Pharmacology Biochemistry and Behavior, Vol. 41, pp. 461-464, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Physical Dependence on Diazepam: Precipitation of Abstinence Syndromes by Peripheral and Central Benzodiazepine Receptor Antagonists

J. A. MARTINEZ*, M. J. FARGEAS† AND L. BUENO†[1]

**Department of Physiology and Pharmacology, Faculty of Medicine, Espinardo, Murcia, Spain*
†Department of Pharmacology-Toxicology INRA, 31931 Toulouse, Cédex, France

Received 10 April 1991

MARTINEZ, J. A., M. J. FARGEAS AND L. BUENO. *Physical dependence on diazepam: Precipitation of abstinence syndromes by peripheral and central benzodiazepine receptor antagonists.* PHARMACOL BIOCHEM BEHAV **41**(2) 461-464, 1992.—This work was performed to compare withdrawal symptoms induced by the administration of the central vs. peripheral benzodiazepine antagonists in rats treated chronically with diazepam (15 mg/kg, SC) for 8 days. Withdrawal was expressed as motor, autonomic, and behavioral signs. Significant withdrawal occurred after the administration of both flumazenil (15 and 20 mg/kg, IP) and PK11195 (5 and 10 mg/kg, IP). With these doses, PK11195 induced diarrhea and decreased motor activity more than did flumazenil. These preliminary results suggest that peripheral benzodiazepine receptors are involved in the withdrawal syndrome in diazepam-dependent rats.

Benzodiazepines Diazepam Central receptors Peripheral type receptors Behavior Rats

BENZODIAZEPINES (BZD's) have been used as anxiolytics, hypnotics, anticonvulsants, and muscle relaxants since the beginning of the 1960's; BZD's exert their pharmacological effects via interaction with specific recognition sites that are part of the macromolecular GABA receptor complex (3). Experimental evidence indicates that BZD recognition site ligands can be considered as a continuum of three overlapping groups, conventionally designated agonists, antagonists, and inverse agonists, according to their modulatory effects on the GABAergic transmission (2). However, it has been recognized that they can induce tolerance and physical dependence (8). Abrupt withdrawal from prolonged treatment with BZD's can lead to an abstinence syndrome with symptoms depending upon the duration of the treatment and their daily dosage (14). Experimental BZD dependence has been produced in different animals species (7); however, the mechanism is not yet clearly understood. The existence of two different types of BZD receptors (central and peripheral) is now well documented (12). It has also been clearly shown that central BZD receptors (CBR) exist in many regions of the brain, are coupled with the GABA receptors, and mediate the acute actions of BZD's in the central nervous system (10). However, peripheral type BZD receptors (PTBR) are also present in the brain (11) and autoradiographic techniques have demonstrated that their distribution differs from that of central type BZD receptors (4). The structure of peripheral BZD receptors has not yet been identified, but unlike the central BZD receptors it does not seem to be closely related to the GABA receptor (13). Moreover, some BZD's such as diazepam bind to both central and peripheral BZD receptors with a high affinity (11).

The overall aim of this investigation was to characterize qualitatively and quantitatively the withdrawal syndromes precipitated by the peripheral and central BZD receptor antagonists in diazepam-treated rats.

[1] Requests for reprints should be addressed to Dr. L. Bueno, Department of Pharmacology, INRA, 180 chemin de Tournefeuille, B.P. 3, 31931 Toulouse, Cédex, France.

METHOD

Animals

Eighty male Wistar rats weighing 200–300 g were used in these experiments. Rats were placed in individual cages and maintained at constant temperature (21°C) and light-dark cycle (lights 7 a.m.–7 p.m.). Standard laboratory chow and water were provided ad lib to all animals.

Procedure

Rats were assigned to 8 groups of 10 animals. Four groups of 10 naive rats were injected with diazepam (15 mg/kg, SC, per day) for 8 days. On day 9, the first two groups received flumazenil (15 and 20 mg/kg, IP, respectively) and the two other groups received PK11195 (5 and 10 mg/kg, IP). The four remaining groups were injected with benzyl alcohol (1.5%), the solvent of diazepam, for 8 days and treated, on day 9, by either flumazenil or PK11195 at the same doses as diazepam-treated rats.

Evaluations of withdrawal signs were done under standard conditions described by Ryan and Boisse (9). Observations were performed by two independent experienced raters for 60 min after the antagonist was given. Eleven motor, autonomic, and behavioral signs were monitored by operationally defined criteria described by Boisse et al. (1). Many signs were graded from 0 to 3, with a grade of 3 indicating the maximal intensity. Some signs that could not be quantitated were recorded as present or absent (O or N). The total withdrawal score (WS) was the sum of the grades of all signs when they were expressed maximally. In our experiments, the maximum possible WS was 29.

