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Diazepam, Pentobarbital, And Methaqualone Effects On Several Behaviors In The Rat And Antagonism By Ro 15-1788

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The sedative hypnotics may exert their effects through a number of different mechanisms. Diazepam interacts with a specific receptor linked to a GABA receptor and a Cl^- ionophore (Skolnick and Paul, 1981) and enhances the binding affinity of the GABA receptor for its ligand. Barbiturates may act at an additional receptor linked to this complex (Olsen, 1981). The sites of action of methaqualone have yet to be defined.

Recently Hunkeler et al. (1981) synthesized a new class of compounds, the imidazodiazepines, the prototype being Ro 15-1788. They showed that Ro 15-1788 inhibits ^{3}H-diazepam binding to brain synaptosomes, reverses diazepam-induced protection against metrazol seizures, and alleviates the disruption induced by diazepam in a horizontal wire test. Ro 15-1788 does not affect the depression induced by phenobarbital, meprobamate or ethanol. In a standard conflict paradigm Ro 15-1788 prevents the antipunishment effect of diazepam. Ro 15-1788 also antagonizes the decrease in rat cerebellar cGMP by diazepam, but not that by barbiturates, ethanol or meprobamate (Mohler et al., 1981), and reverses the effects of 3-methylclonazepam in a number of tests in humans (Darragh et al., 1981).

We have investigated the effects of diazepam (DZ), pentobarbital (PB) and methaqualone (MQ) alone and in combination with Ro 15-1788 in a novel conflict paradigm, conditioned suppression of drinking (CSD), as well as in rotarod performance (RR) and motor activity (MA).

METHODS

Conditioned Suppression of Drinking (CSD). Female Sprague-Dawley rats (150-200 g; Spartan Research Animals, Inc., Haslett, MI) were water-deprived and trained to drink in 10 min daily sessions from a tube protruding through the wall of a 30x56x28 cm plexiglass cage with stainless steel floor (Kilts et al., 1981). The drinking tube was attached to a calibrated (±0.5 ml) polyethylene tube to monitor fluid consumption. When drinking had stabilized, 7-sec tones were presented on a variable interval 21 sec schedule. During the last 5 sec of the tone the drinking tube and cage floor were electrified (0.03 mA current, C.J. Applegate,

Stimulator Model No. 250, Boulder, CO). Animals were tested six days a week at the same time of day.

Drug treatments were administered every 3-4 days. DZ, PB and MQ were administered 10 min and Ro 15-1788 immediately before the session. The number of shocks received (punished responding) and the volume of water consumed (unpunished responding) on 'drug-days' were divided by these measures for the day immediately prior to obtain percent of control shocks taken and water consumed, respectively. Changes in water or shocks were compared using a multi-factorial ANOVA with least significant differences for multiple comparisons; $p<0.05$ was used as the criterion for statistical significance.

Rotarod Performance (RR). Female Sprague-Dawley rats were trained to walk on a rotating rod (RR, 8 rpm). Drugs were tested after animals had reached criterion of walking 180 sec for two consecutive trials on two consecutive days. Thirty mg/kg DZ, 18 mg/kg PB, 18 mg/kg MQ, or saline was administered 15 min before testing and 2.0 mg/kg Ro 15-1788 or saline was administered 5 min before testing. Animals were then placed on the RR for two consecutive trials; the longest walk was recorded. Mean scores for each drug were compared using a one-way ANOVA with least significant differences for multiple comparisons ($p < 0.05$ = level of significance).

Motor Activity (MA). Rats used previously in a RR experiment were randomly divided into groups regardless of previous drug experience. Animals were given 18 mg/kg DZ, 18 mg/kg PB, 18 mg/kg MQ or saline 15 min before and 2 mg/kg Ro 15-1788 or saline 5 min before being placed into motor activity cages. Total counts over 15 minutes were recorded using a Stoelting electromagnetic-field counter. Statistical analysis was done as described for RR performance.

Drugs. All drugs were administered i.p. and doses were randomized. Ro 15-1788 and DZ were gifts from Hoffman-LaRoche, Inc (Nutley, NJ). PB sodium was obtained from Sigma Chemical Co. (St. Louis, MO). MQ free base was a gift from Wm. H. Rorer, Inc. (Fort Washington, PA). Ro 15-1788, DZ and MQ were suspended in 0.5% methylcellulose with two drops/10 ml Tween 80. PB sodium was dissolved in distilled water.

