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ADRENAL MITOCHONDRIAL BENZODIAZEPINE RECEPTORS ARE SENSITIVE TO AGENTS ACTIVE AT THE DOPAMINE RECEPTOR

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Abstract—Male rats were treated for 21 days with drugs known to affect prolactin secretion, in order to assess the effects of these drugs on mitochondrial benzodiazepine receptors (MBRs). Sulpiride, a selective dopamine D_2 receptor antagonist and hyperprolactinemic agent, decreased MBR density in the adrenal gland (49%; $P < 0.005$), whereas metoclopramide, another dopamine antagonist with a preference for dopamine D_2 receptors, increased adrenal gland MBR density (31%; $P < 0.05$). Bromocriptine, a specific dopamine agonist, increased MBR density in this organ (87%; $P < 0.001$). None of the three agents influenced kidney or testicular MBRs. These data indicate that the mechanism of organ-specific alterations in MBRs seems to be prolactin independent.

Benzodiazepines (BZs‖) are widely used as anxiolytics, sedative-hypnotics, and anticonvulsants. They bind to two distinct types of receptors: the central BZ receptors (CBRs), which are localized exclusively in the central nervous system and are coupled to γ-aminobutyric receptors and chloride ion channels [1], and the mitochondrial BZ receptors (MBRs), which are distributed throughout the body and are independent of the γ-aminobutyric receptors [2, 3]. Whereas CBRs are confined to brain synaptosomes [4], MBRs are localized mainly in the outer membrane of the mitochondria [5]. The localization of MBR implies a functional role for these sites in intracellular processes, probably associated with steroid metabolism [6, 7] and dopamine release [8].

It has been found that BZs, especially the MBR-specific ligand Ro 5-4864, affect prolactin (PRL) secretion (a dopaminergic-controlled process) [9, 10], whereas the neuroleptics (which possess anti-dopaminergic properties) alter MBRs in various organs. For example, chronic treatment with phenothiazines induced down-regulation of platelet MBRs in schizophrenic patients [11, 12]. A similar effect was found in cardiac and renal MBRs in rats as a result of chronic treatment with chlorpromazine, accompanied by an increase in the density of these receptors in the cerebral cortex [13]. Chronic haloperidol treatment also increased MBR density in rat cerebral cortex, but had no effect on cardiac MBRs [14]. In contrast to the effect of neuroleptics on platelet MBRs, the chronic use of cocaine (a dopaminergic agonistic agent) augmented platelet MBR binding [15]. Since treatment with anti-dopaminergic agents is associated with hyperprolactinemia, while dopaminergic agonists suppress PRL release, and since these treatments are accompanied by parallel MBR alterations in certain organs, it is hypothesized that PRL may also be regarded as a regulatory hormone of MBRs.

In the present study we addressed this issue by *in vivo* pharmacological manipulation of PRL secretion induced by drugs which primarily affect dopamine and are less potent toward the cholinergic, serotonergic and noradrenergic systems. The following drugs were used: metoclopramide, a potent dopamine (D_2 receptor) antagonist; sulpiride, another dopamine (D_2 receptor) antagonist; and bromocriptine, a specific dopamine receptor agonist.

MATERIALS AND METHODS

Materials. [^{3}H]PK 11195 [1-(2,chlorophenyl)-*N*-methyl - *N* - (1-methylpropyl) - 3 - isoquinoline carboxamide] (92.3 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Unlabeled PK 11195 was kindly donated by Dr A. Bouvier, Rhône-Poulenc Santé (Vitry-sur-Seine, France). Sulpiride, metoclopramide and bromocriptine mesylate were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Lumax was obtained from Lumac (Schaesberg, The Netherlands). All other chemicals were obtained from commercial sources.

Treatment of rats. Male Sprague–Dawley rats (70 days, 200–250 g) were maintained under standard laboratory conditions of 12-hr light/dark cycle at 24°. Food and water were available *ad lib.* Rats were

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‖ Abbreviations: BZ, benzodiazepine; CBR, central BZ receptors; MBR, mitochondrial BZ receptor; PRL, prolactin.

injected once daily intraperitoneally (0.2 mL), for 21 days, with sulpiride, 10 mg/kg (N = 5); metoclopramide, 5 mg/kg (N = 5); bromocriptine, 2 mg/kg (N = 5) or vehicle, 0.2 mL saline (N = 5).

Preparation of membranes. Twenty-four hours after the last injection, the rats were killed by decapitation, blood was collected and the peripheral organs were immediately removed, trimmed of fibrous tissues and fat, and were stored at −70° until assayed for receptor binding.

Before assay, the various organs were thawed and homogenized separately in 50 vol. of 50 mM Tris–HCl buffer, pH 7.4, at 4°, using a Ystral Polytron (setting 10) for 15 sec. Each homogenate was centrifuged at 49,000 *g* for 15 min. The pellets of peripheral organs were resuspended in 200 vol. of Tris–HCl buffer, and the homogenates were used for binding assays.

