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The Effects of Ro 15-1788 on Anxiolytic Self-Administration in the Rhesus Monkey

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JOHANSON, C E AND C R SCHUSTER *The effects of Ro 15-1788 on anxiolytic self-administration in the rhesus monkey* PHARMACOL BIOCHEM BEHAV 24(4) 855–859, 1986 —The ability of a benzodiazepine antagonist, Ro 15-1788, to modify the self-administration of anxiolytics was determined in rhesus monkeys Lever-press responding was maintained in four monkeys under a fixed-ratio 10 schedule of drug delivery in daily sessions of 2 hr duration Responding was maintained either by flurazepam, lorazepam or pentobarbital When responding was stable for each of these drugs, a range of doses of a benzodiazepine antagonist, Ro 15-1788 (0 0001-10 mg/kg, IM), was given 5 min prior to a session At some doses the antagonist increased responding for lorazepam and flurazepam whereas pentobarbital self-administration was largely unaffected or reduced These results suggest that Ro 15-1788 was able to specifically modify the effects of benzodiazepines responsible for drug-maintained performance

Lorazepam Flurazepam Ro 15-1788 Self-administration Reinforcing properties Rhesus monkeys

THE benzodiazepines as well as several non-benzodiazepine anxiolytics exert many but not all of their effects by interacting with specific receptors in the central nervous system Further evidence that these receptors are responsible for the actions of anxiolytics has been obtained using antagonists which block the actions of these drugs at the receptor. For instance Ro 15-1788 (flumazepil), an imidazodiazepine, blocks many of the centrally-mediated effects of benzodiazepines by a competitive inhibition Hunkeler *et al* [8] showed that Ro 15-1788 blocked the neurochemical, sedative, anticonvulsant, muscle relaxant, and anticonflict effects of benzodiazepines in several species. In addition, this effect was specific to the benzodiazepines since the same effects produced by barbiturates were not blocked by Ro 15-1788 [8] Foltin *et al* [5] showed that increases in food intake in rhesus monkeys produced by diazepam were blocked by Ro 15-1788. On the other hand, increases in food intake produced by pentobarbital were unaffected by Ro 15-1788. Similarly, this antagonist can block the discriminative stimulus effects of benzodiazepines [7] For instance, Ator and Griffiths [1] trained baboons to discriminate either between lorazepam and saline or pentobarbital and saline Ro 15-1788 when given in combination with lorazepam resulted in saline-appropriate responding whereas, when given in combination with pentobarbital, the monkeys still responded on the drug lever Finally, in animals treated chronically with anxiolytics such as flurazepam, diazepam and lorazepam, Ro 15-1788 can elicit an abstinence syndrome [3, 13, 14].

In most studies with Ro 15-1788, this antagonist has been devoid of any intrinsic activity of its own For instance, Hunkeler *et al* [8] failed to find any evidence of typical anxiolytic effects for this compound up to doses that were subtoxic. Jensen *et al* [9] found that although Ro 15-1788 reversed the anticonvulsant effects of benzodiazepines in DBA/2 mice susceptible to sound-induced clonic seizures, this antagonist also had anticonvulsant activity of its own at high doses Tang *et al* [15] found that even low doses of Ro 15-1788 increased the intake of hypertonic NaCl solutions in rats, an effect characteristic of benzodiazepines and barbiturates. Dantzer and Perio [4] demonstrated that Ro 15-1788 blocked the antipunishment effects of clorazepate and reversed the blockade by this anxiolytic of the pentylenetetrazol cue in a discrimination paradigm However, in rats trained to discriminate between clorazepate and saline, Ro 15-1788 did not block the effects of clorazepate and actually substituted for the anxiolytic at high doses [4].

In the present study the effect of Ro 15-1788 on the ability of anxiolytics to maintain responding leading to their delivery was investigated. Rhesus monkeys were trained to make a response to receive an infusion either of flurazepam, lorazepam or pentobarbital When responding became stable, a range of doses of Ro 15-1788 was administered prior

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TABLE 1
ORDER OF TESTING

Monkey	Order 1	2	3
2036	0 3 mg/kg pentobarbital	0 01 mg/kg lorazepam	0 1 mg/kg flurazepam
2037	0 01 mg/kg lorazepam	0 03 mg/kg flurazepam	0 3 mg/kg pentobarbital
3044	0 1 mg/kg flurazepam	0 01 mg/kg lorazepam	0 3 mg/kg pentobarbital
9083	0 1 mg/kg pentobarbital	0 01 mg/kg lorazepam	0 03 mg/kg flurazepam