RESULTS

CSD. Baseline responding consisted of 15.5±0.5 (mean ± S.E.M., n = 20) ml of water consumed per session and 17±2 (mean ± S.E.M., n = 20) shocks taken. Both measures were stable across control sessions. Ro 15-1788 (0.5, 1 or 2 mg/kg), administered alone immediately before the sessions, did not alter shock or water scores (zero dose, Fig. 1). DZ (3, 5.6, 10, 18 and 30 mg/kg) caused a significant increase in punished responding (shocks) and, at doses of 18 and 30 mg/kg, caused a decrease in unpunished responding (water intake). Ro 15-1788 caused a dose-dependent attenuation of the effects of DZ on punished responding ($F(3, 182) = 21.4$).

At a dose of 0.5 mg/kg, Ro 15-1788 in combination with DZ significantly reduced the DZ anticonflict effect, although shocks taken were still above baseline with several dose levels. Water intake, reduced by 18 mg/kg DZ

was significantly different from DZ alone after the drug combination. The 1.0 mg/kg dose of Ro 15-1788 nullified the DZ anticonflict effect for all but the 18 mg/kg dose; the DZ-induced decrease in water intake was reversed by the combination at the 30 mg/kg DZ dose level but not at 18 mg/kg DZ. At 2.0 mg/kg, Ro 15-1788 combined with DZ resulted in complete attenuation of the DZ anticonflict effect. The reduction of water intake by DZ was not reversed by combination with 2.0 mg/kg Ro 15-1788.

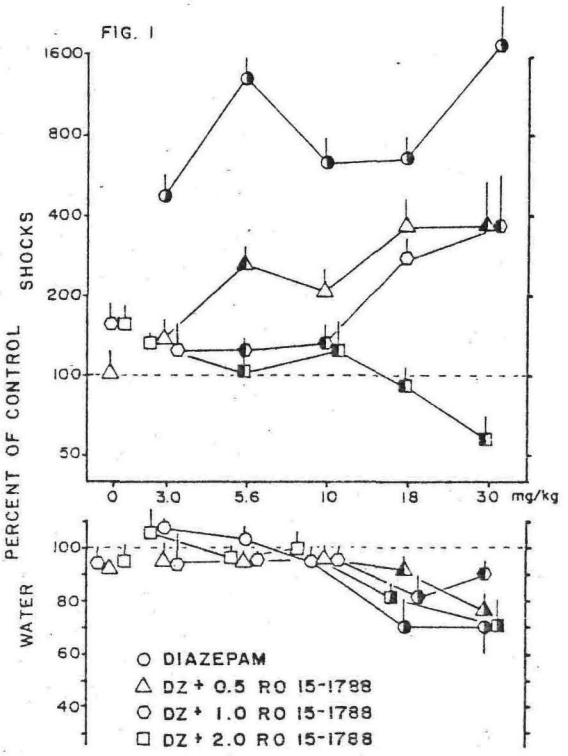

FIG. 1. Effects of diazepam alone and in combination with Ro 15-1788 in CSD. ◑ = significantly different from control, ◭ = significantly different from diazepam alone, $p<0.05$.

PB (3 to 18 mg/kg) also released punished responding (Fig. 2), being maximal at 10 mg/kg. Water intake was significantly decreased at 10 and 18 mg/kg PB. Combination with 1 or 2 mg/kg Ro 15-1788 did not alter the PB effect on the punished component of this behavior. Unpunished behavior, however, was significantly potentiated at 18 mg/kg PB by combination with 1 or 2 mg/kg Ro 15-1788. MQ (5.6 to 30 mg/kg) also caused a release of punished responding, increasing shocks at 10, 18 and 30 mg/kg (Fig. 3). Unpunished responding was decreased by MQ alone at doses of 18 and 30 mg/kg. Combination with Ro 15-1788 (1 mg/kg) did not alter the effects of MQ on either punished or unpunished responding.

RR. The results of RR experiments are seen in Fig. 4. Ro 15-1788 (2 mg/kg) did not alter RR performance. DZ (30 mg/kg) caused a significant

disruption of performance; this effect was reversed by Ro 15-1788 combined with 30 mg/kg DZ. In contrast, the disruption by 18 mg/kg PB was significantly potentiated by combining with Ro 15-1788. Ro 15-1788 had no effect on the disruption of RR walking by 18 mg/kg MQ.

