The Peripheral-type Benzodiazepine Receptor Is Functionally Linked to Leydig Cell Steroidogenesis*

Vassilios Papadopoulos§, Alexey G. Mukhin¶, Erminio Costa®, and Karl E. Krueger††

From the §Department of Anatomy and Cell Biology and the ¶Fidia-Georgetown Institute for the Neurosciences, Georgetown University School of Medicine, Washington, D. C. 20007

Testicular mitochondria were previously shown to contain an abundance of peripheral-type benzodiazepine recognition site(s)/receptor(s) (PBR). We have previously purified, cloned, and expressed an M, 18,000 PBR protein (Antkiewicz-Michaluk, Mukhin, A. G., Guidotti, A., and Krueger, K. E. (1988) J. Biol. Chem. 263, 17317-17321; (Sprengel, R., Werner, P., Seeburg, P. H., Mukhin, A. G., Santi, M. R., Grayson, D. R., Guidotti, A., and Krueger, K. E. (1989) J. Biol. Chem. 264, 20415-20421); and in this report, we present evidence that PBR are functionally linked to Leydig cell steroid biosynthesis. A spectrum of nine different ligands covering a range of 4 orders of magnitude in their affinities for PBR were tested for their potencies to modulate steroidogenesis in the MA-10 mouse Leydig tumor cell line. The K, for inhibition of [3H]1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide binding and the EC50 for steroid biosynthesis for this series of compounds showed a correlation coefficient of r = 0.95. The most potent ligands stimulated steroid production by 4-fold in these cells. This stimulation was not inhibited by cycloheximide, unlike human chorionic gonadotropin- or cyclic AMP-activated steroidogenesis. The action of PBR ligands was not additive to stimulation by human chorionic gonadotropin or cyclic AMP, but was additive to that of epidermal growth factor, another regulator of MA-10 Leydig cell steroidogenesis. Moreover, PBR ligands stimulated, in a dose-dependent manner, pregnenolone biosynthesis by isolated mitochondria when supplied with exogenous cholesterol. This effect was not observed with mitoplasts (mitochondria devoid of the outer membrane). Cytochrome P-450 side chain cleavage activity, as measured by metabolism of (22R)-hydroxycholesterol, was not affected by PBR ligands in intact cells. Similar results were also obtained with purified rat Leydig cells. In conclusion, PBR are implicated in the acute stimulation of Leydig cell steroidogenesis possibly by mediating the entry, distribution, and/or availability of cholesterol within mitochondria.

Benzo diazepines are one of the most widely used class of drugs in therapy due to their anxiolytic and anticonvulsant properties. It is now well established that besides their inter-
androgen production. The use of decapsulated testes also limits the validity of these results, taking into account the regulation of Leydig cell steroidogenesis by paracrine factors of Sertoli cell origin (21, 22).

It is important to note that recently it has been reported that the endogenous DBI polypeptide, present in most central and peripheral tissues, is also expressed in testes (23, 24), and its presence has been primarily localized in Leydig cells. DBI displaces PBR ligands from rat adrenal gland cortex (25, 26), which raises the possibility that DBI may interact physiologically with PBR and elicit an as yet undefined biological activity. Recently, another laboratory (27, 28) has reported the purification of an 8.2-kDa polypeptide from bovine adrenal fasciculata which exhibited the ability to stimulate pregnenolone synthesis in mitochondrial preparations of adrenal glands. This polypeptide was subsequently sequenced and determined to be DBI (29). These findings support the possibility that PBR and DBI may both cooperate in the process of steroid biosynthesis.

The biosynthesis of steroid hormones begins with the transport of the substrate cholesterol from extramitochondrial stores to the first enzyme in the pathway, cytochrome P-450, located on the inner mitochondrial membrane. This is the rate-limiting step of steroidogenesis and the main site for regulation by physiological stimuli during acute stimulation (30, 31). The localization of PBR on the mitochondrial compartment, the above-mentioned preliminary studies, and the finding that DBI is localized in Leydig cells raised the possibility that PBR may participate in the regulation of testicular steroidogenesis and prompted us to examine whether PBR ligands could alter Leydig cell function. For this purpose, two cell models were used: the mouse Leydig cell line MA-10 and purified rat Leydig cells.

