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BRIEF COMMUNICATION

Effects of Naloxone and Picrotoxin on Diazepam- or Pentobarbital-Induced Hyperphagia in Nondeprived Rats

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NARUSE, T., T. ASAMI AND Y. KOIZUMI. *Effects of naloxone and picrotoxin on diazepam- or pentobarbital-induced hyperphagia in nondeprived rats.* PHARMACOL BIOCHEM BEHAV 31(3) 709–711, 1988.—Diazepam and pentobarbital administered intravenously increased food intake in a dose-dependent manner in nondeprived rats. Low doses of naloxone inhibited diazepam-induced feeding, but did not inhibit pentobarbital-induced feeding. On the other hand, picrotoxin inhibited feeding induced by both drugs. These findings suggest that diazepam-induced hyperphagia is related to endogenous opioid mechanisms, but pentobarbital-induced hyperphagia is not. Hyperphagia induced by both drugs may be related to GABAergic neurons.

Diazepam Pentobarbital Hyperphagia Naloxone Picrotoxin Rat

IT is well known that diazepam, a benzodiazepine, produces hyperphagia in rats (1, 14, 17, 19); however, the neurotransmitters underlying the hyperphagia are still unclear. It has been proposed that diazepam-induced hyperphagia may be related to endogenous opioid mechanisms (3, 8, 18) and GABAergic neurons (2). In addition, it is thought that barbiturates induce hyperphagia interacting with the benzodiazepine-GABA receptor complex (10,16). Studies comparing the mechanisms of hyperphagia action of both drugs after intravenous administration have not been carried out. Intravenous (IV) administration of these drugs may be a more suitable method to determine their dynamic actions, since they probably enter the brain rapidly from the blood stream following IV administration. The present study was undertaken to clarify whether the mechanisms of diazepam- and pentobarbital-induced hyperphagia are different by using the opiate receptor antagonist, naloxone and the GABA-A antagonist, picrotoxin.

METHOD

Animals

Male Sprague-Dawley rats, weighing 500–600 g at the beginning of the experiment, were used. To avoid stress during IV injections, a chronic femoral vein cannula of U-shaped silicone tubing was surgically implanted in each animal under pentobarbital anesthesia (40 mg/kg, IP). After surgery, each animal was permanently housed in an operant chamber (20.5×24×27 cm) and fitted with a harness. The harness was connected to a stainless steel tube which was attached to a swivel. The swivel arrangement in the tubing system allowed animals to move freely. All rats were given automatic IV injections of vehicle at 2 hr intervals for 5 to 7 days before the experiment. Animals were allowed free access to water and food. The room housing the animals was maintained at a temperature of 22±2°C and humidity with 55±10% under a 12 hr light-dark cycle (lights on at 7:00 a.m.).

Drugs

Diazepam (Dott Bonapace & Co., Italy) was dissolved in 0.04 N HCl saline solution. Pentobarbital sodium (Nembutal® sodium solution, Abbott Laboratories, USA), naloxone hydrochloride (Naloxone Injection®, Sankyo, Japan) and picrotoxin (Wako, Japan) were diluted or dissolved in 0.9% saline solution. All drugs were administered by a syringe in a volume of 1 ml/kg, and doses were expressed as the free base. All rats were used repeatedly at 5- to 7-day

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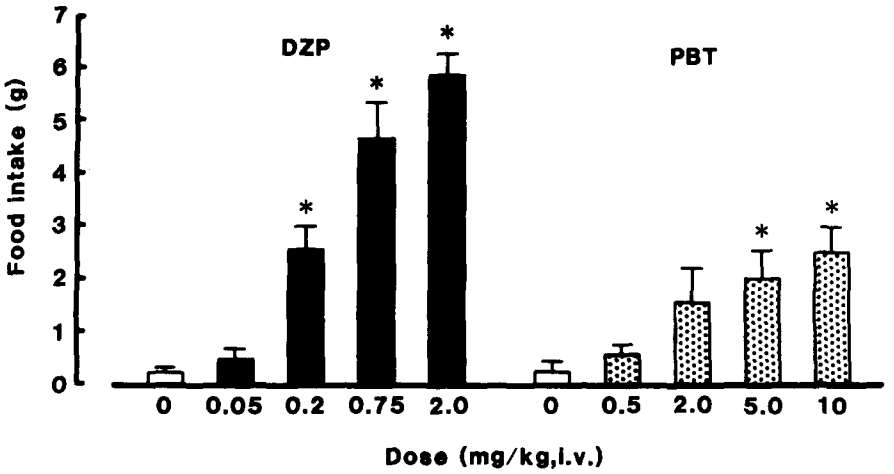