TABLE 2
MEAN (SE) NUMBER OF INFUSIONS DURING HOUR 1

Monkey	Pentobarbital	Lorazepam	Flurazepam
2036	27 4 (5 5)	48 8 (10 1)	13 9 (2 3)*
2037	53 7 (8 2)	35 6 (8 2)	26 1 (10 2)†
3044	30 8‡ (30–31 5)	16 4 (3 4)	41 6 (8 9)*
9083	37 8 (11 8)	55 4 (20 4)	33 0 (10 3)†

*0 1 mg/kg
†0 03 mg/kg
‡Only 2 values for mean–range is given

to an experimental session It was found that Ro 15-1788 increased the rate of lorazepam and flurazepam self-administration in this paradigm but had little effect on pentobarbital-maintained responding

METHOD

Animals

Two male (2036, 2037) and two female (3044, 9083) rhesus monkeys were used in this experiment. All of these monkeys had participated previously in experiments involving anxiolytic self-administration [10] Each monkey was equipped with a single-lumen silicone venous catheter (Rodhelm Reiss Co , Belle Mead, NJ), implanted under sodium pentobarbital anesthesia (up to 30 mg/kg IV, as needed) The proximal end of the catheter was inserted into a major vein (internal jugular, external jugular or femoral) for a distance calculated to terminate in or near the right atrium of the heart, the distal end was threaded under the skin, exiting the body through an incision in the back of the monkey. It was not always possible to maintain a single catheter for the duration of the experiment. When a catheter became dislodged, the monkey was removed from the experiment for several days. A replacement catheter was surgically inserted into one of the remaining veins and the monkey was returned to the experiment 1 or 2 days later

All monkeys had continuous access to water and were given supplemental vitamins several days each week They were given sufficient food (Purina Monkey Chow) following experimental sessions to maintain free-feeding weight In addition, the diet was occasionally supplemented with fresh fruit When necessary, antibiotics were administered to treat a catheter tract infection

Apparatus

Each monkey was housed in a sound-attenuating wooden cubicle (inside dimensions: 70×80×70 cm) that served as the experimental space. Each cubicle was equipped with a ventilation fan that also masked extraneous sounds The front door of each cubicle had a Plexiglas window which allowed the monkey visual access to the room This window was covered during experimental sessions Mounted on the inside of the cubicle door were two metal boxes located 23 cm apart Each box contained a response lever (PRL-001, BRS/LVE, Beltsville, MD) and four Dialco stimulus lights, two covered with white lens caps and two covered with red lens caps The Plexiglas ceiling of the cubicle could be transilluminated by either white or red lights

Each monkey wore a stainless steel harness connected to a spring arm (E & H Engineering Co , Chicago, IL) which was approximately 46 cm long and bolted through the back wall of the cubicle This arrangement allowed the monkey relatively unrestricted movement within the cubicle and protected the catheter which was threaded through the spring arm Outside the cubicle, the catheter was connected to a peristaltic infusion pump (7540X, Cole-Parmer Instrument, Chicago, IL), which delivered solutions at the rate of 6 ml/min Solid state equipment located in an adjacent room controlled stimulus light presentation, drug delivery and recorded lever responses

Procedure

Since all four monkeys had been previously trained to respond under a fixed-ratio 10 (FR 10) schedule of pentobarbital delivery, they were exposed immediately to the terminal conditions Sessions were 2 hr in duration and drug was delivered upon the completion of a FR 10 on the right lever in the presence of an illuminated white ceiling light and white stimulus lights above both levers During each 10-sec infusion, all white lights were extinguished and the red ceiling and red stimulus lights above both levers were illuminated. Responding during the infusion as well as all responding on the left lever had no programmed consequences The drugs used to maintain responding were pentobarbital, lorazepam and flurazepam All monkeys were tested separately with all three drugs but dose and order of the 3 drugs varied as shown in Table 1.