[^{3}H]*PK* 11195 *binding assay.* [^{3}H]PK 11195 binding to MBR was assayed in 50 mM Tris–HCl buffer, pH 7.4, at 4° in a final volume of 500 μL containing 400 μL membranes and 25 μL [^{3}H]PK 11195 (0.2–6 nM final concentration) in the absence (total binding) or presence (non-specific binding) of 75 μL unlabeled Ro 5-4864 (1 μM final concentration). After incubation for 60 min, samples were filtered under vacuum over Whatman GF/B filters and washed three times with 3 mL Tris–HCl buffer. Filters were placed in vials containing 5 mL of xylene-Lumax (3:1) and counted for radioactivity after 12 hr equilibration.

Prolactin immunoassay. Serum PRL was measured by radioimmunoassay employing reagents supplied by the Hormone Distribution Program of the NIDDK. Results are reported in equivalents of NIH rat PRL standard RP-1. The inter- and intra-assay variations were 5.6 and 2.1%, respectively. In order to minimize variation, all the PRL samples were measured in the same assay.

Statistical analysis. The binding parameters were analysed using one-way analysis of variance (ANOVA) and the Scheffe multiple-comparison test for intergroup comparisons. The results are expressed as means ± SEM.

RESULTS

A significant increase in total body weight (18%; $P < 0.05$ compared to controls) was observed only following sulpiride administration for 21 days to adult male rats, whereas metoclopramide and bromocriptine did not affect body weight. As to the weight of the organs tested (kidney, testis and adrenal gland), only the kidney increased in weight (by 34%) following treatment with sulpiride ($P < 0.05$ compared to controls), but the other agents had no significant effect.

Figure 1 illustrates the effects of chronic administration of the various drugs on the maximal binding capacity (B_{max}) of [^{3}H]PK 11195 binding in the peripheral organs. A significant decrease (49%; $P < 0.005$) was observed in the adrenal gland following sulpiride administration, whereas metoclopramide and bromocriptine treatment induced a significant increase in adrenal MBR density (31%, $P < 0.05$ and 87%, $P < 0.001$, respectively). The alterations in B_{max} values were not accompanied by any alterations in equilibrium dissociation constant (K_d: 1.8–2.6 nM). None of the three pharmacologic agents caused a change in MBR density in the kidney or testis or in K_d values (kidney, 1.4–1.9 nM; testis, 1.5–1.9 nM). As expected, both sulpiride and metoclopramide stimulated PRL secretion (217 and 250% of control values, respectively), while bromocriptine suppressed PRL secretion (50% of control values).

In order to exclude a direct effect of the compounds on [^{3}H]PK 11195 binding, we performed competition studies. Sulpiride, metoclopramide and bromocriptine, in final concentrations of up to 10 μM, did not affect the binding of 4 nM [^{3}H]PK 11195 to renal MBRs.

DISCUSSION

One of the issues regarding BZs is their involvement with PRL production. PRL release is primarily a function of the continuous inhibitory tonus exerted by dopaminergic neurons of the arcuate nucleus and of stimulatory serotonergic input [16]. Grandison [9] found that basal secretion of PRL in primary cultures of the rat anterior pituitary was not changed by BZs. However, PRL release produced by apomorphine (a dopaminergic agonist) was inhibited by pretreatment with BZs [9]. The rank order of drug potency with respect to inhibition of PRL release (Ro 5-4864 > diazepam ≫ clonazepam) correlated strongly with ligand specificity to MBRs. This rank order was dissimilar to that of the interaction of BZs with CBRs. Therefore, it is suggested that BZ-induced inhibition of PRL secretion *in vitro* is a MBR-related phenomenon that has also been demonstrated *in vivo* in rats [10].

The present study showed that pharmacological stimulation and suppression of PRL release did not affect MBR levels in the testis and kidney. In the adrenal gland, sulpiride down-regulated, whereas metoclopramide and bromocriptine up-regulated, MBR density. Since both sulpiride and metoclopramide are dopamine receptor antagonists, one could have expected the same direction of effect on MBRs, assuming this receptor is under the regulatory effect of PRL. However, opposite effects were observed with different types of dopamine antagonists, indicating MBRs to be PRL independent.