**EXPERIMENTAL PROCEDURES**

**Cells**—The MA-10 cell line originally cloned from the solid M590P mouse Leydig cell tumor (32) used in these experiments was generously given by Dr. Mario Ascoli (The Population Council, Rockefeller University, New York). Stock cultures were grown in modified Waymouth's MB-752/1 medium containing 10% horse serum, 1.0 g/liter NaHCO₃, and 15% horse serum, pH 7.4, as described by Ascoli (32). Before use, the MA-10 Leydig cells grown in 12 x 22-mm wells were washed three times, at 30-min intervals, with 1 ml of serum-free media to eliminate serum components that may interfere with the assays and were incubated for the indicated periods of time in the presence of the indicated substances. Testicular interstitial cells were prepared by collagenase dissociation (33) of testes obtained from adult Sprague-Dawley rats (300 g). This preparation contained 20-30% 3β-hydroxysteroid dehydrogenase-positive cells (Leydig cells). Leydig cells were further purified using discontinuous Percoll gradient centrifugation as previously described (33). The preparations obtained contained 75-85% Leydig cells as shown by histochemical staining for 3β-hydroxysteroid dehydrogenase (34).

[1,11195 Binding Assays and Photolabeling of PBR—Cells were scrapped from 75-cm² culture flasks into 5 ml of Kreb's buffer (104 mM NaCl, 60 mM RCl, 3 mM CaCl₂, 1.0 mM KH₂PO₄, 1.5 mM MgSO₄, 20 mM NaPO₄, pH 7.4), dispersed by trituration, and centrifuged at 12000 x g for 5 min. The cell pellets were resuspended in buffer, and larger cell aggregates were allowed to settle to the bottom of the tube before the cell suspension was retrieved for experimentation.

[2]°H]PK 11195 binding studies on 5 μg of protein from the cell suspensions were performed in 250-400 μl of Kreb's buffer at 37 °C, essentially the same conditions under which the effects of PBR ligands on steroidogenesis were studied. Nonspecific binding was determined in the presence of 10 μM [1,11195. After 30 min, the assays were stopped by filtration through Whatman GF/C filters and washed with 20 ml of 25 mM Tris-HCl, pH 7.4, containing 1 μM PK 11195. Radioactivity trapped on the filters was determined by liquid scintillation counting. Total binding accounted for <10% of the radioligand introduced, whereas specific binding was >85% of the total binding at all radioligand concentrations used.

Photolabeling of MA-10 cell mitochondria was performed by suspending 2 mg of membrane protein in 2 ml of 25 mM Tris-HCl, pH 7.4, 0.32 M sucrose buffer which contained 9 nM [3H]PK 14105. The membranes were preincubated at 4 °C for 30 min before being placed in a rectangular dish (75 x 52 x 5 mm), continuously rocked for 60 min at 4 °C, and simultaneously irradiated from a distance of 2 cm using an UVCL-58 ultraviolet light (UVP, Inc., San Gabriel, CA) with maximum emission at 366 nm. Following irradiation, 10 μl of 2 mM PK 11195 was added to the membrane suspensions. The samples were incubated for another 30 min, diluted into 10 ml of Tris/sucrose buffer, and centrifuged at 20,000 x g for 10 min; and the pellets were washed once more in 10 ml of buffer.

**Steroid Biosynthesis**—These experiments were performed with the MA-10 Leydig cells plated in 12 x 22-mm wells and incubated for the times shown in the presence of the indicated substances at a final volume of 1 ml of serum-free media at 37 °C. Where purified rat Leydig cells were used, 50,000 cells/500 μl of serum-free media were incubated in borosilicate culture tubes at 32 °C. At the end of the incubation period, the cell media were saved, centrifuged at 1,500 x g for 10 min, and stored at −20 °C until used. Cells were dissolved with 0.1 N NaOH for protein measurement.

**Cholesterol Transport in Mitochondria**—MA-10 Leydig cells were washed with serum-free media as described above followed by washing in phosphate-buffered saline. Mitochondria were then prepared as previously described (35, 36). The effect of different compounds on cholesterol transport and/or on P-450s activity was determined as previously described (28, 35) by incubating mitochondria with the ligands in the presence of exogenous cholesterol (100 μM) and trilutane (0.1 μM), an inhibitor of pregnenolone metabolism (37). The production of pregnenolone was measured after a 10-min incubation with a specific radioimmunoassay (38) using an antibody given by Dr. F. F. G. Rommens (Erasmus University, Rotterdam, Netherlands). In another series of experiments, MA-10 Leydig cell mitochondria were prepared using a method of Goessens and colleagues (39). Anti-testosterone antibody was obtained from ICN Co. (Lisle, IL), and the assay was performed as described by the manufacturer. Analysis of the radioimmunoassay data was performed using the IBM-PC RIA Data Reduction program (version 4.1) obtained from Jaffe and Associates (Silver Spring, MD).

