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Involvement of the "Peripheral" Benzodiazepine Receptor Type (ω_3) in the Tolerance to the Electroencephalographic Effects of Benzodiazepines in Rats: Comparison of Diazepam and Clonazepam

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MASSOTTI, M., L. MELE AND C. DE LUCA. *Involvement of the "peripheral" benzodiazepine receptor type (ω_3) in the tolerance to the electroencephalographic effects of benzodiazepines in rats: Comparison of diazepam and clonazepam.* PHARMACOL BIOCHEM BEHAV **35**(4) 933–936, 1990.—Rapid tolerance to the sedative effect of large doses of diazepam (10 mg/kg IV), but not of large doses of clonazepam (2 mg/kg IV) occurs in rats after 5 days of treatment on a once-a-day regimen. Electroencephalographic (EEG) studies show that such behavioral tolerance is associated with a decreased induction of spindle bursts and with an increased induction of 20–30 Hz waves (β-like activity). Administration of clonazepam plus the agonist of the "peripheral" benzodiazepine receptor type (ω_3) Ro 5-4864 (4 mg/kg IV) for 5 days induces signs of behavioral and EEG tolerance to sedative effects of the benzodiazepine agonist. In animals treated for 5 days with diazepam plus the ω_3 antagonist PK 11195 (5 mg/kg IV), no signs of EEG and behavioral tolerance are observed. These results suggest that ω_3 type activation influences the development of rapid tolerance to the sedative effect of diazepam in rats.

EEG Rats Diazepam Clonazepam Ro 5-4864 PK 11195 ω_3 receptor Chronic administration

EXPERIMENTAL and clinical studies have shown that rapid tolerance can occur to the sedative-hypnotic effect, but not to the anxiolytic effect of benzodiazepines (BZ) [for reference, see (5)]. Electroencephalographic (EEG) studies from our laboratory showed that in rats exposed to large doses of diazepam for 5 days, tolerance to the sedative effect was associated with a decrease of the periods of synchronization and an increase of the periods of high-frequency waves (16,25). More recently, such an effect was also found after administration of large doses of flunitrazepam, but not of clonazepam and the triazolopyridazine derivative Cl 218,872 (26). Consistent with these data, it has been reported that no tolerance to the sedative effects of Cl 218,872 develops after 5–7 days of treatment in rats (12), and that clonazepam induces a slower development of tolerance to the anticonvulsant effect with respect to clobazam in mice (6).

To explain the different abilities of the two groups of BZ receptor agonists to induce such a rapid EEG tolerance, two hypotheses may be proposed. The first derives from the finding that clonazepam and Cl 218,872 behave as partial agonists, whereas diazepam and flunitrazepam act as full agonists (4). It has been suggested that partial agonists require a longer period of treatment than full agonists for tolerance induction (3). The second hypothesis concerns the possibility that selective types of BZ receptor may be involved in tolerance development. If so, according to the current knowledge derived from binding studies (8), tolerance to the sedative effect of BZ might depend on an interaction with the so-called "peripheral" BZ receptor type, recently designated ω_3 (10). The latter has an affinity for diazepam and, to a lesser extent, for flunitrazepam, but not for clonazepam and Cl 218,872 (8).

In this study, we tested the second hypothesis by evaluating the effects of the atypical BZ derivative Ro 5-4864, a presumed ω_3 agonist (2), and the isoquinoline carboxamide derivative PK 11195, a proposed ω_3 antagonist (2), on the EEG changes induced by repeated administration of clonazepam and diazepam, respectively.

