Both high and low molecular weight components were detected (34). Some of these were isolated, such as nicotinamide from brain extracts because of its ability to inhibit ligand binding to central benzodiazepine receptors. However, nicotinamide requires millimolar concentrations to markedly influence central receptors and so does not likely play a physiological role (35). A 10–15 kD protein designated diazepam-binding inhibitor (DBI) or endozepine, has micromolar affinity for central benzodiazepine receptors with similar effects upon peripheral-type benzodiazepine receptors (36). Behavioral effects can be identified when this substance is injected into the brain. The cDNA for DBI (endozepine) has been independently cloned in two laboratories (37, 38). Another 16 kD protein inhibitor of [^{3}H]RO5-4864 binding with micromolar affinity is a phospholipase A_2 isoenzyme (39) capable of directly interacting with components of the receptor or of generating free fatty acids that compete for PBR. Phospholipids and unsaturated fatty acids do inhibit PBR binding at micromolar concentrations (40).

Our efforts to identify an endogenous ligand were prompted by a report of Schoemaker et al (41) that benzodiazepine binding in the kidney was augmented by perfusion, suggesting that blood might contain an endogenous ligand. We observed that red blood cell lysates potently inhibited ligand binding to peripheral receptors. Organic extraction of the inhibitory activity provided a 300-fold purification to apparent homogeneity. Analyses using thin layer chromatography and ultraviolet absorbance indicated the active material in red blood cells was hemin (42). We purified inhibitory activity from perfused rat spleen, harderian gland, kidney, liver, and adrenal, and observed that in all our tissue extracts the peripheral benzodiazepine binding inhibitory activity could be completely accounted for by porphyrins. The exact porphyrin responsible for influences on receptors varies with the tissue. In the spleen, the inhibitory activity predominantly reflects hemin, presumably derived from the red blood cell content of that tissue. On the other hand, in many other tissues protoporphyrin IX is the major substance responsible for influences upon benzodiazepine receptors.

Several features of the actions of porphyrins on the receptors strongly imply that they have a physiological role in association with these sites. First, in contrast to the micromolar potencies of DBI-endozepine and unsaturated

lipids, porphyrins have actions in the nanomolar range. Protoporphyrin IX has a Ki for peripheral receptors of 15 nM, about a thousand times more potent than DBI-endozepine.

Another corroborating feature is that the relative potencies of a wide range of porphyrins examined vary markedly, the most potent having known physiological activity. Thus, heme, protoporphyrin IX, mesoporphyrin IX, and deuteroporphyrin IX are naturally occurring porphyrins with substantial affinity for receptors. By contrast, the precursors coproporphyrin and uroporphyrin are much weaker than protoporphyrin IX, as are also the breakdown products of porphyrins, biliverdin, and bilirubin.

If porphyrins are physiological ligands for the receptor, then one would anticipate evolutionary conservation of their recognition site on the receptor. The affinity of the benzodiazepine RO5-4864 varies markedly in different species and organs (43, 44). For instance, RO5-4864 is about 80 times more potent in rat than rabbit kidney and about 100 times more in rat than bovine heart or brain. Protoporphyrin IX, however, has identical potency at peripheral benzodiazepine receptors in all species and in all organs examined (44).

The concept that porphyrins are endogenous ligands for the receptor fits with a mitochondrial localization of the receptor. Thus, the initial and final steps in porphyrin biosynthesis occur within the mitochondria so that porphyrin precursors of protoporphyrin IX and heme must cross mitochondrial membranes. Cytosolic proteins that use porphyrins as prosthetic groups, such as hemoglobin, myoglobin, catalase, tryptophan pyrrolase, and several peroxidases, are formed as a consequence of porphyrin transport out of mitochondria. Cytochromes all contain heme and occur in both inner and outer mitochondrial membranes. These facts might relate to the high densities of receptors in the adrenal cortex and in the steroid-producing cells of the testes and ovaries, since cytochromes are involved in the biosynthesis of both adrenal and gonadal steroids. Proteins such as tryptophan pyrrolase bind heme noncovalently, with the level of saturation by the porphyrin determining the enzyme's activity (45), and possibly likewise modulating the PBR.

