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BRIEF COMMUNICATION

Different Location of Benzodiazepine Sites Involved in Gut and Behavioral Effects of Benzodiazepine Withdrawal in Rats

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BONNAFOUS, C. AND L. BUENO. *Different location of benzodiazepine sites involved in gut and behavioral effects of benzodiazepine withdrawal in rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 253–256, 1994.—This work was performed to determine if the alterations in gastric emptying induced by precipitated withdrawal are linked to peripherally or centrally located benzodiazepine sites, in rats treated chronically with diazepam (15 mg/kg/day IP) for 7 days. In sham-capsaicin-treated rats, precipitated withdrawal by flumazenil (15 mg/kg IP) induced an increase of gastric emptying, whereas it had no effect in systemic capsaicin-treated rats. Both groups of animals developed withdrawal syndrome expressed as motor, autonomic, and behavioral signs. On diazepam-dependent rats, central administration of flumazenil (0.15 mg/kg ICV) induced withdrawal syndrome but had no effect on gastric emptying. These preliminary results suggest that benzodiazepine receptors located in the central nervous system are involved in behavioral withdrawal syndrome, whereas benzodiazepine receptors located at the peripheral level are responsible for digestive withdrawal syndrome involving capsaicin-sensitive neurons.

Diazepam Benzodiazepine receptors Withdrawal Capsaicin-sensitive nerves Gastric emptying

BENZODIAZEPINES (BZs) are extensively used clinically for their sedative, hypnotic, muscular relaxant, and anticonvulsivant effects. However, chronic administration of benzodiazepines leads to the development of tolerance and dependence with concomitant withdrawal effects (14). Excessive anxiety, tremor, muscle cramps, vomiting, diarrhoea, and epigastric distress were noticed as symptoms of precipitated withdrawal induced by BZ receptor antagonist (flumazenil) in several species (13,15). Recently, Martinez et al. (7) have demonstrated that, in diazepam-dependent rats, precipitated BZ withdrawal induces an acceleration of gastrointestinal transit and gastric emptying with alterations of intestinal myoelectric activity.

Benzodiazepines induce their pharmacological actions through BZ receptors associated to $GABA_A$ receptors located in the central nervous system (10). It has been shown that central-type BZ receptors are also found in guinea pig myenteric neurons (2). Moreover, several studies have demonstrated the involvement of a central relay in alterations of motility in response to peripheral stimulus (3,8). Capsaicin, a neurotoxin that causes a long-term sensory receptor-blocking action, can be used in functional investigations on the sensory pathways (5). The purpose of this study was a) to determine if gastric emptying troubles induced by precipitated diazepam withdrawal in rats, are linked to the involvement of central-type BZ receptors located on afferent neurons using systemic capsaicin treatment, and b) to verify if systemic capsaicin-treated rats can also develop dependance to diazepam, using behavior tests after precipitated withdrawal with flumazenil.

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METHOD

Animals

Sixty-four male Wistar rats weighting 200–250 g were used in these experiments. Rats were placed in individual cages and maintained at constant temperature (21°C) and light : dark cycle (lights 0700 to 1900 h). Standard laboratory chow and water were provided ad lib to all animals.

Procedure

Capsaicin treatment. Four groups of eight rats were used. Groups 1 and 2 were systemically treated for 4 days with increasing doses of capsaicin to reach a total dose of 125 mg/kg: 2 times 5 mg/kg the first day, 10 and 15 mg/kg the second day, 2 times 20 mg/kg the third day, and 2 times 25 mg/kg the fourth day. Groups 3 and 4 were treated with the capsaicin vehicle only. The experiments were performed 2 weeks after completion of capsaicin treatment.

Experimental Procedure

In a first series of experiments, groups 1 and 3 were treated by diazepam (15 mg/kg/day IP) during 7 days. Groups 2 and 4 received 0.9 ml/day IP of dimethysulfoxide (DMSO 100%: diazepam vehicle) during 7 days. On the eighteenth day, all the animals fasted for 16 h, received 15 mg/kg IP of flumazenil (benzodiazepine receptor antagonist).

In a second series of experiments, 16 rats received diazepam (15 mg/kg/day IP) during 7 days; 16 other animals received DMSO (0.9 ml/day IP). On the eighteenth day, all the animals were fasted for 16 h; 8 diazepam-treated and 8 DMSO-treated rats received 0.15 mg/kg intracerebroventricularly (ICV) of flumazenil. These rats were also fitted with a small polyethylene catheter (i.d., 0.3 mm; o.d., 0.7 mm) inserted into a lateral ventricle of the brain using a stereotaxis apparatus (Kopf, Los Angeles, CA) (11). The bone reference marks were determined as follows: (coordinates from the bregma) anterioposterior, 0.6 mm; lateral, 2 mm; ventral, 4 mm. Two screws were inserted in the bone surface. The catheter was then secured to the skull and screws by means of a dental cement. Injections were delivered with 10 μl Hamilton syringe. Vehicle (50% DMSO, 50% NaCl 9%) pH 7.4. No damage to the tissue was observed at the injection site. Eight diazepam-treated and eight DMSO-treated rats received 15 mg/kg IP of flumazenil.