METHOD

Male Sprague-Dawley rats, weighing 250–300 g at the begin-

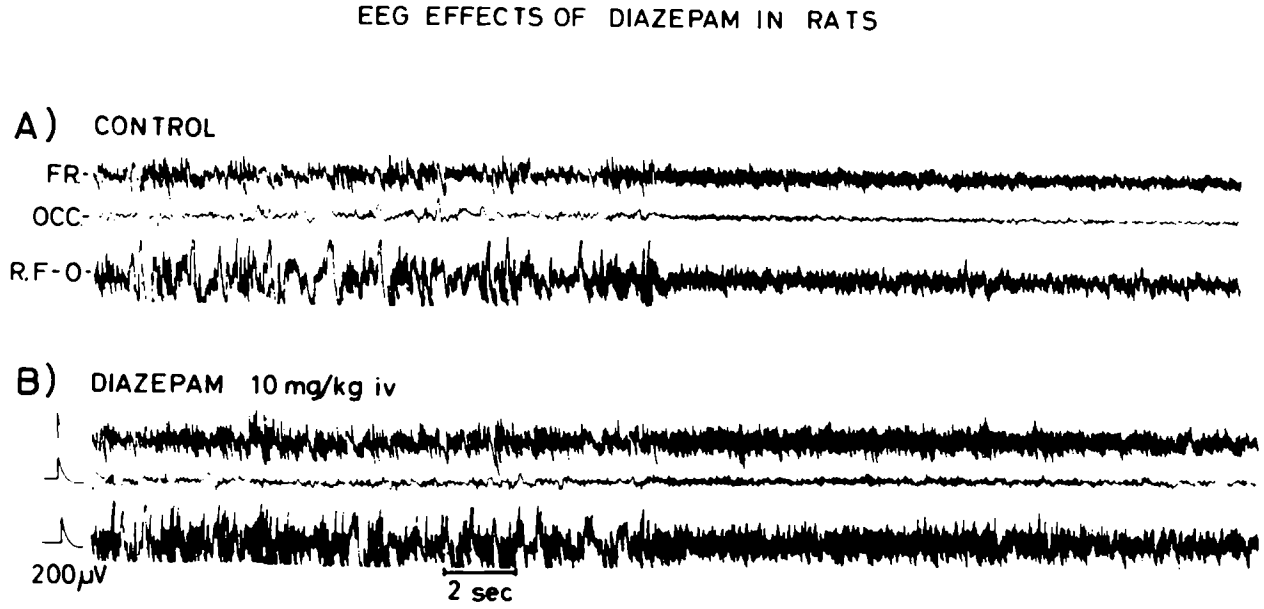

FIG. 1. EEG modifications elicited by single administration of diazepam in rats. The figure depicts the morphological changes of electrical activity observed in cortical sites. (A) Control. Left side: the state of rest is characterized by a synchronized pattern (high-voltage low-frequency waves) with the presence of 7–12 Hz spindle bursts in the sensorimotor cortex. Right side: the state of arousal is characterized by a desynchronized record (low-voltage high-frequency waves). (B) Acute administration of diazepam (10 mg/kg IV). At the level of the sensorimotor cortex an increase of spindle bursts and voltage are clearly observed during the synchronized (left side) and desynchronized (right side) periods, respectively. Leads: FR, right-left sensorimotor cortices; OCC, right-left optic cortices; R.F-O., right sensorimotor-optic cortices.

ning of treatment, were used. The animal care and use followed the recommendations of U.S.D.H.H.S. (25). Surgical procedures were performed under equithesin (2.7 ml/kg IP) anesthesia. A chronic indwelling P 20 catheter was inserted into the external jugular vein to allow intravenous (IV) injection in freely moving animals at a speed of 0.25 ml/min in a volume of 1 ml/kg. Cortical electrodes were then implanted at the level of the sensorimotor and associative cortices of both hemispheres.

After three days of recovery, animals received the first dose of the drug(s), followed by a single daily injection for a total of 5 days. In the case of combined administration, Ro 5-4864 and PK 11195 were injected 1 min after clonazepam and diazepam, respectively, for 5 days. The EEG was recorded the 1st and 5th day. Each session lasted from about 90 min before up to 90 min after drug injection. Measurements of the periods of EEG spindle bursts and of fast frequencies (25–30 Hz) were done in the first hour after injection of drugs(s), as previously described (16,26). All drugs were dissolved with 1–2 drops of 10 N HCl. Diazepam, clonazepam and Ro 5-4864 were diluted with saline, and the solution's pH was adjusted to 3–4 with 0.1 N NaOH. PK 11195 was diluted with polyethylene glycol 300.

RESULTS

Acute administration of diazepam elicits the appearance of 7–12 Hz, 200–400 μV spindle bursts in the electrocorticogram sometimes interrupted by brief periods of 20–30 Hz, 60–80 μV waves (Fig. 1). The frequency range and the localization at the level of the sensorimotor cortex both suggest for the fast frequency waves a homology with the β rhythms described in humans (9). Therefore, we called it β-like activity. Similar EEG features were observed after administration of clonazepam (2 mg/kg IV).

The synchronized pattern is associated with behavioral sedation (lying down or on the side, eyes closed, and myorelaxation), whereas β-like activity is associated with signs of weak behavioral activation (gnawing, eating, slight motor activity and ear twitches).

Acute injection of Ro 5-4864 elicits two dose-dependent changes in the EEG pattern consisting of trains of spike-and-wave complexes in the sensorimotor cortex and trains of slow waves in the optic cortex (2–6 mg/kg IV) and EEG "grand-mal" seizures associated with behavioral convulsions (6–10 mg/kg IV).

