**Differential effects of ovarian steroid hormones on β-adrenoceptor downregulation caused by the antidepressants imipramine and rolipram**

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**Summary.** Downregulation of β-adrenoceptors in response to repeated administration of imipramine or rolipram was investigated in the brain of pregnant rats and ovariectomized animals treated with estrone, progesterone or a combination of both hormones. Ovariectomy alone was without influence on drug responses. Pregnancy and complete hormone replacement of animals prior to drug treatment halved the response to imipramine and obliterated that to rolipram. Administration of only estrone to ovariectomized rats did not affect the extent of adrenergic downregulation caused by imipramine, but fully suppressed the rolipram action. Castrated males with or without treatment with estrone and progesterone were used as further controls. Castration alone diminished the effects of both antidepressants. Treatment of gonadectomized rats with both ovarian steroid hormones voided the actions of rolipram. The data indicate that imipramine and rolipram share a common pathway for their mechanism of action which can be attenuated by ovarian steroid hormones. In addition, imipramine has a second major site of action which is not subject to modulation by female steroids.

**Key words:** Antidepressants – β-Adrenoceptors – Steroid hormones – Pregnancy – Ovariectomy

**Introduction**

Steroid hormones are known to cause considerable mood fluctuations in humans (Lewis and Smith 1983). These appear particulary prominent during pregnancy, postpartum period, and after onset of menopause. The details of the chain of events initiated by steroids in the brain which ultimately are responsible for these effects, are presently not understood. From experiments with rats it is apparent that steroid hormones modulate among other processes the function of central monoaminergic receptors (for a review see Maggi and Perez 1985). For example, treatment of female rats for two weeks with progesterone results in an increase in the number of β-adrenoceptors in frontal cortex while a treatment with estrogen leads to a significant reduction (Wagner et al. 1979; Biegon et al. 1983; Maggi et al. 1985). Ovariectomy or orchidectomy of rats is reported to be followed by an upregulation of hypothalamic and pituitary β-adrenoceptor density (Wilkinson et al. 1979; Petrovic et al. 1984) while chronic exposure of ovariectomized rats to 17α-ethynyl-estradiol produces a significant reduction of β-adrenoceptor density in frontal cortex (Wagner et al. 1979). The biochemical mechanisms which are responsible for these steroid hormone effects have not yet been elucidated.

Independent from the effects of steroid hormones on catecholaminergic receptors in the CNS, many psychoactive drugs have been demonstrated to cause similar adaptive changes (for reviews see Charney et al. 1981; Sulser et al. 1978). It is well established that prolonged treatment of animals with antidepressant drugs from different chemical classes induces significant, yet quantitatively different changes in adrenoceptor density in the CNS (Kopanski et al. 1983). Currently, this is considered to be directly related to the mechanism of action of antidepressant drugs. Not surprisingly, in many studies possible correlations between the prevailing hormonal status and long term effects of antidepressants on catecholamine receptor responsivity have been investigated (Enna and Duman 1983; Heal et al. 1988; Kendall et al. 1982; Mobley et al. 1983; Mishra and Sulser 1981). Furthermore, downregulation of 5-HT2 receptor density by imipramine was abolished in ovariectomized rats. Steroid replacement therapy with estradiol and progesterone antagonized the effect of ovariectomy (Kendall et al. 1981).

The present study was undertaken to systematically characterize β-adrenoceptor responses to combined changes in female steroid hormonal status and two different antidepressant treatment regimens in an attempt to better understand these interactions. Rolipram [4RS-(3-cyclopent-3-yl-oxy-4-methoxyphenyl)-2-pyrrolidone, ZK 62711] and imipramine were chosen as antidepressants.
because these drugs supposedly have disparate biochemical mechanisms of action (Schultz and Schmidt 1986; Wachtel 1983). The results indicate that the actions of rolipram on \( \beta \)-adrenoceptor changes in cerebral cortical membranes are completely cancelled under conditions of high estrogen plasma levels while those of imipramine are only reduced by half under the same conditions. These findings further support the possibility of major differences in the mechanisms whereby chemically very different antidepressant compounds can modify neurotransmitter receptor binding and function.

Materials and methods

Animals. Female and male Wistar rats of 200–220 g were obtained from SAVO GmbH, Kiellegg, FRG. The rats were housed under temperature- and light-controlled conditions (lights on from 0700–1900 h) with free access to a standard laboratory chow and tap water. Body weight was monitored daily.