Gastric emptying studies. Fifteen minutes after flumazenil administration (IP or ICV), 2 ml of a test meal containing 1 μCi/ml of ^{51}Cr sodium chromate were administered by gavage. Thirty minutes after the test meal, animals were killed by cervical dislocation. The stomach and small intestine were excized and placed into tubes. Radioactivity was determined by placing tubes in a gamma counter (MR 252C, Kontron, Basel, Switzerland) for 5 min. Gastric emptying was calculated as the percentage of total counts found in the small intestine and the colon.

Withdrawal test. For all animals studied, evolution of withdrawal signs were done under standard conditions described by Ryan and Boisse (9). Observations were performed by two independent experienced raters for 30 min after the antagonist (flumazenil) was given. Eleven motor, autonomic, and behavioral signs were monitored by operationally defined criteria described by Boisse et al. (1) Signs were graded from 0 to 3; the total withdrawal score (WS) was the sum of the grades of all signs.

Statistical analysis. Values are given as mean ± standard error. Comparison of gastric emptying percent between sham- and capsaicin-treated rats were analyzed statistically using ANOVA followed by unpaired Student's *t*-test. Comparison of parameters of behavioral withdrawal was performed using step-wise discriminant analysis with DMDP statistical softwave. Differences were considered significant for $p < 0.05$.

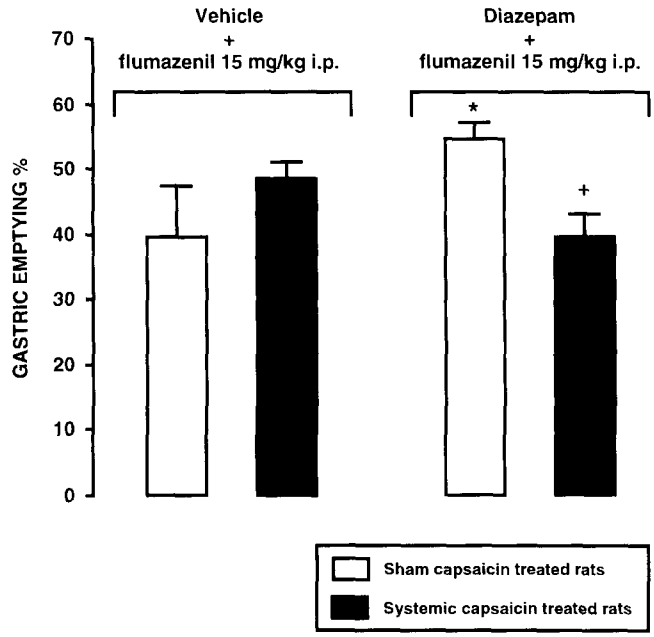

FIG. 1. Effect of peripheral flumazenil-precipitated withdrawal in systemic capsaicin- or sham-treated rats that have received either diazepam or its vehicle. *Significantly different ($p < 0.05$) from vehicle values ($n = 8$). +Significantly different ($p < 0.05$) from sham + diazepam + flumazenil ($n = 8$).

RESULTS

There was no difference of the percentage of gastric emptying between sham- and capsaicin-treated rats that had received IP DMSO and flumazenil. In sham-treated rats made diazepam dependent, the precipitated withdrawal induced a strong acceleration of the gastric emptying, whereas in capsaicin-treated rats, the IP administration of flumazenil had no effect on the percentage of gastric emptying; values were not significantly ($p < 0.05$) different from the control (Fig. 1).

Sham- and capsaicin-treated rats both developed withdrawal syndrome. The overall WS were not significantly different between the two groups of animals (WS: 20.8 ± 1.1 and 21.0 ± 0.4), and no significant differences were noted on each different motor, autonomic, and behavioral signs (Table 1).

Diazepam-dependent rats withdrawn by flumazenil administered ICV, developed withdrawal syndrome. The overall WS (21.1 ± 0.7) was not significantly different from those of sham (21.0 ± 0.4)- and capsaicin (20.8 ± 1.1)-treated rats (Table 1). On the other hand, ICV administration of flumazenil had no effect on the percentage of gastric emptying; value was not significantly different from the control, i.e., vehicle-treated rats + flumazenil ICV, whereas flumazenil IP induced a strong stimulation of gastric emptying in diazepam-dependent rats (Fig. 2).