When responding maintained by the delivery of a drug was stable for 2 sessions, an intramuscular injection of Ro 15-1788 or its vehicle was given 5 minutes prior to the next experimental session. Doses of Ro 15-1788 varied between 0 0001 and 10 mg/kg and Ro 15-1788 dose-response functions were determined under each drug maintenance condition Treatments with Ro 15-1788 were given no more than two times a week with at least 2 sessions intervening The effects of each dose were determined once with occasional repetitions

Data Analysis

During each session, the number of infusions delivered and total responses were recorded Due to the short duration

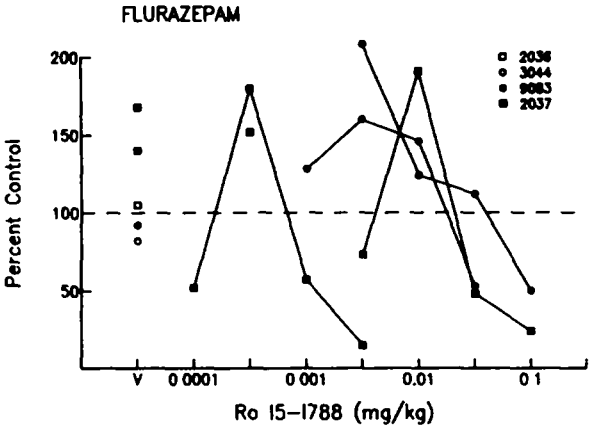

FIG 1 The number of infusions of flurazepam self-administered during the first hour of a 2-hr session following IM treatments of Ro 15-1788 given 5 min presession. Effects are expressed as a percent of the mean number of hour 1 infusions during the two previous sessions The doses of Ro 15-1788 given are shown on the abscissa on a log scale The points above V are the results following vehicle treatment If a treatment was repeated, the second determination is shown as an unconnected point

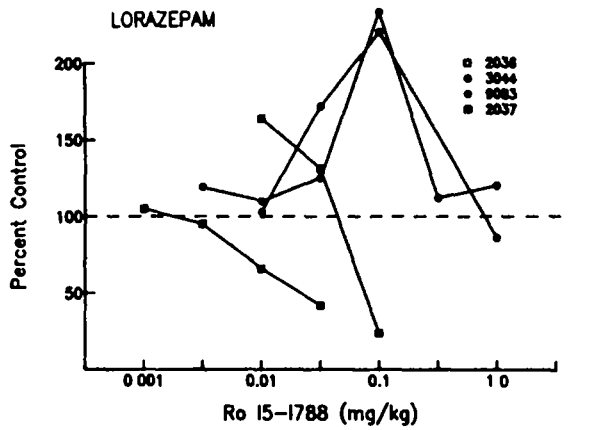

FIG 2 The number of infusions of lorazepam self-administered during the first hour of a 2-hr session following IM treatments of Ro 15-1788 given 5 min presession Other details as in Fig. 1

of action of Ro 15-1788 [12] only data from the first hour of the sessions are presented. Because number of infusions self-administered varied across baseline drugs, the effects of Ro 15-1788 are expressed as a percentage of the mean of the 2 previous sessions (first hour only).

Drugs

Flurazepam hydrochloride (a gift of Abbott Laboratories) and sodium pentobarbital were dissolved in sterile physiological saline and doses refer to the salt Lorazepam (a gift of Wyeth Laboratories) and Ro 15-1788 (a gift of Hoffmann-La Roche, Nutley, NJ) were prepared using a suspension system suitable for water insoluble compounds [2]. Specifically, they were dissolved in a small quantity of 95% ethyl alcohol to which polyoxyethylated vegetable oil (Emulphor EL-620, GAF) was added in a 1:1 ratio. The concentration was 20 mg/ml for lorazepam and 10 mg/ml for Ro 15-1788. Presession vehicle treatments used the suspension system alone. Lorazepam solutions were prepared by adding saline to the suspension to achieve final concentration.

RESULTS

For monkeys 9083, 2036 and 2037, the doses of flurazepam and lorazepam used to maintain responding were selected on the basis of dose-response functions generated in a previous study using a 3 hr session [10] These doses are shown in Table 1. For monkey 9083, the dose of both drugs in this experiment was one-half log unit higher than the dose of flurazepam (0.01 mg/kg) and lorazepam (0.003 mg/kg) that had maintained peak levels of responding in the previous experiment For monkey 2036, the dose of flurazepam in the present study was also higher than the dose (0.03 mg/kg) that had maintained peak responding in the previous study but for lorazepam, the dose (0.01 mg/kg) producing peak levels in the previous study was used. A lower dose (0.003 mg/kg) had maintained only slightly lower rates Monkey 2037 had only previously been tested with flurazepam and only the dose (0.03 mg/kg) used in the present study had maintained

