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Is Inhibition by Diazepam and β-Carbolines of Estrogen-Induced Luteinizing Hormone Secretion Related to Sedative Effects?

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GARGIULO, P. A. AND A. O. DONOSO. *Is inhibition by diazepam and β-carbolines of estrogen-induced luteinizing hormone secretion related to sedative effects?* PHARMACOL BIOCHEM BEHAV **40**(2) 335–338, 1991.—The effects of diazepam (DZ) and the β-carbolines ZK-91296 and ZK-93423 on luteinizing hormone (LH) release evoked by β-estradiol were studied in estradiol-primed ovariectomized rats. Acute treatment with 2.5, 5 and 10 mg/kg DZ and ZK-91296 significantly blunted the LH response. ZK-93423 (1, 5 and 10 mg/kg) produced a similar effect. The inverse agonist DMCM (2 mg/kg) was unable to modify plasma LH levels in estrogen-primed, ovariectomized rats. Sedative effects were observed with doses of DZ (1 mg/kg) ineffective to blunt the LH response. In contrast, ZK-91296 (5 mg/kg) produced inhibition of LH surges in nonsedative doses. These results indicate that diazepam and β-carboline agonists prevent the LH surge evoked by estrogen. They suggest, in addition, that the actions on LH release and their sedative effects are not directly related.

Diazepam and β-carbolines Estrogen-induced LH release Sedation

CURRENT evidence indicates that GABA is the major inhibitory neurotransmitter in the brain. Benzodiazepines (BDZ) are ligands for an allosteric modulatory site in the GABA macromolecular complex where they enhance the activity of GABA on the chloride-ionophore by increasing the frequency of Cl^- channel opening (14). Furthermore, the BDZ receptor of the GABA A complex has the property of influencing GABAergic transmission by mediating opposite effects of different ligands, as the β-carbolines, a large number of compounds, agonists and inverse agonists, having a wide spectrum of pharmacological properties (2).

Previous work from our laboratory and others has demonstrated that GABA and drugs that increase endogenous GABA levels are able to blunt the positive feedback action of estrogen on luteinizing hormone release (LH) in rats, a neuroendocrine mechanism involved in the ovulatory process (1,3). The aim of the present study was to examine the ability of BDZ agonists, diazepam (DZ) and two β-carbolines, to prevent the rise of LH in the plasma induced by estrogen. The inverse agonist DMCM was employed in order to verify whether the GABA/BDZ receptor complex has a bidirectional effect on LH release.

Another purpose of the work was to determine whether sedation, an effect associated with anxiolysis produced by BDZ agonists (10), is involved in their endocrine actions.

METHOD

Adult female Sprague-Dawley rats (200–250 g) were ovariectomized under ether anesthesia and kept in a 14-h light, 10-h dark cycle (light on 0500–1900 h) at 22±2°C. Animals were provided with rat chow and water ad lib.

Two weeks after ovariectomy, all the animals were primed SC at noon with 20 μg β-estradiol benzoate dissolved in corn oil. Estradiol injection was repeated 48 h later. The 2nd SC injection of estradiol (at noon) was followed at 1300 h by the IP injection of the drugs under study. Controls received vehicle or saline. Rats primed with a single dose of estradiol 48 h previously were used for the DMCM experiment. All animals were killed by decapitation at 1700 h and blood from the trunk was collected for LH assay.

Behavioral Experiments

Observations of behavior were done between 1400 and 1500 h. Locomotion, nonambulatory activity (grooming) and rearing (air exploration), were scored. Naive rats were placed in the experimental room 4 h before the experiment. One hour after IP injection of the diazepam or the β-carboline agonists, activities were visually controlled and scored for 5 min from 1400 h, in an Opto-Varimex electronic device (OVM; Columbus Instruments, OH) under light provided by a 40-W fluorescent tube placed 4 m above. A complementary experiment was performed in order to ascertain sedation and to determine the validation of OVM scores. This was done by means of a protocol that delayed in 3 h the time schedule for estrogen and drug injections. At the time of observations (1700–1800), the animal colony exhibited higher ambulatory scores than at early afternoon. Behav-

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ior was evaluated with a hole-board device which was placed inside the OVM for automatic recording of ambulatory. Number of holes explored were scored visually. In this experiment, DZ and ZK-91296 (but not ZK-93423, because there was not enough drug) were tested.

LH Radioimmunoassay

Plasma LH levels were measured by a double-antibody RIA using the kits provided by the National Hormone and Pituitary Program, USA, employing NIDDK r-LH-S10 as the antibody. The inter- and intra-assay coefficients of variation were 11.3% and 9%, respectively, and the correlation coefficient in the standard curves was better than .9. Values are expressed in terms of RP-1 standard preparation.

Statistics

Comparisons were made by one-way variance analysis followed by Duncan's new multiple range test for multiple comparisons. Differences were considered significant if $p<0.05$.