**Protein Measurement**—Protein was measured by the method of Bradford (40) using bovine serum albumin as a standard.
cell culture supplies were purchased from Gibco, and cell culture plasticware was from Corning. All other chemicals were of analytical quality and were obtained from commercial sources.

RESULTS

Characterization of PBR in MA-10 Cells—Kinetics of [3H]PK 11195 binding at 37 °C to MA-10 cells revealed that specific binding of this ligand was rapid, reaching steady-state conditions after 10 min (data not shown). Furthermore, if 10 μM PK 11195 was subsequently added, specific radioligand binding was completely displaced within 15-20 min. Scatchard analysis demonstrated a single class of binding sites with a dissociation constant of 1.8 nM at a density of 56 pmol/mg of protein (Fig. 1). Photolabeling of MA-10 cell mitochondrial fractions with [3H]PK 14105 verifies that these cells contain the 18-kDa protein which constitutes the binding domains for PBR ligands (9).

Specificity of these binding sites was determined using nine different ligands from several classes of organic compounds known to interact with PBR. These ligands exhibited a rank order potency to compete against [3H]PK 11195 binding which is consonant with their relative potencies reported by other groups (Fig. 2). The most notable observations are that the isoquinoline carboxamide PK 1195 was the most potent compound of this series. The quinoline propranamide PK 14067 was about 200-fold more potent than its enantiomer, PK 14068 (41). Of the benzodiazepines, Ro5-4864 was the most potent derivative, whereas clonazepam and flumazenil, an antagonist of central benzodiazepine actions, exhibited low affinities for PBR.

Effect of PBR Ligands on MA-10 Leydig Cell Steroid Bio- synthesis—To investigate whether the different ligands affected Leydig cell steroidogenesis, increasing concentrations of these compounds were incubated for 4 h with MA-10 cells, and their effects on progesterone production were measured (Fig. 3). The three most potent compounds of this series were very efficacious at stimulating progesterone synthesis, whereas the less potent compounds either achieved a lower level of maximal stimulation or did not have an effect on steroid production. PK 1195 and PK 14067 were the most potent and produced a 3-4-fold stimulation of progesterone production. Among the benzodiazepines used, Ro5-4864 was the most potent, giving a 3.5-fold increase in steroidogenesis, whereas diazepam attained only a 2-fold maximal stimulation. The other compounds used significantly stimulated steroid production (1.5-fold) only at concentrations higher than 10⁻⁶ M, whereas clonazepam and flumazenil were completely inactive at concentrations above 10⁻⁶ M.

When comparing the EC₅₀ values of the stimulatory effect of the different PBR ligands with the inhibitory constants with which these ligands compete for [3H]PK 11195 binding to the MA-10 cells (Fig. 4), an excellent correlation was observed (r = 0.95). This provides strong evidence that the effects of these drugs on steroidogenesis are consequent to their binding to PBR. PK 11195 (10⁻⁷ M) also stimulated 20α-hydroxyprogesterone production by MA-10 Leydig cells to similar extents as observed with progesterone (data not shown), indicating that their actions are not unique to progesterone and that there is an increase of all steroidogenic products of these cells. This implies an action at the level of the initial substrate (cholesterol) and its processing. It is important to note at this point that all compounds used in these studies did not cross-react with the antibodies used in the different radioimmunoassays.

Effect of PBR Ligands on hCG- and EGF-stimulated Ste-
Leydig Cell Steroidogenesis Mediated by PBR

Log \( K_r \) (Binding) vs. log EC50 (stimulation)

**FIG. 4.** Correlation between binding affinities and stimulatory potencies of PBR ligands. The \( K_r \) estimated for each compound from the analysis of Fig. 2 is compared to the concentration of ligand required to exhibit one-half of the respective maximal stimulation as was determined in Fig. 3. Point 1, PK 11195; point 2, PK 14067; point 3, Ro5-4864; point 4, zolpidem; point 5, PK 14068; point 6, diazepam; point 7, flunitrazepam.

**FIG. 5.** Effects of PBR ligands on hCG- and EGF-stimulated steroidogenesis. MA-10 Leydig cells were incubated for 4 h in the presence of \( 10^{-6} \) M PK 11195 (A) or \( 10^{-5} \) M Ro5-4864 (B) with increasing concentrations of hCG (A, ○) or EGF (B, ○). Cell media were collected, and progesterone accumulation was measured by radioimmunoassay. Values are means ± S.D. of two independent experiments (n = 4). Note the different units used on the abscissas for both panels.