BIOLOGICAL EFFECTS OF PERIPHERAL TYPE BENZODIAZEPINES

Some understanding of the physiological role of the peripheral receptor may be derived from examining the effects of peripherally selective benzodiazepines upon a variety of biological processes. We have already reviewed the hormonal actions of these drugs that fit with an endocrine role for the receptors highly localized to endocrine organs.

Their numerous effects have been described in a variety of biological systems. Some require relatively high concentrations in vitro or high doses in

intact animals. Since benzodiazepines can influence numerous sites, such as calcium antagonist receptors, one must be cautious before concluding that an observed effect reflects interactions with PBR. Extremely potent effects favor selective receptor influences. Another gauge of specificity involves comparing agonist and antagonist effects.

The effects of peripherally selective benzodiazepines occurring at micromolar concentrations include inhibition of the in vitro growth of thymoma cells (46), depression of cardiac muscle contractility (47), and induction of melanogenesis in melanoma cells (48). The effects of RO5-4864 on cardiac muscle contractility are blocked by PK11195, suggesting that they reflect specific receptor interactions.

Most of the potent actions of peripheral benzodiazepines that seem to be biologically relevant are not direct effects, but involve modulation of actions of other substances. For instance, in PC-12 cells derived from pheochromocytomas, nerve growth factor enhances expression of the *c-fos* oncogene (49). RO5-4864 in nanomolar concentrations markedly augments this effect, and PK1115 then blocks the actions of RO5-4864, which alone does not influence oncogene expression (49).

A nervous system effect of peripheral benzodiazepines that illustrates these features involves seizure thresholds. RO5-4864 of itself produces convulsions only at high doses in rats, although at much smaller doses it reduces the threshold for seizure elicited by the convulsant drug pentylenetetrazole. PK11195 blocks the proconvulsant effects of RO5-4864 (47). Pyrethroid insecticides, such as deltamethrin and permethrin, produce convulsions in mammals and are extremely potent in lowering the seizure threshold for pentylenetetrazole. Their proconvulsant effects are blocked by PK11195, which does not alter seizure activity produced by pentylenetetrazole alone (50). These pyrethroids bind to PBR in nanomolar concentrations (51). Thus, these major insecticides may act via PBR in the brain. In contrast to the proconvulsant effect of the peripheral benzodiazepine RO5-4864, central type benzodiazepines are all anticonvulsants.

Arachidonic acid influences oxidative metabolism in P388D1, a murine cell line with macrophage-like properties, producing an oxidative burst with the formation of superoxide anions. Nanomolar concentrations of the peripheral benzodiazepine RO5-4864 markedly enhance this effect and the actions of RO5-4864 are blocked by PK11195 (52). As in the PC-12 cells and in seizure regulation, macrophage oxidative burst is not directly altered by RO5-4864. Similarly, PK11195 does not reverse oxidative stimulation caused by non-benzodiazepines.

The oxidative burst in macrophages elicited by arachidonic acid is thought to be mediated by lipoxygenase products. Accordingly, benzodiazepines might act at sites where these arachidonic acid metabolites are generated or

exert their effects. As discussed below, one candidate might be the glutathione-S-transferase localized to the outer membranes of mitochondria and binding leukotriene-C_4 at low nanomolar concentrations.

The considerable potency and selectivity of peripheral benzodiazepines on macrophage oxidation (52) are also apparent in certain effects on monocyte (53) and lymphocyte (54) behavior. Also, in these examples, benzodiazepines modulate the effects of other agents rather than having intrinsic activity themselves. This phenomenon may be analogous to the modulatory actions of central-type benzodiazepines at neuronal GABA-A receptors. Despite the disparate systems regulated by peripheral benzodiazepines, interactions at the receptor may involve common intracellular mechanisms that may relate to porphyrins and arachidonic acid metabolites.