Statistical analysis of variance (ANOVA) was performed using stepwise discriminant analysis with BMDP statistical software and the results considered significantly different for $p \leq 0.05$.

Drugs

Diazepam (Valium, Roche, France) dissolved in benzyl alcohol (1.5%) was used to induce dependence. Flumazenil (RO 151788) (Hoffman La Roche, Switzerland) and PK11195 (Rhône Poulenc, France) were dissolved in dimethylsulfoxide (DMSO).

Doses of diazepam and flumazenil were chosen from similar studies, the dose of 10 mg/kg flumazenil generally producing maximal withdrawal effects. For PK11195, doses were chosen based on studies of binding (5).

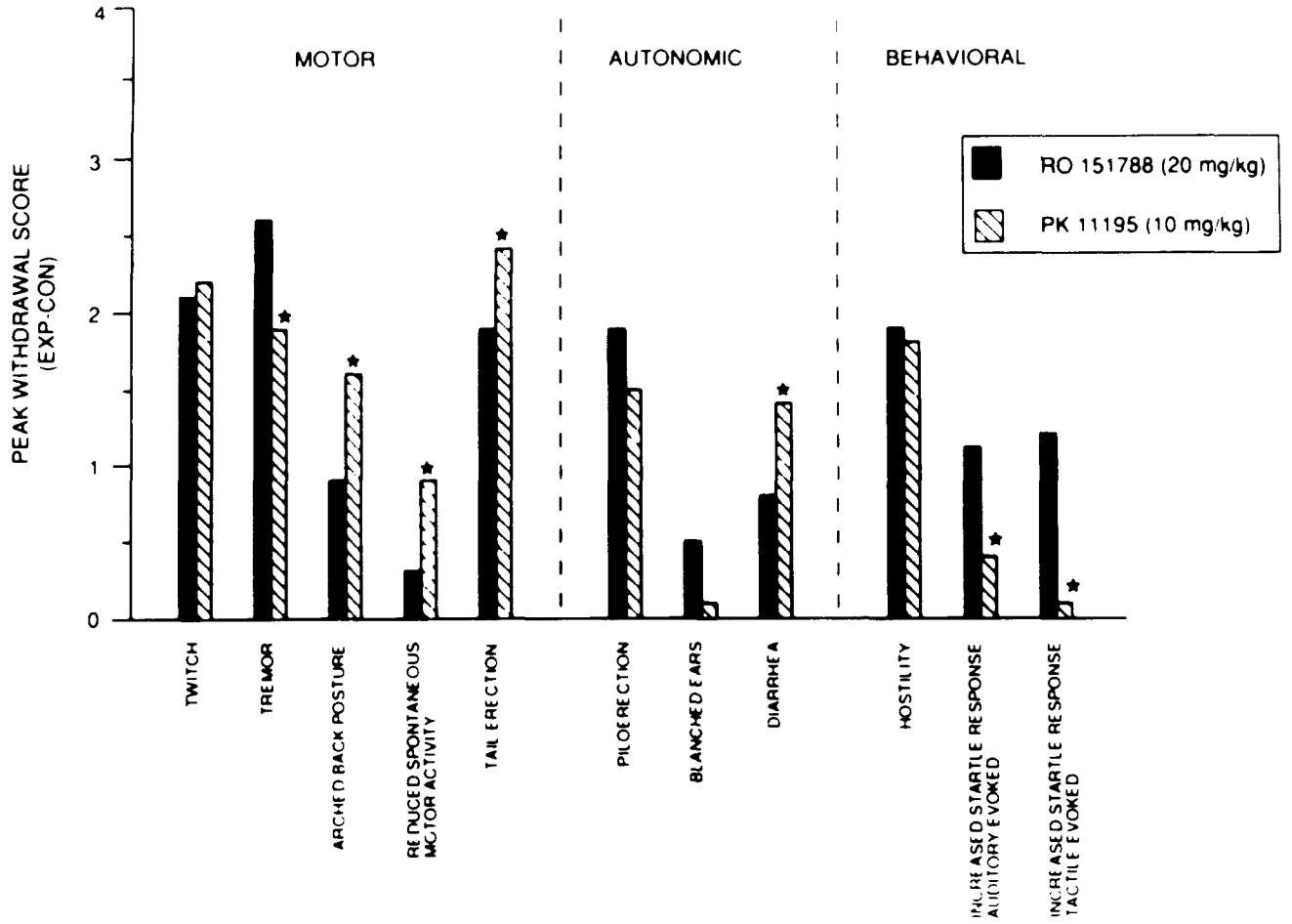

FIG. 1. Effects of flumazenil (20 mg/kg) and PK11195 (10 mg/kg) on several parameters of withdrawal syndrome. Peak withdrawal score expressed the difference between mean values from animals treated with diazepam (15 mg/kg daily for 8 days) and those of vehicle-treated ones. Stars represent significant differences between RO151788- and PK11195-injected animals. (Stepwise discriminant analysis, $n = 10$.)

