*Pharmacology Biochemistry & Behavior*, Vol. 39, pp. 559–561. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

# Diazepam-Induced Feeding in Captive Gray Wolves (*Canis lupus*)

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KREEGER, T. J., A. S. LEVINE, U. S. SEAL, M. CALLAHAN AND M. BECKEL. *Diazepam-induced feeding in captive gray wolves* (Canis lupus). PHARMACOL BIOCHEM BEHAV **39**(3) 559–561, 1991.—Diazepam doses of 0.2, 0.4, and 0.8 mg/kg induced feeding in sated gray wolves in a dose-dependent manner ($p<0.001$). Neither 0.8 mg/kg of the benzodiazepine antagonist, β-CCP ($p=0.36$), nor 0.8 mg/kg of the benzodiazepine inverse agonist, β-CCE ($p=0.85$), decreased the diazepam-induced hyperphagia. Five of 6 naive wolves ($p=0.003$) ate dry dog food within 15.4 ± 1.9 min of being given 0.4 mg/kg diazepam and freely chose dog food after the single diazepam administration.

Feeding behavior Gray wolf Benzodiazepines Diazepam β-Carbolines Eating

IT has been well documented that administration of benzodiazepines can induce hyperphagia in laboratory animals as well as carnivores such as cats (2,5). Such increased food consumption could be due to an enhancement of appetite similar to that produced by food deprivation (2) or to the attenuation of hyponeophagia induced by novel foods or environment (9).

Although the search for the biochemical control of feeding is important for human health, animal managers have a pragmatic need for inducing food consumption in captive wild animals. It is not uncommon for wild animals, particularly predators, to become aphagic when captured and placed into captivity. This aphagia could be due to a hiding instinct overwhelming a drive to eat, or it could be due to the animal not recognizing a novel, human-processed food as something edible. Despite many publications addressing the biochemical basis of feeding behavior, we have not found a systematic study on the pharmacological inducement of eating in nondomestic carnivores.

We have managed gray wolves (*Canis lupus*) in captivity for 15 years. Several of these animals have been removed from the wild because of livestock depredation. Some of the wild-caught wolves have adapted rapidly to captivity and different foods; others have refused to eat anything except natural prey. Herein, we present experiments designed to test the hyperphagic abilities of diazepam in wild and captive gray wolves.

## METHOD

### *Subjects*

These studies took place in east central Minnesota (Wildlife Science Center, Forest Lake, MN). A total of 12 (7 male, 5 female) adult (≥3 year) wolves were used, with some animals being used for more than one experiment. The wolves were housed either in groups of two in outdoor kennels with cement runs, or in 0.14-ha enclosures. Wolves were routinely fed commercial dry dog food (Purina, St. Louis, MO) ad lib supplemented with vehicle-killed white-tailed deer (*Odocoileus virginianus*) and provided water ad lib. All wolves were vaccinated annually for rabies, canine distemper, canine parvovirus, infectious canine hepatitis and leptospirosis. All were regularly treated for ecto- and endoparasites and maintained on heartworm (*Dirofilaria immitis*) prophylaxis (6). One week prior to testing, wolves were immobilized, brought indoors, weighed and physically examined, and blood samples were taken for hematologic evaluation.

### *Procedure*

The general experimental protocol was as follows. Experiments were conducted 14 days apart between 0830–1000 from October through January in 1989–90 and 1990–91. All food was removed from the kennels at 0730 on the day of testing. Wolves were separated within their kennels during testing, but could see and smell their mates. Drugs were given intramuscularly (IM) in the proximal hip muscles either by pole syringe or hand injection. Ten min after drug administration, wolves were presented with 1.0 kg of their normal dry dog food. Humans then observed the wolves from a blind for 60 min, after which the food was removed and weighed.

Data were recorded as gm food eaten per kg body weight (b.wt.). Statistical analyses were by one-way ANOVA and Fisher's PLSD or Contingency Table Analysis at a significance of

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$p<0.05$. Means are reported with standard errors (SE).

To test if benzodiazepines induced feeding in sated wolves, 11 wolves (6 male, 5 female) were given either 0.2, 0.4, or 0.8 mg/kg diazepam (Valium®, Hoffmann-La Roche Inc., Nutley, NJ) or 5.0 ml propylene glycol as the control vehicle and presented with food for 60 min. Each animal was randomly assigned an initial treatment, then received all other treatments 14 days apart in a crossover design.