Direct effects of peripheral benzodiazepine receptor ligands on mitochondrial function have been recently demonstrated. RO5-4864 and PK11195 both decrease respiratory control in isolated rat kidney mitochondria by increasing and decreasing the rates of respiratory states IV and III respectively (55). These effects occur with deuteroporphyrin IX and mesoporphyrin IX, but not clonazepam or RO15-1788, and correlate closely with affinity for PBR. The drugs do not act as uncouplers, nor do they alter the ADP:O ratio, adenine nucleotide flux, or the mitochondrial $\Delta\Psi$ (55). Furthermore, the effect of RO5-4864 on mitochondrial respiratory control is greatest in adrenal gland and intermediate in kidney, paralleling variations in PBR density in these organs.

MOLECULAR PROPERTIES OF PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS

Isolation and molecular characterization of peripheral receptors may clarify their function. After detergent solubilization the receptors tend to lose their ability to bind ligands reversibly (56). Accordingly, the principal efforts in isolating these receptors have used ^{3}H-ligands that bind covalently to the receptors. The benzodiazepine ^{3}H-flunitrazepam has 50 times higher affinity for central than peripheral receptors but does bind selectively to peripheral receptors in tissues outside the brain (3, 33). Flunitrazepam is a photoaffinity ligand that has been extensively employed in covalently labeling central receptors. When we photolabeled peripheral receptors with ^{3}H-flunitrazepam, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the labeled protein in purified outer mitochondrial membranes indicated two bands at 35 and 30 kD. The two bands both displayed the pharmacological specificity of PBR. This molecular weight corresponded to similar values obtained in irradiation analysis of the receptors (57).

The photoaffinity isoquinoline carboxamide ligand ^{3}H-PK14105 binds

irreversibly to PBR and labels a single protein whose apparent molecular weight is 15–18 kD (58–61). Many properties of the binding of isoquinoline and benzodiazepine ligands to the receptor suggest that they interact with different sites or perhaps different subpopulations of receptors. Thus, potencies of isoquinoline carboxamides are the same in various tissues and species, whilc relativc potencies of benzodiazepines in the same tissues and species markedly differ (43, 44). Accordingly, the different molecular weight proteins labeled by ^{3}H-flunitrazepam and ^{3}H-PK14105 may reflect different subunits or other receptor variations.

The outer mitochondrial membrane contains relatively few proteins. One candidate for the protein labeled by ^{3}H-flunitrazepam may be the voltage dependent anion channel (VDAC), which is also referred to as mitochondrial porin. VDAC allows transport of metabolites between mitochondria and the cytoplasm. VDAC can be selectively labeled with ^{14}C-DCCD (N,N'-dicyclohexyl carbodiimide). To compare PBR and VDAC, mitochondrial kidney extracts were photoaffinity labeled with ^{3}H-flunitrazepam and affinity labeled with ^{14}C-DCCD (3, 33). Both solubilized VDAC and PBR were not retained by calcium phosphate gels and displayed identical molecular masses in SDS-PAGE analysis. Moreover, purified VDAC, labeled with ^{3}H-flunitrazepam, corresponded to the 35-kD band. The intensity of labeling of this band was, however, only about one third that of the 30-kD band, the identity of which remains unknown.

Few known outer mitochondrial membrane proteins display a molecular weight of about 15–18 kD, corresponding to the PBR protein labeled with [^{3}H]PK14105. One such protein is an isozyme of phospholipase A_2 which displays preferential enzymatic selectivity for phosphatidylethanolamine over other phospholipids (62). Phosphatidylethanolamine is the most potent phospholipid inhibitor of PBR binding in rat kidney (40). Arachidonic acid, a product of phospholipase A_2, curiously inhibits binding of the benzodiazepine [^{3}H]RO5-4864 but not the isoquinoline [^{3}H]PK11195 (63). The phospholipase A_2 associated with the outer mitochondrial membrane contains a chemically modifiable histidine residue (64). Selective modification of histidine residues with low levels of diethyl pyrocarbonate abolishes [^{3}H]PK11195 binding preferentially with much less effect on [^{3}H]RO5-4864 binding (63).

Another 15-kD protein of the outer mitochondrial membrane, a glutathione-S-transferase like PBR, has nanomolar affinity for porphyrins. Glutathione-S-transferases exist in soluble and membrane-bound forms. A membrane-bound form of this enzyme occurs both in microsomes and the outer membrane of mitochondria where it may protect against membrane lipid peroxidation (65). Porphyrins bind to these enzymes with nanomolar affinity constants similar to their affinities for PBR (66).