Preparation of animals. Female rats were ovariectomized and males were orchidectomized under anesthesia induced by a mixture of atropine/ketamine/xylazine (50 \( \mu \)g/400 \( \mu \)g/30 \( \mu \)g/animal, respectively) and allowed 6 days to recover from surgery. 1 \( \mu \)g Estrone (dissolved in ethanol/0.9% NaCl, 1:10) and 2 mg progesterone (dissolved in 250 \( \mu \)l sesame oil) were s.c. administered between 0900 and 1030 h. This treatment sequence of treatments of the different experimental groups of animals is indicated in Table 1. The general physical well-being of the drug-treated animals did not seem to be affected. The reasons for the absence of body weight gains were noticed when animals concurrently received either rolipram or imipramine. Most prominent was an initial drop in body weight observed 24 h after the first rolipram or imipramine administration (\(-11.8 \pm 0.5 \text{ g} [n = 44]\) and \(-12.4 \pm 0.5 \text{ g} [n = 34]\), respectively; \(P < 0.005\) in both instances). During the 7 d period of continued antidepressant treatment the rats started to gain weight from those reduced levels. Accordingly, at the end of the drug administration periods antidepressant-treated animals generally were significantly lighter than untreated controls. On the average, the differences to untreated rats were 24 g for rolipram-, and 30 g for imipramine-treated animals (\(P < 0.02\) for all applicable groups). The general physical well-being of the drug-treated animals did not seem to be affected. The reasons for this effect are not known.

Results

Changes in body weight gain during drug treatment

Ovariectomy, orchidectomy or pregnancy alone did not impair the usual body weight gain compared to sham-operated controls. Similarly, treatment of operated animals with progesterone, estrone or the combination of both hormones was without significant effect on this parameter, the averaged weight gain was 28 \( \pm \)0.4 g (\(n = 42\)) within 8 days. Large and significant differences in body weight gain were noticed when animals concurrently received either rolipram or imipramine. The brains were rapidly removed, dissected on ice and stored at \(-20^\circ\text{C}\). Membranes from the cerebral cortex were prepared and binding of the \( \beta \)-adrenoceptor antagonist [\( ^3H \)]dihydroalprenolol (DHA) was determined in triplicates at six concentrations ranging from 0.5 to 10 nM as described (Alexander et al. 1975; Bylund and Snyder 1976). Unspecific binding was routinely measured in duplicates in the presence of 10 \( \mu \)M \((\pm)\)-propranolol. It was not affected by up to 100 \( \mu \)M \((\pm)\)-propranolol. Scatchard plots for individual experiments were calculated by computer (correlation coefficients were > 0.97 throughout). \( B_{\text{max}} \) and \( K_D \) values were obtained from those graphs (not depicted).

As further controls, we investigated possible direct effects of rolipram, imipramine, and 2-hydroxyestrone, a major estrogen metabolite in the brain (Paden et al. 1982), on DHA binding. These data excluded artifacts due to these possibilities (not shown).

Drugs and chemicals. Estrone and progesterone were from Sigma, Munich, FRG. Imipramine was a gift from Ciba-Geigy, Basle, Switzerland, racemic rolipram from Schering, Berlin, FRG, and \((\pm)\)-propranolol from ICI, Frankfurt, FRG. All radiochemicals were purchased from Amersham International.

Statistical methods. Data were analyzed with Student’s \( t \)-test. Multiple comparisons were made with an analysis of variance (ANOVA) combined with Newman-Keuls analysis using the applicable tables of Pearson and Hartley (1976) for \( P \)-values. Differences were considered statistically significant when \( P \leq 0.05\). All numbers are given with one SEM.

Determination of adrenoceptor density. Animals were killed by decapitation 20–24 h after the last drug treatment, i.e. on day 21 (see Fig. 1). The brains were rapidly removed, dissected on ice and stored at \(-20^\circ\text{C}\). Membranes from the cerebral cortex were prepared and binding of the \( \beta \)-adrenoceptor antagonist [\( ^3H \)]dihydroalprenolol (DHA) was determined in triplicates at six concentrations ranging from 0.5 to 10 nM as described (Alexander et al. 1975; Bylund and Snyder 1976). Unspecific binding was routinely measured in duplicates in the presence of 10 \( \mu \)M \((\pm)\)-propranolol. It was not affected by up to 100 \( \mu \)M \((\pm)\)-propranolol. Scatchard plots for individual experiments were calculated by computer (correlation coefficients were > 0.97 throughout). \( B_{\text{max}} \) and \( K_D \) values were obtained from those graphs (not depicted).

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\( \beta \)-Adrenoceptors in pregnant, gonadectomized and steroid hormone treated animals

Steroid hormones have been reported to affect \( \beta \)-adrenoceptor density in various brain regions to different extents (Roberts and Bloom 1981; Heal et al. 1988; Wagner et al. 1979; Biegon et al. 1983; Maggi et al. 1985). Therefore,