To assess the role of benzodiazepine receptors as mediators of diazepam-induced feeding, 8 wolves (5 male, 3 female) were given either 1) 0.8 mg/kg diazepam only; 2) 0.8 mg/kg diazepam plus 0.8 mg/kg of the benzodiazepine antagonist, propyl-β-carboline-3-carboxylate (β-CCP; Research Biochemicals Inc., Natick, MA); 3) 0.8 mg/kg diazepam plus 0.8 mg/kg of the benzodiazepine inverse agonist, ethyl-β-carboline-3-carboxylate (β-CCE; Research Biochemicals Inc., Natick, MA); 4) 0.8 mg/kg β-CCP only; or 5) 3.0 ml dimethyl sulfoxide only (DMSO). Both β-CCP and β-CCE were prepared by reconstituting in warmed DMSO to a concentration of 100.0 mg/ml. Diazepam and β-CCP or β-CCE were administered at separate sites within a 1-min time period. Each animal was randomly assigned an initial treatment, then received all other treatments 14 days apart in a crossover design.

Three wild-caught, adult wolves (2 male, 1 female) and 3 captive-raised yearlings (2 male, 1 female) who had never eaten dry dog food were transferred to our facilities in July–September. All were initially fed deer meat for 7 days, after which the deer meat was removed, and the wolves given dry dog food for 3 days. At the end of the 3 days, if the wolves had not eaten the dog food, they were again given deer meat for 7 days to reestablish normal nutrition. At the end of this period, the wolves were presented with dry dog food for 1 h. If a wolf had not eaten the food after 1 h, it was given 0.4 mg/kg diazepam IM and observed for 60 min. If the wolf did not eat after diazepam administration, the previous process was repeated.

## RESULTS

Diazepam induced feeding in sated wolves in a dose-dependent manner (Fig. 1). All treatments were different from each other ($p<0.05$) with the exception of control values versus the 0.2-mg/kg diazepam dose. Wolves appeared moderately sedated only at the 0.8-mg/kg diazepam dose. Regardless of dose, however, all wolves responded in their normal fashion to human presence by pacing rapidly while maintaining eye contact with the person. Wolves given the 0.8-mg/kg diazepam dose would often feed immediately upon presentation of the dog food despite appearing anxious about the presence of the human still in the kennel. On these occasions, the animal would eat ravenously from the dish, but it would maintain a tense body posture and would continually follow the human with its eyes. This behavior was deemed highly unusual, since, in our experience of thousands of similar feeding situations, we never observed an undrugged wolf eating in our immediate presence.

No wolves given either β-CCP or DMSO alone ate dog food during the 60-min test period. There was no difference ($p=0.36$) in the amount of food eaten between wolves given 0.8 mg/kg diazepam ($6.5\pm3.2$ g/kg b.wt.) and wolves given diazepam plus β-CCP ($3.1\pm1.6$ g/kg b.wt.). There was also no difference ($p=0.85$) in the amount of food eaten between wolves given 0.8 mg/kg diazepam ($7.9\pm4.2$ g/kg b.wt.) and wolves given diazepam plus β-CCE ($6.7\pm3.7$ g/kg b.wt.).

All of the new wolves brought to our facilities ate deer meat within 24 h of arrival. None of the wolves ate any dry dog food during the initial 3-day exposure to this novel food. All resumed eating deer meat when it was again presented. No wolf ate any dry dog food after it was presented a second time for 60 min. Five of six wolves then ate the dry dog food within $15.4\pm1.9$ min after diazepam administration ($p=0.003$). The amount of food eaten was not measured. The five wolves who ate after this initial exposure to dog food continued to eat dry dog food on a daily basis thereafter. The one male wolf who did not eat after diazepam administration never ate the dog food despite repeated trials.

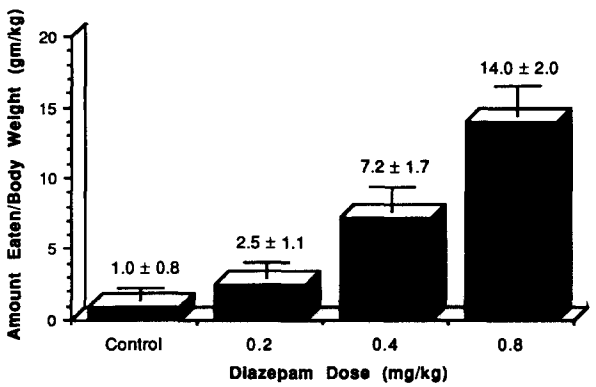


FIG. 1. Dose-dependent hyperphagia induced by diazepam in 11 captive gray wolves (6 male, 5 female) presented with 1.0 kg dry dog food for 60 min. Control animals were given 5.0 ml propylene glycol only. All wolves received all treatments. Means are shown with standard errors. All treatments significantly different from each other ($p<0.05$) except for Control versus 0.2-mg/kg Dose values.