What might be the relationship of the photoaffinity labeled 30-35-kD and

the affinity labeled 15-18-kD receptor proteins? Studies of physical links between the outer and inner mitochondrial membranes provide some suggestions inasmuch as they fuse at specific contact points where VDAC and glutathione-S-transferase are localized (67, 68). The selective ability of phospholipase A_2 to aggregate VDAC molecules (69) could play a role in this compartmentation. It is conceivable that these proteins are associated in a molecular complex at the site of fusion of the two mitochondrial membranes.

A molecular complex with such a localization could influence transactions between cytosolic and mitochondrial compartments. For instance, the outer and inner membranes cooperate during protein import into mitochondria (70). Several cytosolic kinases partition to the outer mitochondrial surface and bind specifically to VDAC at the points of contact between the two membranes (67, 68, 71, 72). By linking outer membrane VDAC and the adenine nucleotide carrier of the inner membrane, these fusion sites provide a physical path for cytoplasmic ADP exchange with mitochondrial ATP, mediated by the bound kinase and the adenine nucleotide carrier (71). This association allows the kinase privileged access to mitochondrial ATP and also improves the efficiency of mitochondrial respiratory control through the supply of ADP by the kinase action. Such a mechanism may be of importance in the metabolism of tumors (72). The observed decrease in respiratory control exerted by PBR ligands (55) may reflect a disruption of this physical path.

The binding and subsequent activation of glycerol kinase by the VDAC molecular complex (73) is of particular importance since its product, glycerol-3-phosphate, serves as the substrate for the glycerol phosphate shuttle that transfers reducing equivalents between the cytosol and mitochondria. Glycerol-3-phosphate is also the parent compound for de novo phospholipid synthesis, and the outer membrane contains the enzymatic ability to synthesize phospholipids (74). Conversely, phospholipase A_2 and glutathione-S-transferase are outer membrane enzymes that can regulate lipid peroxidation (75, 76) and provide potential messenger molecules. Glutathione-S-transferase generates leukotriene C_4 and also binds it with nanomolar affinity. This binding occurs to both the cytosolic and the mitochondrial outer membrane form of the enzyme and is potently displaced by porphyrins (77, 78). Leukotrienes serving as common intermediates in oxidative burst activity (79) and in the cyclic AMP dependent and independent activation of mitochondria in adrenal steroidogenesis (80) may facilitate the interactions at the receptor complex. The rate-limiting reaction in steroid biosynthesis, conversion of cholesterol to pregnenolone, occurs via the mitochondrial inner membrane cytochrome P-450 side-chain cleavage enzyme (P-450 scc) and is regulated by steroidogenic hormones such as ACTH. The action of such agents, mediated through cAMP and/or Ca^{++}, results in rapid changes in phospholipid metabolism reflecting de novo synthesis and turnover (81). The outer

mitochondrial membrane association of glycerol kinase and other enzymes responsible for phospholipid turnover permits an integrated microcompartmentation of phospholipid turnover and steroidogenesis (73). The effect of benzodiazepines on steroidogenesis and oxidative burst may reflect their modulation of such enzymatic compartmentalization. Indeed, potent effects of peripheral benzodiazepines on glycerolipid metabolism may be their underlying mechanism of action on cytosolic or mitochondrial processes. (82, 83).

While these speculations attempt to synthesize various lines of evidence, it is apparent that the role of the outer mitochondrial membrane in cell biology is relatively unappreciated. Modulation of phospholipid metabolism is widely studied in signal transduction, but relatively little thought has been given to the modulation of this activity in intracellular membranes.

Over the past decade studies of "central-type" benzodiazepine receptors have elucidated many principles of synaptic transmission by GABA-mediated chloride ion channels and their modulation by drugs as diverse as benzodiazepines, barbiturates, cage convulsants, and ethanol (1). Future studies of PBR might offer new insights into the interactions of biological signals with intracellular membranes.

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