Drugs

Diazepam was provided by Roche-Argentina. The β-carbolines, ethyl 5-benzyloxy-4-methoxymethyl-β-carboline-3 carboxylate (ZK-91296), 6-benzyloxy-4-methoxymethyl-β-carbolin-3-carbon-ethylester (ZK-93423) and methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) were a generous gift of Schering AG (Berlin, Germany). Drugs were suspended in a vehicle of saline-Tween 80.

RESULTS

Treatment of ovariectomized rats with estrogen (OVX-E2) resulted, as expected, in high plasma LH levels (around 150 ng LH/ml plasma; Fig. 1).

Figure 1 shows that DZ in acute doses produced a significant inhibition of estrogen-induced LH release ($p<0.05$). Plasma LH levels were low in OVX-E2 injected with 2.5–5 mg/kg doses. The lowest dose of DZ, 1 mg/kg, was ineffective. It can be seen, in addition, that the largest dose of DZP, 10 mg/kg, did completely prevent the stimulatory action of estrogen on LH release. As shown in Fig. 1, the β-carboline ZK-91296 over a dose range of 2.5–10 mg/kg produced marked inhibitory effects ($p<0.05$). The effect of compound ZK-93423 on LH release was complex. At low and high doses it inhibited LH release, whereas at the intermediate dose, 2.5 mg/kg, it was ineffective (Fig. 1). The inverse agonist DMCM in doses of 2 mg/kg did not increase plasma LH levels in estrogen-primed, ovariectomized rats (plasma LH: 82.3 ± 7.5 ng/ml; n = 10). At that dose level 3 out of 10 animals displayed occasional jerks.

The behavioral scores recorded in the OVM and the absence of drowsiness in the animals indicated that both DZP and ZK-91296 had no sedative effects over a dose range of 1–10 mg/kg. Similar ambulatory, nonambulatory and rearing scores were found in drug-treated rats and controls. By contrast, ZK-93423 did impair the animals' performance at doses of 10 mg/kg (Fig. 2). The complementary experiment with DZ indicated, however, that OVM recordings lacked sensitivity to detect low degrees of sedation. In fact, DZ in doses of 1 mg/kg produced a consistent decrease in hole-board exploration ($p<0.05$) and lower locomotion scores ($p<0.05$) compared with controls. ZK-91296 (5 mg/kg) and estrogen alone did not modify spontaneous motility and exploratory behavior (Fig. 3).

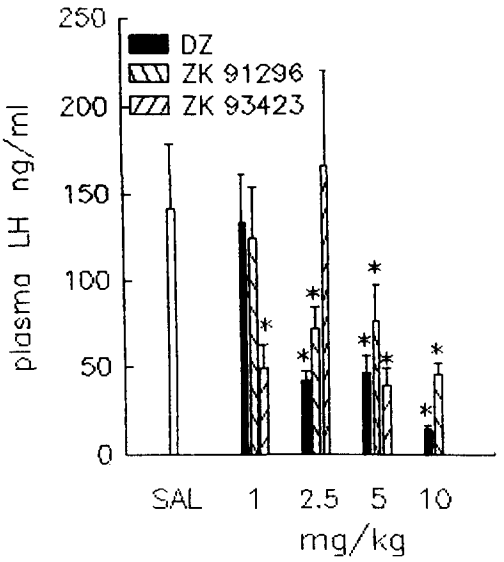

FIG. 1. Inhibition by diazepam (DZ) and β-carbolines of estradiol-induced luteinizing hormone release in ovariectomized, estrogen-primed rats. Each value is the mean ± SEM for each group with 7–12 rats. SAL: vehicle (n = 10 rats). *$p<0.05$ vs. SAL.

DISCUSSION

Present results demonstrate for the first time that compounds acting selectively on the BDZ recognition site of the GABA A

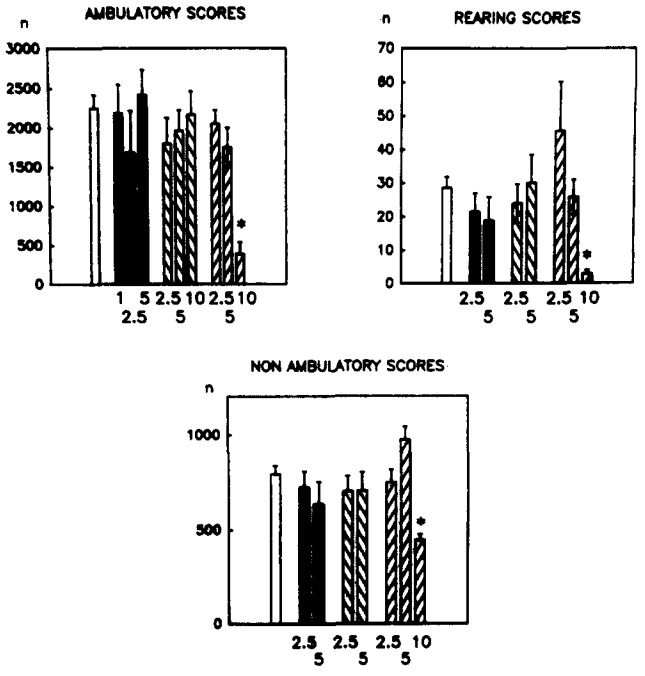

FIG. 2. Behavioral scores in the Opto-Varimex of ovariectomized, estrogen-primed rats injected with vehicle (SAL) (open bars) (n = 24 rats), diazepam (DZ) (filled bars) or the β-carbolines ZK-91296 (left diagonal crossed bars) and ZK-93423 (right diagonal crossed bars). Each value is the mean ± SEM for each group with 7–10 rats. Doses (mg/kg) are given below each bar. *$p<0.05$ vs. vehicle.