MA. When compared to saline controls, 2 mg/kg Ro 15-1788 did not have an effect by itself on MA measured over 15 min (Fig. 5). DZ (18 mg/kg) caused a significant reduction in MA which was almost completely reversed by combination with Ro 15-1788. When Ro 15-1788 was given to animals receiving either 18 mg/kg PB or 18 mg/kg MQ, their MA was not significantly different from that of animals receiving the same dose of PB or MQ alone.

FIG. 2

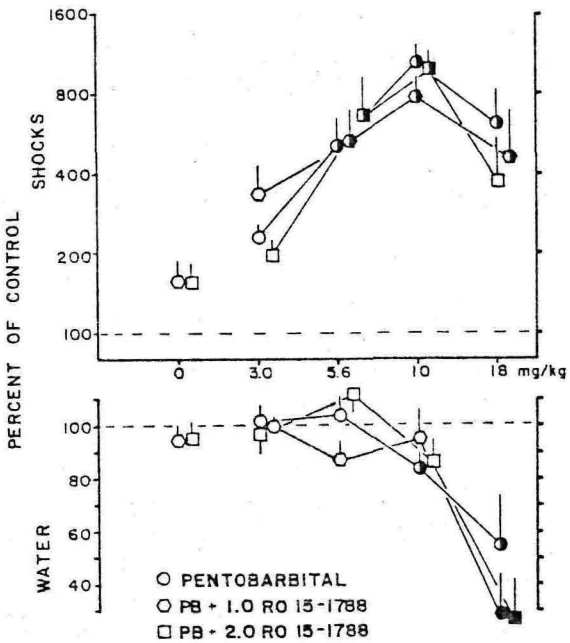

FIG. 2. Effects of pentobarbital alone and in combination with Ro 15-1788 in CSD. ● = significantly different from control, ■ = significantly different from pentobarbital alone, $p<0.05$.

DISCUSSION

In agreement with Kilts et al. (1981) DZ caused a release of punished responding in this conditioned suppression paradigm. Only at higher doses (18 and 30 mg/kg) were depressant effects of DZ observed on water intake. Since water intake is insignificant during tone periods, it serves as a good measure of unpunished responding in the CSD. For example, 5.6 mg/kg DZ increased punished responding by 1400% without altering the level of intake from control (Fig. 1).

Ro 15-1788 caused a dose-dependent attenuation of the release of punished responding elicited by DZ. However, Ro 15-1788 may not

FIG. 3

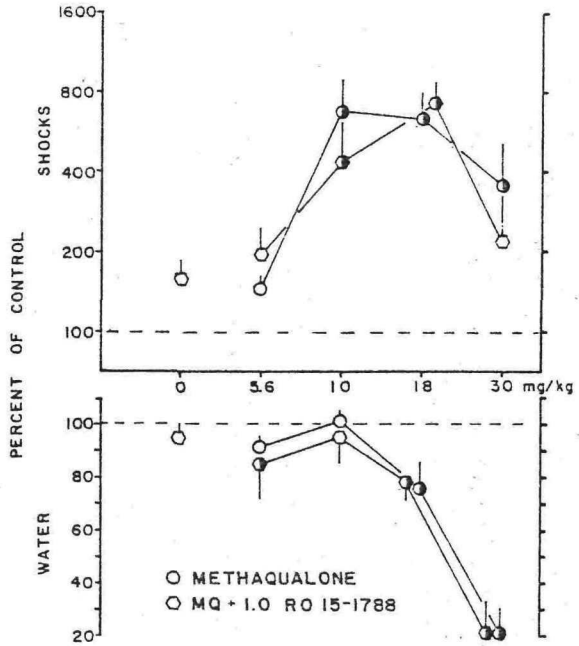

FIG. 3. Effects of methaqualone alone and in combination with Ro 15-1788 in CSD. ◐ = significantly different from control, $p<0.05$.