The acute injection of PK 11195 (0.5–10 mg/kg IV) does not affect the EEG pattern. At these doses, however, the drug antagonized the EEG changes due to the acute administration of Ro 5-4864 (data not shown).

In a first series of chronic studies, we observed that tolerance to the sedative effect of clonazepam occurs when the drug is coadministered with Ro 5-4864 for 5 days. Figure 2 shows that on the first day, clonazepam (2 mg/kg IV) alone or in combination with Ro 5-4864 (4 mg/kg IV) elicited a preponderance of spindle bursts over β-like activity in the EEG pattern. In contrast, on day 5 of treatment, a significant decrease of spindle bursts was found only with combined administration. This effect was associated with a trend towards an increase of β-like activity, which, however, did not attain statistical significance when compared with the value found on day 1 using the paired *t*-test (Fig. 2). The overt sedation and myorelaxation were replaced by signs of behavioral excitation.

One can argue that this effect might depend either on the excitatory effect of Ro 5-4864 (15,17) probably related to its ability to bind to the receptor site for pentamethylenetetrazol (PTZ) and picrotoxin (24). This possibility was ruled out since the combined administration of clonazepam (2 mg/kg IV) and a subconvulsant dose of PTZ (15 mg/kg IV), which induces trains of spike-and-wave complexes in rats, did not induce signs of EEG tolerance. Throughout the 5-day treatment, in no case did trains of spike-and-wave complexes due to either Ro 5-4864 or PTZ emerge after combined administration with clonazepam (data not shown).

In a second series of chronic experiments, we observed that

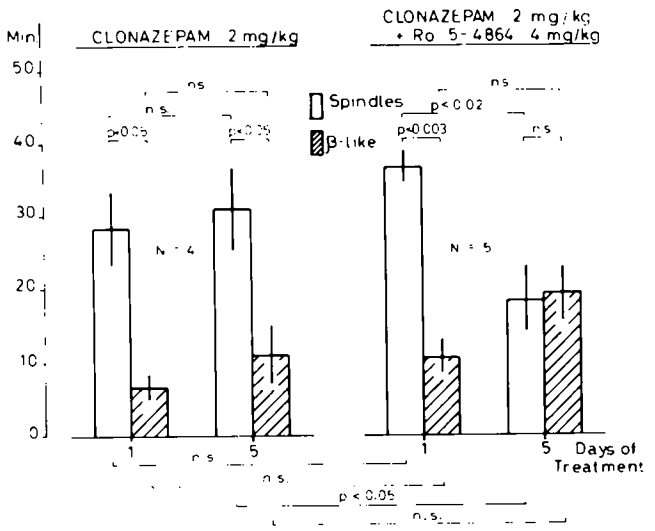

FIG. 2. Occurrence of spindle bursts and β-like activity in the EEG recording after short-term exposure to combined administration of clonazepam plus Ro 5-4864 in rats. The histogram shows the total duration of spindle bursts and β-like activity periods observed at the level of the sensorimotor cortex on day 1 and 5 of treatment within the first hour after the injection of clonazepam alone (2 mg/kg IV) (left side) and with Ro 5-4864 (4 mg/kg IV) (right side). Statistical significance of the difference between the two patterns and between the two days for each treatment are calculated according to paired *t*-test. For comparisons between the two treatments, statistical significances were calculated with the Student's *t*-test.

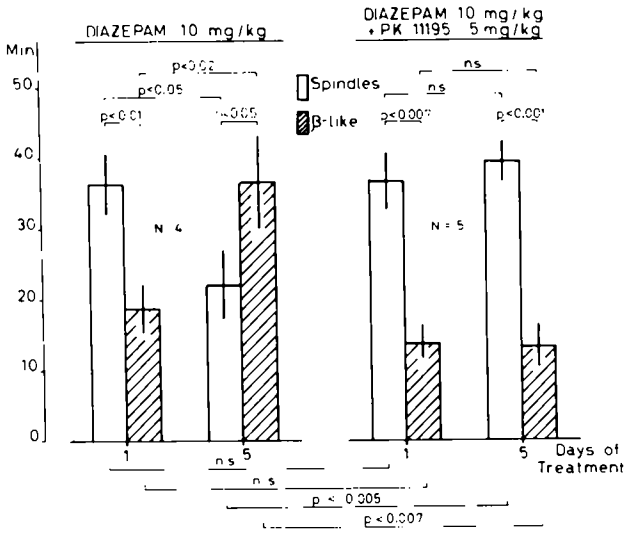

FIG. 3. Occurrence of spindle bursts and β-like activity in the EEG recording after short-term exposure to combined administration of diazepam plus PK 11195 in rats. The histogram shows the total duration of spindle bursts and β-like activity periods observed at the level of the sensorimotor cortex on day 1 and 5 of treatment within the first hour after injection diazepam alone (10 mg/kg IV) (left side) and in combination with PK 11195 (5 mg/kg IV) (right side). Statistical analysis as reported in Fig. 2.