TABLE 1
COMPARATIVE EVALUATION OF WITHDRAWAL SCORES PRODUCED BY FLUMAZENIL AND PK11195 IN RATS DAILY TREATED WITH DIAZEPAM FOR 8 DAYS (MEAN ± SEM, n = 10)

	Withdrawal Score* (arbitrary units)			
	Flumazenil		PK11195	
Dose (mg/kg, IP)	15	20	5	10
Control (vehicle)	4.2 ± 0.5	3.9 ± 1.1	3.6 ± 0.6	3.7 ± 0.9
Diazepam (15 mg/kg, ID)	19.3 ± 1.7†	20.4 ± 2.7†	16.5 ± 2.1†	17.9 ± 3.0†

*Withdrawal score corresponds to the sum of motor, autonomic, and behavioral analyzed parameters (see Fig. 1).

†Values significantly different ($p < 0.01$) from corresponding control values. Variance analysis did not show any interaction between doses and compounds.

RESULTS

In diazepam-dependent rats, flumazenil and PK11195 produced various abstinence signs (Fig. 1). Overall WS's were significantly greater in experimental groups than control groups at both doses tested for each agent (Table 1).

The withdrawal syndrome developed very rapidly after flumazenil. It was expressed maximally at the 5-min observation time and decayed more slowly than it developed. The duration of withdrawal was brief: It lasted about 45 min. The withdrawal syndrome induced by PK11195 began later (maximal effect 20 min after administration) and lasted roughly 30 min. At any dose tested, the overall WS seemed more pronounced in flumazenil-treated rats (WS: 19.3 ± 1.7 and 20.4 ± 2.7) as compared to that of PK11195 ones (WS: 16.5 ± 2.1 and 17.9 ± 3.0) (Table 1). However, these values were not significantly different from each other. Nevertheless, some withdrawal signs, such as diarrhea, decreased motor activity, and arched back posture, were more marked in PK11195-treated rats regardless of dose (Fig. 1). In contrast, behavioral signs, particularly increased startle response, were not different in PK11195-treated vs. control rats, but they are significantly ($p < 0.01$) higher for flumazenil at 20 mg/kg than for PK11195 at 10 mg/kg.

DISCUSSION

Benzodiazepines and related compounds interact with high affinity at two distinct receptors in the mammalian CNS. The better defined is the CBR (6). It is through this receptor that the anxiolotyic and anticonvulsant properties of CBR agonists have been shown to be mediated by enhancement of γ-aminobutyric acid-gated-chloride conductance (10). The second receptor, the PTBR, has been detected in the membranes of several tissues of mammals, including heart, lung, kidney, and testis, as well as in the brain (11), in which it has a different regional distribution than the CBR (13).

Our results confirm previous observations of others (8) that flumazenil produces a precipitated abstinence syndrome when administered to diazepam-dependent rats. Moreover, this study provides unequivocal evidence for the induction of withdrawal syndrome after the administration of the PTBR antagonist PK11195 in diazepam-dependent rats. The different doses tested for each agent produce nearly the same intensity in withdrawal signs, showing that the lower doses used induce maximal response.

Withdrawal syndromes precipitated by RO151788 or PK11195 have many motor, autonomic, and behavioral signs in common although there are some noticeable differences between the two antagonists. These preliminary results do not permit to establish a clear comparison between the two agents. But, these results suggest that the pattern of withdrawal signs may differ for the two antagonists, central and peripheral type receptors mediating different syndromes of withdrawal.

As the behavioral signs produced by PK11195 cannot be explained by its action on PTBR outside the CNS, data from the present study strongly suggest that PK11195 acts on the PTBR located in the CNS. Moreover, despite their name, the PTBR's also occur in the brain (11). On the other hand, withdrawal precipitated by the doses of PK11195 used in this study resulted in a more pronounced diarrhea than that induced by flumazenil. This could be explained by a possible action of PK11195 on PTBR's in the gastrointestinal tract. However, PTBR's are not yet described in the enteric nervous system (13), although studies in our laboratory indicate that the benzodiazepine withdrawal precipitated by PK11195 produces electromygraphic alterations in the gastrointestinal tract of rats (unpublished data). In conclusion, the results of the present study show that chronic administration of diazepam for 8 days using modest doses can produce dependence manifested by withdrawal signs after the administration of CBR as well as PTBR antagonists.

ACKNOWLEDGEMENTS

The authors thank C. Betoulieres for technical assistance, S. Sayavong for manuscript preparation, and M. Campos for statistical analysis. They also thank Ministerio de Education y Ciencia, Spain, and INRA, France, for their financial support.

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