FIG. 1. Food intake after intravenous administration of diazepam (DZP) or pentobarbital (PBT) in nondeprived rats. Food intake was measured for 30 min after drug administration. Open columns indicate the control groups treated with vehicle. Results are presented as mean±S.E. Each group consists of 5 to 12 rats. *Significantly different from the control group, $p<0.05$.

intervals. The experiment was performed between 10:00 a.m. and 3:00 p.m.

Procedure

To test food intake following diazepam or pentobarbital, diazepam (0, 0.05, 0.2, 0.75 and 2.0 mg/kg, n=6 to 12) and pentobarbital (0, 0.5, 2.0, 5.0 and 10 mg/kg, n=5 to 6) were administered intravenously. After drug administration, a preweighed amount of food pellets were placed into the individual cage. Food intake (g) was measured for 30 min and calculated by weighing uneaten food and crumbs. During the test, rats were allowed free access to water. To determine the effect of naloxone or picrotoxin on diazepam- or pentobarbital-induced feeding, the minimum effective dose of diazepam or pentobarbital which produced hyperphagia were used. Naloxone (0, 0.1, 0.2 and 0.4 mg/kg, n=5 to 11) or picrotoxin (0, 0.5, 1.0 and 2.0 mg/kg, n=7 to 9) was administered intraperitoneally 10 min prior to diazepam (0.2 mg/kg, IV) or pentobarbital (5.0 mg/kg, IV) administration. After diazepam or pentobarbital administration, food intake was measured as described above.

Statistical Analysis

Differences between the groups were studied by analysis of variance followed by Dunnett's t-test.

RESULTS

Food intake after IV administration of diazepam or pentobarbital is shown in Fig. 1. Food intake following diazepam increased in a dose-dependent manner. Significant increases in food intake were observed after doses of 0.2 mg/kg or greater. Food intake following pentobarbital also increased in a dose-dependent manner. Significant increases in food intake were observed at doses greater than 5.0 mg/kg, IV.