## DISCUSSION

Diazepam is a benzodiazepine agonist having a moderate hyperphagic potency (3). Diazepam induced a dose-dependent food intake in sated wolves, which was consistent with other species (2,4). The mechanism for this hyperphagia is unknown but thought to be due to either appetitive actions or anxiolytic properties of benzodiazepine tranquilizers (10).

Benzodiazepines act by facilitating the inhibitory effects of gamma-aminobutyric acid (GABA) in the central nervous system. The benzodiazepines enhance the ability of GABA to increase chloride ion channel permeability rather than having their own direct action on the benzodiazepine receptor (8). β-CCE is a benzodiazepine receptor ligand extracted from human urine (1). Derivatives of β-CCE span the spectrum from full agonists to full inverse agonists. β-CCP is a high-affinity ligand for the benzodiazepine receptor lacking intrinsic activity in behavioral tests, but which potently and completely antagonizes the effects of benzodiazepine agonists (8).

β-CCE and its derivatives have been shown to eliminate or reduce diazepam-induced feeding in laboratory animals (3,7). We are unable to explain the lack of a similar effect in our experiments. The doses and test duration that we used were similar to other reports demonstrating an effect (7). Since we did not know how rapidly β-carbolines were metabolized in wolves, we had conducted pilot trials where we would administer the β-carbolines 10 min prior or 10 min after the diazepam administration as well as reduce the food exposure time to 30 min. No effects differing from the data reported herein were observed in these iterations. We did feel that the β-carbolines were having some effects, however, based on observations of wolves receiving both diazepam and either β-CCP or β-CCE. Wolves receiving 0.8 mg/kg diazepam alone always demonstrated some degree

of sedation, whereas wolves receiving diazepam plus a β-carboline subjectively appeared normal. We are left to conclude that, within the constraints of this experimental design, neither β-CCP nor βCCE reduced diazepam-induced hyperphagia in wolves.

The results of the last portion of these studies demonstrate a useful management tool for animal managers faced with the problem of converting wild animals to unnatural food. Not only were we able to "force" 5/6 wolves to eat a novel food, but also to convert them to this food base after only a single trial. A subjective interpretation of this experiment was that naive wolves did not recognize dry dog food as a food source, despite being deprived of deer meat for 3 days. Diazepam then induced consumption of this novel food, after which the wolves "recognized" dry dog food as being palatable and would eat it readily.

The failure of the one wolf to respond to diazepam is inexplicable. This wolf was different than the other five in that it came from a zoo and was fed commercial meat by-products as opposed to deer meat. Why this would affect diazepam-induced feeding is unknown. A genetic difference was also unlikely because this wolf was the offspring of a pair of wolves that did eat after diazepam administration. Nonetheless, we are encouraged by the results of this experiment and suggest that hyponeophobia in captive wild animals may be overcome through diazepam administration.

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## REFERENCES

1. Braestrup, C. M.; Nielsen, M.; Olson, C. E. Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. Proc. Natl. Acad. Sci. USA 77:2288–2292; 1980.
2. Cooper, S. J. Benzodiazepines as appetite-enhancing compounds. Appetite 1:7–19; 1980.
3. Cooper, S. J. β-Carbolines characterized as benzodiazepine receptor agonists and inverse agonists produce bi-directional changes in palatable food consumption. Brain Res. Bull. 17:627–637; 1986.
4. Foltin, R. W.; Ellis, S.; Schuster, C. R. Specific antagonism by RO 15-1788 of benzodiazepine-induced increases in food intake in rhesus monkeys. Pharmacol. Biochem. Behav. 23:249–252; 1985.
5. Fratta, W.; Mereu, G.; Chessa, P.; Paglietti, E.; Gessa, G. Benzodiazepine-induced voraciousness in cats and inhibition of amphetamine-anorexia. Life Sci. 18:1157–1166; 1976.
6. Kreeger, T. J.; Seal, U. S.; Callahan, M.; Beckel, M. Treatment and prevention with ivermectin of dirofilariasis and ancylostomiasis in captive gray wolves (*Canis lupus*). J. Zoo Wildl. Med. 21:310–317; 1990.
7. Mansbach, R. S.; Stanley, J. A.; Barrett, J. E. Ro 15-1788 and β-CCE selectively eliminate diazepam-induced feeding in the rabbit. Pharmacol. Biochem. Behav. 20:763–766; 1984.
8. Nutt, D. J. Benzodiazepine receptor ligands. Neurotransmissions 2:1–5; 1988.
9. Shephard, R. A.; Estall, L. B. Anxiolytic actions of chlordiazepoxide determine its effects on hyponeophagia in rats. Psychopharmacology (Berlin) 82:343–347; 1984.