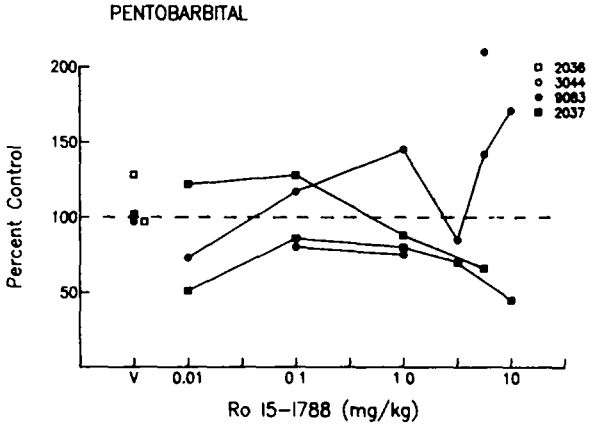

FIG. 3 The number of infusions of pentobarbital self-administered during the first hour of a 2-hr session following IM treatments of Ro 15-1788 given 5 min presession Other details as in Fig 1

adequate rates of responding. Monkey 3044 had not been tested with these drugs previously.

Table 2 shows the mean number of infusions of each drug used to maintain responding during the first hour of sessions prior to treatments with Ro 15-1788. The means for pentobarbital were similar for three monkeys but higher for 2037. Means for 0 01 mg/kg lorazepam varied more across monkeys. For monkey 3044 the mean was low but above saline levels reported in the previous experiment (range 0–5). For 0.03 mg/kg flurazepam, the means were similar for the two monkeys tested. At the higher dose the mean was both higher (3044) and lower (2036).

In three of the four monkeys (2036, 3044, 9083), Ro 15-1788 doses of 0.003 and 0.01 mg/kg clearly increased flurazepam self-administration (Fig. 1). Despite differences in baseline rates and the maintenance dose, the magnitude of rate increases was similar across monkeys. Vehicle treatment had no effect. Self-administration also increased in the fourth monkey (2037) after a low dose of Ro 15-1788 but vehicle also produced similar increases (Fig 1). Responding was below baseline levels at both a lower and higher dose of Ro 15-1788 in this monkey.

The effects of Ro 15-1788 on lorazepam self-administration were similar. Three of the monkeys (2036, 3044, 9083) had rate increases when pretreated with Ro 15-1788 at doses between 0.01 and 0.1 mg/kg (Fig 2) The dose-response functions of the two monkeys with the lowest (3044) and highest rates of lorazepam self-administration were almost identical. Monkey 2037 showed no rate increases but as with flurazepam, doses of Ro 15-1788 which produced increases in other monkeys decreased responding

In contrast to the results with responding maintained by lorazepam and flurazepam, a wide range of doses Ro 15-1788 had little effect on pentobarbital self-administration (Fig 3) At doses above 0.1 mg/kg there were occasional rate increases in a single monkey (9083) Responding was reduced to 50% of control levels at 10 mg/kg for monkey 2037

DISCUSSION

Previous studies have demonstrated that opiate antagonists naloxone and naltrexone increase the rate of self-administration of morphine by rhesus monkeys [6]. These rate increases occur even when access to the opiate has been limited to prevent the development of physical dependence. Since rates of drug self-administration are typically inversely related to dose [11], such results can be interpreted as evidence of partial antagonism which is functionally equivalent to reducing the dose of the opiate. Since naloxone and naltrexone exert their antagonist effects by blocking opiate receptors, the results of the self-administration studies with these antagonists have been interpreted as demonstrating that the response-maintaining properties of opiates involve a drug action at the receptor level

A similar strategy was used in the present experiment to determine whether actions at a benzodiazepine receptor site mediate the response-maintaining effects of flurazepam and lorazepam If this were true, a drug which blocked this site of action would be expected to modify self-administration responding. More specifically partial blockade would functionally lower the dose of the benzodiazepine acting at the receptor Depending on the shape of the dose-response function and the magnitude of the dose used to maintain responding, rates of self-administration would increase or decrease following the administration of an antagonist If the dose of the anxiolytic maintaining responding was on the descending portion of an inverse or U-shaped dose-response curve, responding would increase at some dose of the antagonist On the other hand, if the dose of the anxiolytic maintaining responding was on the ascending portion of dose-response function or at the peak, reducing the effective dose with the antagonist should decrease responding.

