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Hormonal Regulation of Peripheral-type Benzodiazepine Receptors

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Central benzodiazepine (BZ) receptors are located only in the central nervous system and mediate the clinical effects obtained by various BZs. In addition, there is another receptor that binds BZs with different drug specificities, which is located mainly on the outer mitochondrial membrane of various peripheral tissues. Peripheral BZ receptors (PBR) are composed of three subunits: an isoquinoline binding site, a voltage-dependent anion channel, and an adenine nucleotide carrier, with molecular weights of 18, 32, and 30 kDa, respectively. Complementary DNA of the isoquinoline binding subunit has been cloned in rat, calf, and human. The major role of PBR is in the regulation of steroid biosynthesis. Various PBR ligands stimulate the conversion of cholesterol into pregnenolone and the production of steroid hormones. The naturally occurring diazepam-binding inhibitor stimulates *in vivo* steroidogenesis via binding to PBR. In the female, PBR density is increased in rat and human ovary proportional with greater cell maturation and differentiation. In the male, testosterone modulates PBR density in the genital tract. These results show the strong relationship between PBR and the endocrine system.

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Benzodiazepines (BZs) are used clinically as muscle relaxants, anticonvulsants, anxiolytics, and sedative-hypnotics. These effects are mediated via specific receptors located in the central nervous system which are coupled with the γ-aminobutyric acid (GABA) receptors [1]. In addition to the central-type BZ receptors (CBR), peripheral-type BZ receptors (PBR) have been identified in peripheral tissues such as lung, liver, and kidney [2], mast cells [3], platelets [4], and in the genital tracts of female [5] and male [6] rats. PBR differ from CBR in their lack of coupling to GABA receptors and in their ligand specificity. CBR exhibit high affinity to the BZ clonazepam, but not to the BZ Ro 5-4864 or the isoquinoline carboxamide derivative PK 11195. The reverse is true with regard to PBR, which exhibit high affinity to Ro 5-4864 and PK 11195, but low affinity to clonazepam. The binding of [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 to membrane preparations from cerebral cortex and peripheral tissues of various species has been studied [7]. [^{3}H]PK 11195 binds with high affinity to rat and calf cerebral cortex and kidney membranes. [^{3}H]Ro 5-4864 also successfully labels rat cerebral cortex and kidney membranes, but in calf cerebral cortex and kidney membranes its binding is negligible. Ro 5-4864, diazepam, and flunitrazepam are much more potent in displacing [^{3}H]PK 11195 from rat cerebral cortex and kidney membranes than from calf tissues. The potency of unlabeled Ro 5-4864 in displacing [^{3}H]PK 11195 from PBR of various species is in the rank order of rat = guinea pig > cat = dog > rabbit. In human, Ro 5-4864 is one order of magnitude more potent than in calf in displacing [^{3}H]PK 11195 from PBR [8].

PBR are composed of three subunits: binding sites for PK 11195, a voltage-dependent anion channel, and an adenine nucleotide carrier, with molecular weights of 18, 32, and 30 kDa, respectively [9]. The site for isoquinoline binding has been purified to apparent homogeneity from rat adrenal mitochondria [10]. The protein that includes this site has a molecular weight of 17 kDa. A full-length complementary DNA (cDNA) comprising 781 bp and encoding this protein has been cloned [11] and encodes a 169 amino acid open reading frame with five putative transmembrane regions [11]. Subsequently, the amino acid homology of human PBR has been found to be 79% identical to that reported for rat PBR [12]. Using the cDNA of human PBR as a probe, the PBR gene has been located in the q13.3 region of the long arm of human chromosome 22 [12].

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Calf PBR has also been cloned using rat cDNA as a probe [13]. In this case we have found a 95% nucleotide homology between human and calf cDNAs (unpublished results).

Early studies showed that BZ ligands such as diazepam increase *in vivo* corticosterone [14] and testosterone [15] secretion in rat, apparently through their binding to mitochondrial PBR. Moreover, plasma testosterone and plasma 11-hydrocorticoid levels are also increased by these ligands in human [16]. A direct effect of Ro 5-4864 on testicular testosterone secretion has been observed in the rat. Decapsulated testis shows a significant increase in basal human chorionic gonadotropin-stimulated testosterone secretion [17]. PBR-specific ligands stimulate steroidogenesis; it has been found that this effect is mediated by PBR [18–20]. It has been suggested that PBR plays a role in the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in steroidogenesis [21].