**FIG. 6.** Time course on effects of PBR ligands, hCG, and EGF on MA-10 steroid biosynthesis. MA-10 Leydig cells were incubated for the times shown in the absence (○) or presence of \( 10^{-6} \) M PK 11195 (△), \( 10^{-5} \) M Ro5-4864 (□), \( 10 \) ng/ml EGF (●), or \( 50 \) ng/ml hCG (●). At the end of the incubation period, media were collected, and progesterone was measured by radioimmunoassay. Values are means ± S.D. from two independent experiments (n = 4) plotted on two separate scales. In parallel experiments, incubation of the cells with 1 mM BrdU gave results which were equivalent to those with hCG.
may be included in the multiple cellular events affected by hCG.

**Studies on Mechanism of Action of PBR Ligands on Leydig Cell Steroidogenesis**—In search for the site of action of PBR ligands on steroid biosynthesis, we first examined the possibility that PBR ligands may affect protein synthesis, which in turn will affect steroidogenesis. In the presence of the protein synthesis inhibitor cycloheximide (0.2 mM), hCG or Bt-cAMP-stimulated steroidogenesis is almost completely inhibited, although a slight but significant stimulation remains (Table I). In contrast, progesterone production stimulated by 10^{-6} M PK 11195 or 10^{-5} M Ro5-4864 was not inhibited by cycloheximide. At submaximal concentrations of hCG (0.5 ng/ml), steroid production was still greater than that observed with the PBR ligands; cycloheximide, however, completely inhibited steroid production at these hCG concentrations (Table I). These findings demonstrate that cycloheximide does not inhibit the stimulation by PBR ligands, unlike the stimulation observed with hCG.

Table I also shows that stimulation of steroid biosynthesis by hCG, Bt-cAMP, or PBR ligands is inhibited by the P-450, inhibitor aminoglutethimide (42, 43). Furthermore, progesterone production under conditions of unlimited substrate, as in the presence of exogenous pregnenolone, was not affected by hCG, Bt-cAMP, or PBR ligands (data not shown), indicating that their acute regulatory effects on steroidogenesis are prior to pregnenolone metabolism.

At this point, we also examined the possibility that ligand binding to PBR may affect cholesterol metabolism to pregnenolone by directly acting on the mitochondrial P-450, enzyme. The metabolism of (22R)-hydroxycholesterol to progesterone by MA-10 cells was used as an index for P-450, activity (44). Ro5-4864 (10^{-5} M) did not affect progesterone production in the presence of 0.25 μM (22R)-hydroxycholesterol (244.67 ± 42.52 versus 239.67 ± 30.33ng of progesterone/mg of protein/4 h; means ± S.D., n = 3) or in the presence of 100 μM (22R)-hydroxycholesterol (maximal concentration) (5047.00 ± 238.54 versus 4977.33 ± 213.30ng of progesterone/mg of protein/4 h; means ± S.D., n = 3). Similar results were observed using other (22R)-hydroxycholesterol concentrations (data not shown), showing that the mechanism of stimulation by PBR is not by direct activation of P-450, enzyme. Therefore, from these findings, it can be deduced that PBR are most likely involved with cholesterol uptake from intracellular stores into mitochondria and/or promoting cholesterol availability to P-450,.

**Regulation of Steroidogenesis by PBR Ligands in Leydig Cells Purified from Rat Testis**—To further explore the possibility that the involvement of PBR in steroidogenesis may be specific to the MA-10 cell line rather than representative of normal Leydig cells, we also examined the action of PBR ligands on purified rat Leydig cell steroidogenesis. Table II shows that...
with [3H]PK 14105. This extreme abundance of PBR is comparable with the levels found in adrenal gland mitochondria (6) and is consistent with the high density of these receptors found in apparently all steroidogenic cells (5).

PK 11195 and Ro5-4864 stimulate, by about 2-fold, testosterone production by purified rat Leydig cells, whereas diazepam was less potent, and clonazepam had no effect. Moreover, stimulation of rat Leydig cell steroidogenesis was stereoselective for PBR, as demonstrated using the optical isomers PK 14067 and PK 14068. The hCG-stimulated testosterone production was not affected by PBR ligands (Table II). The site of PBR ligand action in rat Leydig cell steroid biosynthesis was also located, as was performed in MA-10 cells, at the level of cholesterol transport into mitochondria, the rate-limiting step of steroidogenesis (data not shown). These results verify that the effects on steroidogenesis mediated by PBR are also found in normal rat Leydig cell preparations. Hence, the MA-10 cell line has proven to be a suitable model system to examine the role of PBR in Leydig cell function.

Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>hCG (50 ng/ml)</td>
</tr>
<tr>
<td></td>
<td>ng/10^6 cells/4 h</td>
</tr>
<tr>
<td>Control</td>
<td>1.30 ± 0.20</td>
</tr>
<tr>
<td>PK 11195 (10^-6 M)</td>
<td>2.33 ± 0.21</td>
</tr>
<tr>
<td>PK 14067 (10^-6 M)</td>
<td>2.20 ± 0.30</td>
</tr>
<tr>
<td>PK 14068 (10^-5 M)</td>
<td>1.16 ± 0.15</td>
</tr>
<tr>
<td>Ro5-4864 (10^-5 M)</td>
<td>2.40 ± 0.30</td>
</tr>
<tr>
<td>Diazepam (10^-6 M)</td>
<td>1.50 ± 0.10</td>
</tr>
<tr>
<td>Clonazepam (10^-6 M)</td>
<td>1.16 ± 0.11</td>
</tr>
</tbody>
</table>

Among the ligands within the series which were tested, PK 11106, PK 14067, and Ro5-4864 exhibited both the highest affinities for PBR and the greatest maximal stimulation of steroid production. Our data reveal only one class of PBR in MA-10 cells, yet the ligands with lower affinities for PBR elicit a lesser maximal stimulation despite the fact that they completely displace [3H]PK 11195 from PBR, with a Hill coefficient of near unity. This implies that the affinity a ligand has for PBR is an important determinant for the magnitude of the steroidogenic response which is achieved. It should also be noted that none of the compounds acted as antagonists, unlike the reports of others (13, 15) examining different functions for PBR. This difference might indicate structural or functional heterogeneity of PBR in different cell systems.

Since hCG produces a very robust stimulation (>300-fold) of steroidogenesis in MA-10 cells (32), a response which is atypical for normal Leydig cells (10-fold stimulation), one might argue that the effects of PBR ligands are very modest and possibly insignificant. This argument was ruled invalid because purified rat Leydig cells also exhibited a significant stimulation of testosterone synthesis in response to PBR ligands. In every respect which we examined, the effects of PBR ligands in purified Leydig cells or their isolated mitochondria were equivalent to the effects observed in MA-10 cells.

For these studies, we used the accumulation of extracellular progesterone as an index of steroid biosynthesis for MA-10 cells (32). The observations that the production of 20a-hydroxyprogesterone, another steroid product of MA-10 cells, was increased at the same level as progesterone and that the metabolism of exogenous pregnenolone and hydroxysterol was not altered after addition of ligands indicated that the site of action of PBR ligands was likely to be prior to pregnenolone formation.

Steroid biosynthesis begins with the transport of cholesterol from extramitochondrial stores into mitochondria, where it is metabolized to pregnenolone by P-450, located on the inner mitochondrial membrane (30, 46, 47). This delivery is the rate-limiting step of steroidogenesis (data not shown). These results verify that the effects on steroidogenesis mediated by PBR are also found in normal rat Leydig cell preparations. Hence, the MA-10 cell line has proven to be a suitable model system to examine the role of PBR in Leydig cell function.

Discussion

The experiments reported here were designed to investigate the role of PBR in Leydig cell function. It has been previously shown (3, 5–7) that PBR are abundant in steroidogenic tissues and are predominantly localized on the outer mitochondrial membrane. A number of preliminary reports (18–20, 29, 45) show that some PBR ligands can increase steroidogenesis; however, a detailed pharmacological analysis was lacking to establish whether the mediator of this action was PBR. This study establishes that PBR play an important role in steroid biosynthesis and that their subcellular localization is consistent with current knowledge on the mitochondrial compartments participating in steroidogenesis.

In vitro studies using decapsulated testes or interstitial cell suspensions have shown that benzodiazepines (diazepam and Ro5-4864) stimulate androgen production (18–20). In view of the complexity of testicular structure and the role of cell-cell interactions in the testis (21, 22), we decided to examine the participation of PBR in Leydig cell steroidogenesis. For this purpose, two model systems, the mouse tumor MA-10 cell line and Leydig cells purified from rat testis, were used to demonstrate an excellent correlation between the affinities of nine ligands for PBR and their potencies to stimulate steroidogenesis.