TABLE 1

PARAMETERS OF WITHDRAWAL SYNDROME OF SHAM AND SYSTEMIC CAPSAICIN-TREATED RATS WITHDRAWN BY PERIPHERAL ADMINISTRATION OF FLUMAZENIL (15 mg/kg), AND OF RATS WITHDRAWN BY CENTRAL ADMINISTRATION OF FLUMAZENIL (0.15 mg/kg)

	Treatments		
Peak Withdrawal Score	Sham Capsaicin-Treated Rats + Diazepam + Flumazenil IP	Systemic Capsaicin-Treated Rats + Diazepam + Flumazenil IP	Diazepam-Treated Rats + Flumazenil ICV
Motor			
Twitch	2.2 ± 0.2	2.6 ± 0.2	2.2 ± 0.1
Tremor	1.0 ± 0.3	1.2 ± 1.2	2.0 ± 0.1
Arched back posture	2.4 ± 0.2	2.4 ± 0.2	1.6 ± 0.2
Reduced spontaneous motor activity	2.6 ± 0.2	2.4 ± 0.2	1.8 ± 0.2
Tail erection	2.8 ± 0.3	2.8 ± 0.3	2.0 ± 0.4
Autonomic			
Piloerection	1.0 ± 0.2	1.0 ± 0.3	1.6 ± 0.2
Blanched ears	1.6 ± 0.6	2.0 ± 0.4	1.7 ± 0.1
Diarrhea	1.4 ± 0.4	1.4 ± 0.3	1.1 ± 0.2
Behavioral			
Hostility	2.0 ± 0.3	1.2 ± 0.3	2.2 ± 0.1
Increased startle response auditory evoked	1.2 ± 0.3	1.8 ± 0.5	2.2 ± 0.2
Increased startle response tactile evoked	2.6 ± 0.2	2.0 ± 0.3	2.3 ± 0.1
Withdrawal score	21.0 ± 0.4	20.8 ± 1.1	21.1 ± 0.7

Control animals (diazepam-vehicle treated) have withdrawal score equal to 0.
$n = 8$.

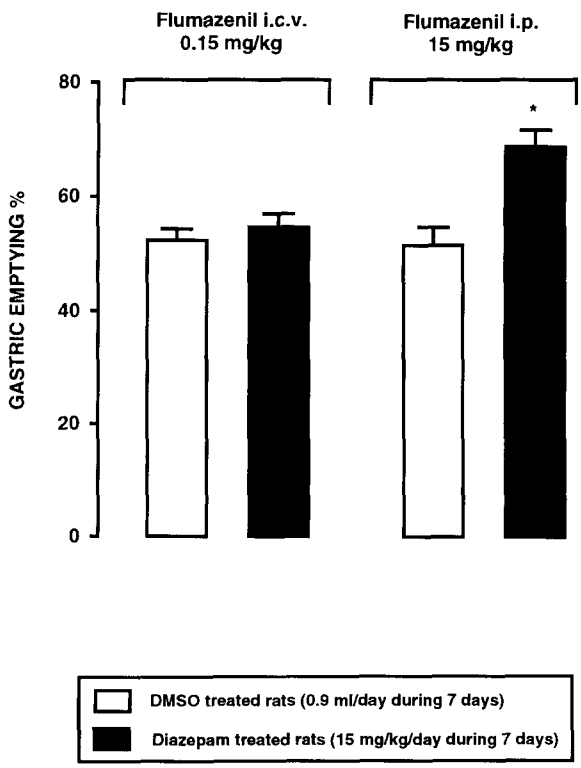

FIG. 2. Effect of central flumazenil-precipitated withdrawal in rats treated with diazepam or its vehicle. *Significantly different ($p < 0.05$) from corresponding vehicle values ($n = 8$).

DISCUSSION

Our findings are in agreement with a previous work (7) showing that precipitated diazepam withdrawal induces an increase of gastric emptying. Systemic capsaicin treatment suppresses this effect but has no consequence on behavioral diazepam dependency. Indeed, systemic capsaicin-treated rats develop a withdrawal syndrome as sham capsaicin-treated do. In the digestive tract, capsaicin-sensitive afferent innervation participates in nociception and intestino-intestinal activation of inhibitory reflexes (4,6). Consequently, we can hypothetize that digestive withdrawal syndrome is linked to activation of receptors and/or to release of neuropeptide activating capsaicin-sensitive afferent neurons. Then, a central relay involving different neurotransmitter-release (CCK, 5-HT) can be implicated, leading to peripheral effects, as it has yet been demonstrated for withdrawal effect on small intestine motility mediated by central release of CCK (unpublished data).

Recent studies have demonstrated the presence of GABA-receptors in guinea pig myenteric plexus and that myenteric neurons themselves are endowed with benzodiazepine sites that are akin to central-type sites (12). The fact that central administration of flumazenil (at 100 lower dose than by peripheral route) induces behavioral withdrawal syndrome but has no effect on gastric emptying shows that when peripheral benzodiazepine sites are not activated, digestive withdrawal manifestations are not observed.

We can conclude that benzodiazepine sites located on the central nervous system are mainly involved in the behavioral withdrawal syndrome, whereas benzodiazepine sites located peripherally are implicated in digestive diazepam-withdrawal manifestations. When withdrawal is precipitated, these benzodiazepine sites are activated and induce via capsaicin-sensitive afferent neurons a stimulation of CNS leading to gastric emptying disturbances.

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