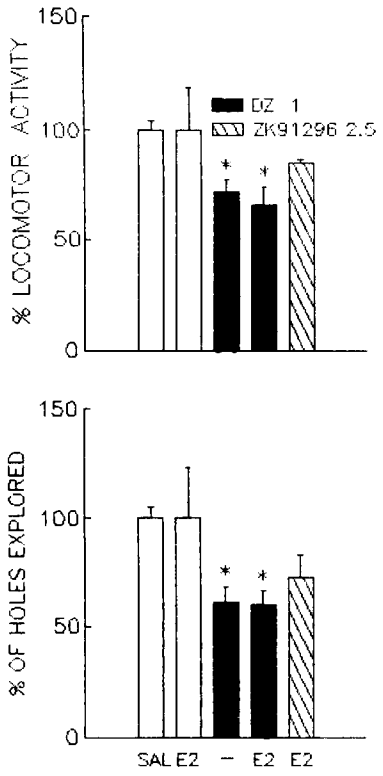

FIG. 3. Locomotor activity and number of holes explored in a hole-board (for details see the Method section). Scores are expressed in percent with respect to vehicle-injected rats (SAL). E2: estradiol-primed rats not receiving drugs. DZ: diazepam, 1 mg/kg; ZK-91296, 2.5 mg/kg. Each value is the mean ± SEM for each group with 7 rats. *$p<0.05$ vs. vehicle.

receptor impair the enhanced release of LH induced by estradiol in ovariectomized rats. There is little available information on BDZ in relation to gonadotropin secretion. In contrast with our results, the short-acting compound triazolam failed to modify the LH surges induced by estradiol in ovariectomized hamsters maintained in constant light (15). Such differences can be due to the protocols used in these experiments. In contrast to the acute doses we injected, triazolam was administered at successive days.

BDZ, in a similar manner to GABA and GABA mimetics [(3, 5, 6, 8, 9), and Donoso, unpublished results], did not modify resting secretion of LH. These results suggest the lack of a tonic GABAergic control on LH release. The failure of DMCM to increase plasma LH levels, as found in the present work, further supports such a view.

Results suggest, though they do not provide conclusive evidence, that LH blockade by the GABA mimetics, that was observed at doses higher than those producing anxiolysis (11–13), is unrelated to sedation. The results obtained with the β-carboline ZK-91296 strongly support this view. As previously demonstrated in normal male rats through different tests, and supported by present results, the anxiolytic drug ZK-91296, which seems to qualify as a partial agonist at central BDZ receptors, exhibits no sedative actions in a wide range of doses (10,11). Sedation has been observed only after large doses of ZK-91296 (10). In a similar manner, this β-carboline at nonsedative, nonataxic doses displays a partial attenuating effect on stress-induced corticosterone release (13).

It appears to be difficult to dissociate the neuroendocrine action and the sedative effects of both DZ and ZK-93423. Low doses of DZ may discriminate sedation and LH blockade. At 1 mg/kg, DZ produced sedation in the hole-board test while not impairing the estrogen-induced LH release. When the DZ doses were increased, both actions overlapped. Although in our experiments ZK-93423, a full agonist of BDZ, was able to inhibit locomotor activity only at high doses (5 mg/kg), both ZK-93423 and DZ are known to sedate at similar low doses (12,13).

Present experiments also indicate that automatic recordings in the OVM, based on the classical open field test, of the ambulatory activities of rats in the early afternoon, lack adequate sensitivity to evaluate sedation. In coincidence with other authors, a significant sedative effect of DZ was detected only when the behavioral activities were scored in a hole-board device near the lights-off time. Lack of sedative effects of large doses of diazepam in evaluations around noon and using open field devices (7), can also be explained by these findings.

Based on current evidence with GABA mimetics, the neuroendocrine impairment produced by DZ and β-carbolines most likely reflects inhibition of the neurons producing LHRH (luteinizing hormone-releasing hormone). In support of this hypothesis it has been shown that estrogen-receptive GABAergic neurons are involved in an inhibitory mechanism that when activated impairs evoked LH surges (Seltzer and Donoso, in press). The possibility cannot be ruled out that DZ would have a direct effect on LHRH action at the pituitary since certain BDZ structures are antagonists of LHRH (4).

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NOTE ADDED IN PROOF

Recent results from our Laboratory showed that single doses of diazepam (5 mg/kg) or ZK-91296 (5 mg/kg) did not modify LH release induced by LHRH (100 ng/rat).