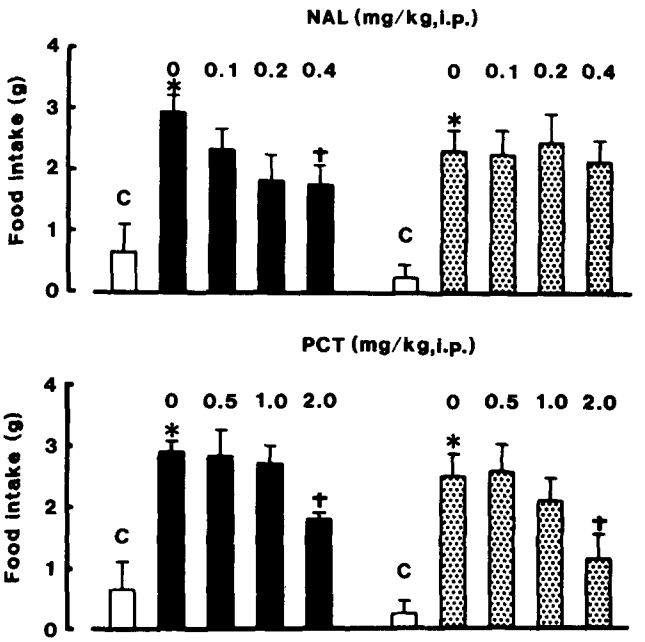

FIG. 2. Effect of pretreatment with naloxone (NAL) or picrotoxin (PCT) on diazepam or pentobarbital-induced feeding in nondeprived rats. NAL (0 to 0.4 mg/kg) or PCT (0 to 2.0 mg/kg) was administered intraperitoneally 10 min prior to administrations of diazepam (0.2 mg/kg, IV) and pentobarbital (5.0 mg/kg, IV). Open columns (C) indicate the control groups treated with vehicle for diazepam or pentobarbital. Cross-hatched columns and dotted columns indicate groups treated with diazepam and pentobarbital, respectively. Food intake was measured for 30 min after administration of diazepam or pentobarbital. Results are presented as mean±S.E. Each group consists of 5 to 12 rats. *Significantly different from the control group, $p<0.05$. †Significantly different from the group treated with diazepam or pentobarbital alone (not pretreated with NAL or PCT), $p<0.05$.

Pentobarbital at a dose of 10 mg/kg, IV also produced ataxia. Therefore, food intake at 10 mg/kg did not increase markedly from the dose of 5.0 mg/kg.

Naloxone inhibited diazepam- (0.2 mg/kg, IV) induced feeding in a dose-dependent manner although blockade only reached statistical significance at a dose of 0.4 mg/kg, IP. Naloxone had no effect on pentobarbital-induced feeding (Fig. 2). Picrotoxin (2.0 mg/kg, IP) significantly inhibited both diazepam and pentobarbital-induced feeding (Fig. 2). In another experiment, we showed that the doses of naloxone and picrotoxin used did not produce significant changes in food intake when compared with the vehicle-treated control group. These data are not shown in Fig. 2. The mean amount consumed following each naloxone dose (n=7) was: 0.1 mg/kg, IP (0.86±0.40 g, mean±S.E.), 0.2 mg/kg, IP (0.50±0.24 g) and 0.4 mg/kg, IP (0.71±0.36 g). The mean amount consumed following each picrotoxin dose (n=7) was: 0.5 mg/kg, IP (0.57±0.37 g), 1.0 mg/kg, IP (0.43±0.30 g) and 2.0 mg/kg, IP (0.29±0.29 g).

DISCUSSION

This is the first report studying diazepam- or pentobarbital-induced feeding after IV administration. Both drugs given IV produced feeding in a dose-dependent manner. These effects of diazepam and barbiturates have been reported following administration by various different routes (1, 9, 14, 17, 19). The possible mechanism of diazepam-induced hyperphagia may be related to GABAergic neurons (2). Similarly, the possible mechanism of pentobarbital-induced hyperphagia may be related to GABAergic neurons, since barbiturates interact with the benzodiazepine-GABA receptor complex (16). Based on the present data indicating that picrotoxin reduced hyperphagia induced by either diazepam or pentobarbital, feeding behavior induced by both drugs may be related to activation of GABAergic neurons.

Recently, it has become clear that endogenous opioid mechanisms play an important role in feeding behavior (9, 13, 15). In addition, there are some reports suggesting that diazepam-induced feeding may be related to endogenous opioid mechanisms (8,18). However, the issue of whether endogenous opioid mechanisms modulate barbiturates-induced feeding has not been addressed. Interestingly, it has been also reported that chlordiazepoxide antagonized picrotoxin- or naloxone-induced suppression of drinking (6) and chlordiazepoxide-induced hyperdipsia was inhibited by naloxone, but phenobarbital-induced hyperdipsia was not (7). This evidence suggests that hyperdipsia induced by chlordiazepoxide may be related to endogenous opioid mechanisms, but hyperdipsia by phenobarbital is not. Thus, the different mechanism between diazepam- and pentobarbital-induced hyperphagia may exist. In the present study, we used low doses of naloxone to avoid its GABA antagonistic effect (11) and the inhibitory effect on food intake of naloxone by itself (4, 5, 12). Low doses of naloxone inhibited diazepam-induced feeding in a dose-dependent manner which is consistent with a previous report (18). However, feeding by pentobarbital was unaffected by low doses of naloxone. These findings suggest that diazepam-induced hyperphagia may be related to endogenous opioid mechanisms, whereas pentobarbital-induced hyperphagia is not. Hyperphagia induced by both drugs was blocked by picrotoxin suggesting the involvement of GABAergic neurons in both drug responses.

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