PBR have also been localized in the rat ovary [5]. Higher PBR densities have been detected in granulosa cells and in interstitial theca cells from immature rats as a result of whole-animal treatment with pregnant mare serum gonadotropin (PMSG) (unpublished results). Furthermore, PBR have been detected in ovarian granulosa cells from Chinese hamster [22] and human [23], as well as in rat granulosa cell lines transformed by SV40 T-antigen and Ha-*ras* oncogene [24].

In the immature rat ovary, PBR density increases with age [5]. Hypophysectomy prevents this age-dependent increase and causes a significant decrease in ovarian PBR density associated with follicular atresia and a decrease in ovarian weight [25]. The effect of hypophysectomy can be abolished by either 2 days' treatment with PMSG or 4 days' treatment with the synthetic estrogen diethylstilbestrol (DES) [25]. In intact rat, PMSG or DES also significantly increases ovarian PBR density [5]. Whereas short-term (4 days) treatment with either progesterone or testosterone increases PBR density in the ovary, long-term (10 days) treatment results in a significant decrease in PBR density [26].

In the adult rat, ovarian PBR density increases to maximum on the day of proestrus [27]. On the day of proestrus, follicular size is maximal and the follicle is ready to ovulate. Maximal PBR densities are already seen on the night following diestrus, that is, 14 h before the onset of the luteinizing hormone surge [27].

In granulosa cells obtained from Chinese hamster ovary, PBR have been identified as a protein located in the mitochondrial outer membrane [22]. Using the photoaffinity probe [^{3}H]PK 14105, a nitrophenyl derivative of [^{3}H]PK 11195, a 17 kDa mitochondrial protein was purified which presumably contained at least part of the PBR recognition site [22]. Also, in granulosa cell lines transformed by SV40 T-antigen and Ha-*ras* oncogene, [^{3}H]Ro 5-4864 demonstrated saturable binding to the isolated mitochondria. This specific binding could be displaced by specific PBR ligands such as unlabeled Ro 5-4864 or Ro 5-2807, but could not be displaced by specific CBR ligands such as Ro 15-4513 or Ro 15-1788 [24]. In these *in vitro* conditions a significant increase in PBR density was observed in response to various agents which increase intercellular cyclic AMP level [24].

The association between degree of differentiation and PBR densities in the ovarian cells of various rodents encouraged us to study PBR density in the human ovary. PBR levels were demonstrated in human granulosa-lutein cells obtained at *in vitro* fertilization/embryo transfer (IVF/ET). It appears that PBR densities are significantly greater in granulosa-lutein cells obtained from larger follicles or when morphological luteinization of follicular cells has been observed [23]. Furthermore, when PBR densities were studied in individual follicles, a high correlation was found between egg cell performance (in terms of completion of meiotic maturation to the second metaphase stage, fertilization and embryonic cleavage) and PBR densities in the corresponding granulosa cells [23]. PBR densities in human ovarian granulosa cells were significantly higher in women with high plasma estradiol-17β levels (>1400 pg/ml) compared with women with lower levels (<1000 pg/ml). Moreover, granulosa cell PBR densities were 1.8-fold higher in women who conceived following IVF/ET treatment than in those who did not conceive [23]. Thus, in the ovary, PBR density is under endocrine, paracrine, and autocrine regulation, and PBR-specific ligands also appear to modulate ovarian follicular steroidogenic activity.

High PBR levels have been detected in human term placenta [28, 29]. Exposure of placental explants to Ro 5-4864 (10^{-8} M) increases progesterone and estradiol secretion 2.4- and 1.4-fold, respectively [29]. Treatment of placental explants with diazepam (10^{-7} M) and PK 11195 (10^{-6} M) causes a significant increase in progesterone and estradiol secretion, whereas clonazepam has no effect on steroid secretion [29]. These effects are probably modulated via PBR.

PBR are also involved in the regulation of testicular androgen production [17]. We have identified and characterized PBR in prostate, seminal vesicles, and Cowper's glands [6]. Adrenal and testicular PBR are depleted in rats following hypophysectomy [30]. We found that following removal of the testes, a significant decrease in PBR density was observed in Cowper's glands, and testosterone prevented the decrease in PBR density obtained following castration [31].

From the data just discussed within the body of literature that exists, it is clear that peripheral BZ receptors interact with and are acted upon by different aspects of the endocrine system. This is true for both the female and the male of various species [32].

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