The drug used to test these predictions in the present study was Ro 15-1788. Ro 15-1788 has been shown to be a specific antagonist at the benzodiazepine receptor in numerous studies The anticonvulsant, sedative, antipunishment and discriminative stimulus actions as well as effects on food intake of many benzodiazepines but not barbiturates are blocked by Ro 15-1788 [1, 5, 7, 8] Agonist properties of Ro 15-1788 have usually only been detected at high doses [9], although even at low doses there is evidence of certain agonist effects [15]. In the present study presession treatment with Ro 15-1788 increased the rate of self-administration of both benzodiazepines In contrast, pentobarbital self-administration was largely unaffected, indicating that the interaction between Ro 15-1788 and the anxiolytics was specifically related to the benzodiazepine receptor

One interpretation of these results is that the administration of the antagonist precipitated withdrawal which in turn increased the rate of anxiolytic self-administration Previous studies have clearly shown that chronic administration of both lorazepam and flurazepam can produce a state of physical dependence which is revealed by the administration of Ro 15-1788 [3, 13, 14] While this interpretation cannot be ruled out, access to the anxiolytic in the present study was limited to only 2 hr per day, decreasing the likelihood that a state of physical dependence had been produced Furthermore while withdrawal from opiates has been shown to result in increased rates of drug-seeking behavior [16], this has not been shown for other classess of drugs including ethanol and barbiturates

A more plausible interpretation of the changes in anxiolytic self-administration produced by Ro 15-1788 is that the reinforcing properties of these drugs are mediated by the benzodiazepine receptor A partial blockade by the antagonist would be equivalent to reducing the dose Since dose-response functions for flurazepam and lorazepam had previously been obtained for three of these monkeys (2036, 2037 and 9083), it is possible to determine whether partial blockade is a tenable hypothesis For instance monkey 9083 was maintained on 0 03 mg/kg flurazepam and 0 01 mg/kg lorazepam which in the previous experiment were on the descending limb of the dose-response function, i e , lower doses maintained higher rates This was also the case with flurazepam for monkey 2036 For both these monkeys, increased rates of self-administration produced by Ro 15-1788 could be interpreted as partial antagonism which is equivalent to reducing the dose of the self-administered drug For monkey 2036, lorazepam at the dose of 0.01 mg/kg in a previous experiment had maintained peak levels of responding In that case Ro 15-1788 should only lower rates of lorazepam self-administration. The rate increases found (Fig 2) were in fact more modest than in monkeys 9083 and 3044 and occurred at lower doses of Ro 15-1788 This result is difficult to interpret but could also be viewed as a decrease in effective dose if it is assumed that a smaller decrease in the dose of lorazepam in the previous study (less than the standard 1/2 log unit) would have resulted in an increase in rate

Although there was a dose of Ro 15-1788 that also increased flurazepam self-administration in monkey 2037, this increase can be assumed to be an artifact of this monkey's nonspecific response to presession injections (rate increases were also seen following vehicle given presession) On the other hand, doses of Ro 15-1788 which increased rates of flurazepam self-administration in the other monkeys decreased responding in this case However, monkey 2037 was also tested with flurazepam in a previous study The dose of 0 03 mg/kg flurazepam used in the present study had maintained the highest rates of responding whereas doses 1/2 log unit higher and lower maintained precipitously lower rates. Therefore, it is possible to conclude that lowering the effective dose of flurazepam with Ro 15-1788 should have resulted in rate decreases as were observed

Although the results of this study can be interpreted to demonstrate that Ro 15-1788 antagonizes the reinforcing properties of anxiolytics, a more modest conclusion is more justified As discussed by Johanson and Schuster [11], reinforcing properties alone do not determine rate of drug self-administration An alteration in this rate could be due to a specific antagonism of reinforcing actions but is more likely

due to an antagonism of other properties as well. For instance, rates of anxiolytic self-administration could be low not only because of minimal reinforcing properties but also because of direct rate-decreasing effects on any performance The effects of the antagonist could be to reverse these latter effects rather than specifically to alter the reinforcing properties. It is even more likely that the increased rates of responding produced by Ro 15-1788 were a product of its ability to antagonize the reinforcing actions of flurazepam and lorazepam as well as their rate decreasing effects.

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