MA-10 cells were found to contain a density of PBR which corresponds to ~6 × 10^7 binding sites/cell, representing about 0.1% of the total cell protein based on the M~18,000 determined from our earlier PBR purification and cDNA cloning studies (8, 9) and verified here by photolabeling experiments with [3H]PK 14105. This extreme abundance of PBR is comparable with the levels found in adrenal gland mitochondria (6) and is consistent with the high density of these receptors found in apparently all steroidogenic cells (5).
blocks hormone-stimulated steroid production and that the inhibition occurs at the site of cholesterol transport into mitochondria. The results in Table I show that cycloheximide does not inhibit ligand-stimulated progesterone production by MA-10 Leydig cells, whereas it essentially abolishes hCG-stimulated steroidogenesis, in agreement with previous results (61). This suggests that promotion of cholesterol transport elicited by PBR ligands is not sensitive to cycloheximide, presumably due to a direct activation of PBR. The kinetics of ligand binding and steroidogenic activity we observed support this proposal. These findings might help account for the observations of others (52–54) that hCG-stimulated cholesterol transport is not entirely dependent on a protein synthesis-mediated step. In retrospect, it is also possible that the hormone response may include a cycloheximide-sensitive step to recruit PBR for steroid biosynthesis, and receptor occupancy by ligands merely overrides this physiological control.

In contrast to their relationship with the stimulation by hCG, PBR ligands have an additive effect on steroidogenesis with EGF, another modulator of Leydig cell steroidogenesis (55, 56). It has been reported by Ascoli et al. (55) that EGF and submaximal concentrations of hCG stimulate progesterone production by MA-10 Leydig cells using two different pathways. Our results show that PBR ligands and EGF also use different pathways to increase steroid biosynthesis. Moreover, the kinetics of drug action on progesterone production is much more rapid than that of EGF, indicating a more direct action on a regulatory step of steroidogenesis.

While the experiments presented here were in progress, a paper was published by Besman et al. (29) showing that diazepam, a ligand for central and peripheral benzodiazepine recognition sites, stimulates pregnenolone formation by bovine adrenal gland mitochondria at a concentration of ~10 μM. Our studies on Leydig cells with diazepam are in agreement with these findings, except that in our model, the EC_{50} of the stimulation obtained with diazepam is ~1 μM. Since we obtained similar values with rat and mouse adrenal gland cells (data not shown), this discrepancy with bovine adrenal gland cells is probably due to the known species differences in receptor affinity for benzodiazepines (57).

In search for physiological endogenous ligands for benzo-diazepine receptors, another laboratory at this institution has found that DBI, a polypeptide abundant in steroidogenic tissues, displaces the PBR ligands from rat adrenal gland mitochondrial membranes (25, 26). In addition, the workers found that DBI is expressed in the testis (23, 24), where it primarily localizes in Leydig cells.2 We have also measured high levels of immunoreactive DBI in MA-10 Leydig cells (data not shown). Furthermore, there are recent reports (28, 29) that a protein from bovine adrenal fasciculata cells which stimulates steroidogenesis was identified as being DBI. These new developments raise the possibility that DBI may interact physiologically with PBR mediating cholesterol delivery into mitochondria, thereby activating steroidogenesis. The uncovering of these potential components in the steroidogenic pathway may prove to be an important step in elucidating the molecular systems which regulate steroid biosynthesis.

Acknowledgments—We are indebted to the following investigators and agencies for the generous gifts of different materials used in these studies: Dr. Mario Ascoli for his generous gift of the Leydig cell line MA-10 and for the helpful instructions on the establishment and use of this cell line; Dr. G. Nieswender for the gift of the 20α-hydroxyprogesterone antiserum; Dr. F. F. G. Rommert for his generous gift of pregnenolone antiserum (gR1Δ4-PR2); the National Hormone and Pituitary Program (National Institute of Child Health and Human Development, National Institutes of Health) for the gift of hCG; and Dr. C. Gueremy Dr. S. Z. Langer, and Hoffmann-La Roche for the gift of different PBR ligands. We also wish to thank Dr. M. Dym (Georgetown University, Washington, D. C.) for his continuous support of this project.

REFERENCES


The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis.

V Papadopoulos, A G Mukhin, E Costa and K E Krueger


Access the most updated version of this article at [http://www.jbc.org/content/265/7/3772](http://www.jbc.org/content/265/7/3772)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/265/7/3772.full.html#ref-list-1) to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/265/7/3772.full.html#ref-list-1](http://www.jbc.org/content/265/7/3772.full.html#ref-list-1)