diazepam-induced tolerance is abolished by simultaneous administration of PK 11195 for 5 days. Figure 3 shows that on the first day, the preponderance of spindle burst periods over β-like activity periods occurs after diazepam alone (10 mg/kg IV). A slight, but not statistically significant decrease of β-like activity incidence occurred when the BZ was administered with PK 11195 (5 mg/kg IV). At the fifth day, rats treated with diazepam alone showed signs of EEG tolerance (i.e., decrease of the spindle bursts and increase of β-like activity), whereas animals receiving diazepam plus PK 11195 still showed a preponderance of spindle bursts. From the behavioral point of view, the ω_3 antagonist did not modify overt sedation and myorelaxation induced by the large dose of diazepam given acutely, and this response was maintained during the 5 days of treatment.

DISCUSSION

Acute administration of BZ induces both sedative-hypnotic effects and signs of behavioral stimulation (21, 23, 27). EEG studies clearly discriminate these two components in laboratory animals (11) and in humans (7,22). They also showed that after repeated administration of BZ, the EEG synchronization decreases and β-like activity increases as tolerance to the sedative effect develops (16,26). This study shows that common EEG and behavioral effects can be observed after short-lasting administration of large doses of either clonazepam plus Ro 5-4868 or diazepam in rats. On the basis of distinct binding profiles of these compounds (8), these findings suggest the possible involvement of ω_3 receptor in the occurrence of rapid tolerance in rats. The apparent failure of diazepam to induce tolerance when coadministered with PK 11195, a proposed ω_3 antagonist (2), is consistent with this hypothesis. This possibility seems to be strengthened by the recent observation that in rats receiving Ro 5-4864 (4 mg/kg IV once a day) for 5 days, single injection of diazepam (10 mg/kg IV) on day 5 elicits in the EEG an increase of β-like activity and a decrease of the synchronization (De Luca and Massotti, manuscript in preparation).

EEG tolerance to the effects of clonazepam occurs only when the drug is coadministered on a chronic basis with Ro 5-4864, but not with PTZ at equiactive doses. It has been reported that both drugs can elicit their convulsant effects acting at picrotoxinin receptor site (19,24). Consistent with this hypothesis, common EEG patterns were observed with both drugs in laboratory animals [(14,15), this study]. However, binding sites for Ro 5-4864 have been found at the level of γ-aminobutyric acid oligomeric complex (24), of mitochondrial membrane (1) and nuclear membrane (13). Therefore, one can speculate that the effect obtained with Ro 5-4864 in this study is mediated at the level of the receptor located in mitochondrial and/or nuclear membrane.

An increase of β-like activity was described in the EEG pattern of rats [(16,26), present study], rabbits (20) and humans (22) subjected to repeated administration of diazepam. Previous studies in rats and rabbits suggested the possibility that the EEG and behavioral stimulatory signs elicited by the BZ are originated by activation of the neuronal BZ receptor type 2 (ω_2) (11). If so, according to the present results, the possibility exists that in tolerant animals increase of β-like activity is due to repeated and concurrent activation of ω_2 and ω_3 receptor types.

In this study, no tolerance to the capability of clonazepam to antagonize the spike-and-wave complexes due to Ro 5-4864 and PTZ was observed. Instead, previous reports indicate that tolerance to the anti-PTZ effect of BZ does occur. This discrepancy might be due to the low dose of the convulsant drug and/or to the different protocol we used (coadministration on a chronic basis) with respect to other studies (challenge with BZ plus convulsant drug at end of treatment period with BZ) [for references, see (5,18)].

In conclusion this study shows that: 1) tolerance to the sedative effect of diazepam disappears when the drug is coadministered

with PK 11195 on a chronic basis; 2) tolerance to the sedative effect of clonazepam is observed when the drug is coadministered with Ro 5-4864 on a chronic basis, but not when clonazepam is given alone. These findings seem to favor the hypothesis that prolonged activation of ω_3 receptor influences the adaptive mechanism responsible for rapid tolerance to the EEG and behavioral effects of diazepam in rats.

Whether chronic activation of ω_3 receptor type is additional to either chronic activation of neuronal BZ receptors (ω_1 and/or ω_2) or to the intrinsic efficacy of the BZ requires further studies.

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