How did sulpiride down-regulate and metoclopramide up-regulate MBR in the adrenal gland? Recently Lin *et al.* [17] showed that sulpiride-induced hyperprolactinemia inhibited ovulation and steroidogenesis in rabbits. We have shown that MBRs in the organs of the female genital axis are highly dependent upon gonadotropins and estrogen [18, 19]. Since the adrenal gland is involved in steroid synthesis and MBRs are steroid regulated, it could be that sulpiride-induced steroidogenesis dysfunction is manifested as MBR depletion in the adrenal gland. Testicular as well as renal MBRs, however, are spared despite PRL alteration, suggesting a PRL/MBR-related effect on steroidogenesis to be confined to the adrenal gland. On the other hand, metoclopramide stimulates aldosterone production [20, 21], which is an established regulator of adrenal

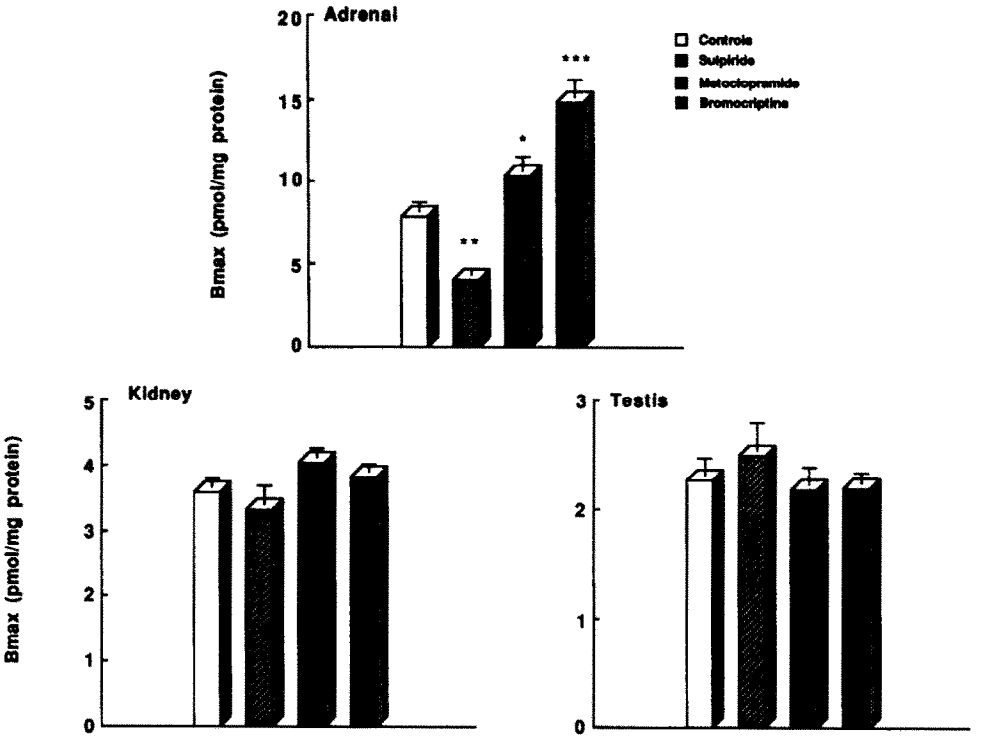

Fig. 1. Maximal binding capacity (B_{max}) of [^{3}H]PK 11195 binding sites (fmol/mg protein) in various peripheral organs following 21 days treatment with sulpiride (10 mg/kg), metoclopramide (5 mg/kg), and bromocriptine (2 mg/kg) treatment in adult male rats. [^{3}H]PK 11195 binding was assayed at six concentrations (0.2–6 nM) in the absence (total binding) or presence (non-specific binding) of 1 μM unlabeled Ro 5-4864. The results of each animal were analysed individually. Results are expressed as means ± SEM. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$, compared to controls. The statistical analysis was performed using the one-way ANOVA and the Scheffe multiple-comparison test.

MBRs [22]. Thus, increased aldosterone plasma level due to metoclopramide administration could account for the up-regulation in adrenal MBRs observed in the present study. With the exception of sulpiride, none of the drugs examined are ultimately specific for dopamine receptors. Metoclopramide acts on 5-hydroxytryptamine-3/4 receptors, and bromocriptine affects noradrenaline and 5-hydroxytryptamine systems [21, 23–25]. Thus, the divergent effects of the drugs could also be explained by their different pharmacological profiles.

The selective depletion of MBRs induced in the adrenal gland by the D_2 blocker sulpiride accords with the down-regulatory effect of chlorpromazine in the heart and kidney [11]. The possible involvement of PRL in the regulation of MBRs appears at first glance to be further supported by the up-regulatory effect of bromocriptine, a dopamine agonist, on the adrenal MBRs. However, metoclopramide, which stimulates PRL release by blocking the dopamine D_2 receptor, also induced an increase in adrenal MBR density (an effect which is opposite to that achieved by the dopamine antagonist sulpiride). Thus, it seems that PRL does not play a role in the regulation of MBRs. This conclusion is supported by the lack of any effect of the dopamine antagonists and agonist on renal or testicular MBRs. Nevertheless, the marked sensitivity of adrenal MBRs to agents affecting the dopaminergic system is as yet unclear and merits further investigation.

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