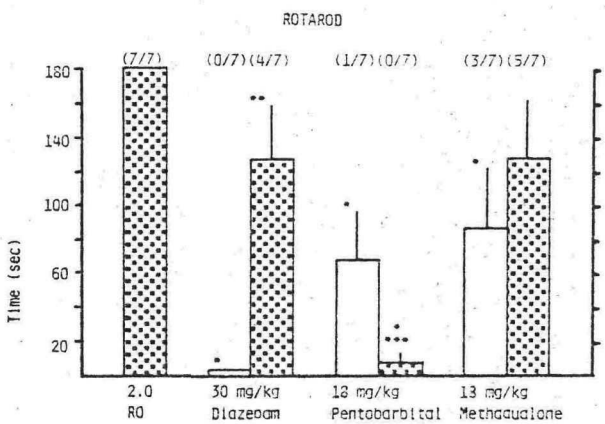

FIG. 4. Effects of diazepam, pentobarbital and methaqualone alone (open bars) or in combination with 2.0 mg/kg Ro 15-1788 (filled bars) on rotarod performance. * = significantly different from Ro 15-1788 alone, ** = significantly different from diazepam alone, *** = significantly different from pentobarbital alone, $p<0.05$.

antagonize some depressant effects of DZ, as evidenced by the inability of Ro 15-1788 to reverse in a clear dose-dependent manner the decrease in unpunished responding after higher doses of DZ. This is in contrast to the findings of Darragh et al. (1981) that Ro 15-1788 is capable of reversing the depressant side effects of 3-methylclonazepam in humans. It may be that higher doses of Ro 15-1788 would be capable of reversing these depressant effects in the CSD.

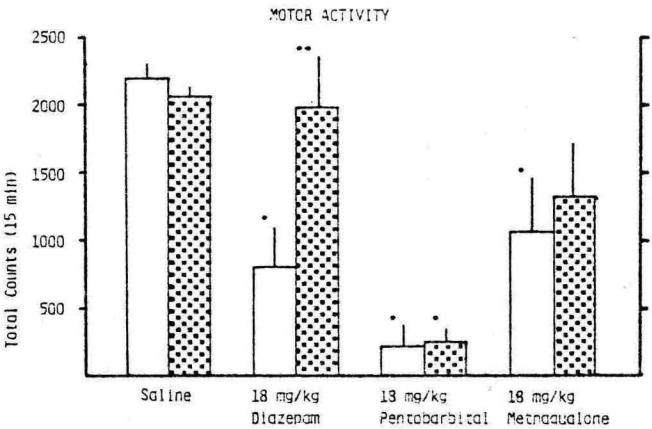

FIG. 5. Effects of diazepam, pentobarbital and methaqualone alone (open bars) or in combination with 2.0 mg/kg Ro 15-1788 (filled bars) on motor activity. * = significantly different from saline alone, ** = significantly different from diazepam alone, $p<0.05$.

The apparent lack of effect of Ro 15-1788 on the release of punishment-suppressed behavior by PB would suggest that the anti-anxiety effects of PB are not related to a specific benzodiazepine effect, in agreement with other investigators, Barrett and Brady (1982), Brady (this volume), and Gorodetzky (this volume). The potentiation by Ro 15-1788 of the depression in water consumption by higher doses of PB may indicate some interaction between these two drugs, however. This has also been suggested by Barrett and Brady (1982): Ro 15-1788 potentiated the effects of PB in another conflict test. Ro 15-1788 also did not reverse the anti-conflict effects of MQ, suggesting that this compound is similar to PB in not interacting with the benzodiazepine receptor to produce its effects.

Ro 15-1788 reversed the disruptive effects of DZ on RR and MA, a paradox when contrasted with the lack of a clear-cut antagonism by Ro 15-1788 of the DZ decrease in the unpunished component of the CSD. This suggests that these depressant actions may be working through different mechanisms. The potentiation by Ro 15-1788 of PB disruption of RR further supports an interaction between these drugs. The current study has not ruled out pharmacokinetic interaction. The lack of effect of Ro 15-1788 on MQ disruption of RR and MA suggests that this drug works by mechanisms that differ from both DZ and PB.

These experiments indicate that these examples of the sedative-hypnotic class of drugs exert their effects through a number of different mecha-

nisms. The anticonflict effects of DZ are clearly mediated through a mechanism which is antagonized by Ro 15-1788. This may not be the case for the decrease in water intake by DZ in the CSD paradigm. The anticonflict effects of PB and MQ, however, are clearly not mediated through a Ro 15-1788-blockable mechanism. The effects of Ro 15-1788 on RR and MA depressant actions of DZ, PB and MQ further separate these drugs as to mechanisms. Obviously, further study of these interactions is desirable to further define differences in the mechanisms of action of these sedative-hypnotic agents.

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