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BRIEF COMMUNICATION

Stereospecific Reduction by Narcotic Antagonists of Clonidine-Induced Food Intake

NORMAN L. KATZ,[1] R. FRANCIS SCHLEMMER, JR.* AND DONALD P. WALLER

Department of Pharmacodynamics, College of Pharmacy
University of Illinois at Chicago Health Sciences Center, Chicago, IL 60612
**Illinois State Psychiatric Institute, Department of Research*
1601 West Taylor Street, Chicago, IL 60612

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KATZ, N. L., R. F. SCHLEMMER, JR. AND D. P. WALLER. *Stereospecific reduction by narcotic antagonists of clonidine-induced food intake.* PHARMACOL BIOCHEM BEHAV **22**(4) 649–651, 1985.—The present study examined the effect of opiate antagonists on clonidine-induced feeding in rabbits. The change in food intake induced by clonidine was blocked by naltrexone. The active (−)-isomer of the antagonist 5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan had an effect similar to naltrexone. Similar doses of the (+)-isomer were inactive, except at the highest dose used in the study. The results suggest that opiate antagonists block feeding elicited by a specific noradrenoreceptor agonist and that this inhibition is due to a direct interaction with opiate systems.

Clonidine Naltrexone Stereospecific opiate antagonism Appetite Rabbits Opiate receptors Feeding

IN addition to its use as a centrally-acting antihypertensive agent [1], clonidine exerts a variety of psychopharmacological effects [11]. For example, in several species, clonidine increases feeding behavior, and studies suggest that α_2-noradrenoreceptors mediate increased food intake in the clonidine-treated animals [4, 5, 10].

Opiate antagonists, such as naloxone and naltrexone, have a suppressive effect both on normal feeding behavior and various types of hyperphagic conditions [7]. In the present paper, we demonstrate the suppressive effect of naltrexone on clonidine-induced feeding in rabbits. The effect of the opiate antagonist appears to be stereospecific, since the (−)-isomer of 5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan (Mr2266) had an effect similar to naltrexone, whereas the (+)-isomer (Mr2267) was much less active. In this enantiomeric pair, opiate antagonism resides mainly in the (−)-isomer (Mr2266) [13]. The results suggest opiate antagonists reduce clonidine-induced feeding response by blockade of opiate systems.

METHOD

Male New Zealand rabbits (2.15–3.35 kg) were acclimated, for at least 7 days, in individual metal cages (58×61×42 cm) in an environmentally controlled room maintained on a 12 hr dark-light cycle. Food (Purina rabbit chow) and water were provided ad lib. Clonidine HCl (Boehringer Ingelheim Ltd.) and naltrexone HCl (Endo Laboratories, Inc.) were dissolved in normal saline in concentrations adjusted so as to be administered as single intramuscular injections of 0.1 ml/kg of body weight. Solutions of Mr2266 and Mr2267 were prepared by dissolving the bases with the equivalent amount of 0.1 M HCl and sufficient water to make final concentrations which could be administered as single injections of 0.1 ml/kg of body weight.

Each experiment commenced at 1300 hr, at which time both the rabbits and food trays were weighed. After receiving drug or control treatment, rabbits were returned to their home cages where they were observed for 1 hr. The amount of food consumed was measured at the end of the observation period, spilled food being collected from paper placed beneath the grid floor just before the experiment began. Experiments were conducted 3 times weekly at 48 hr intervals. Rabbits were selected and dosed at random.

In the first study, in order to establish a dose-response effect, 4 sets of paired rabbits received either vehicle or 1 of 4 doses of clonidine HCl ranging from 0.025–0.25 mg (salt)/kg. During the course of the study, each animal received each dose of clonidine HCl.

[1]Requests for reprints should be addressed to N. L. Katz, University of Illinois, Health Sciences Center, College of Pharmacy, Department of Pharmacodynamics, P.O. Box 6998, Chicago, IL 60680.

TABLE 1

EFFECT OF CLONIDINE ON FOOD INTAKE AND ANTAGONISM BY NALTREXONE

A. Dose Effect of Clonidine (Clon)	
Vehicle	1.85 ± 0.97
Clon (0.025)	7.62 ± 1.92*
Clon (0.05)	9.10 ± 1.33*
Clon (0.10)	12.42 ± 1.81*
Clon (0.25)	5.70 ± 1.59
B. Antagonism by Naltrexone (Nal)	
Vehicle + Vehicle	0.375 ± 0.26
Vehicle + Clon (0.075)	13.780 ± 1.94
Nal (1) + Clon (0.075)	2.560 ± 0.16*
Nal (2) + Clon (0.075)	0.210 ± 0.14*

Each value is the mean ± SEM of the total amount of food consumed (g) during a 1 hr observation period. N=8. Statistical difference from vehicle control (A) or vehicle + Clon (0.075) (B) is denoted by: *$p<0.01$. Numbers in parentheses are doses in mg/kg.

In the second study, another 8 rabbits received either a 1 or 2 mg (salt)/kg of naltrexone HCl or vehicle control, 10 min before receiving a 0.075 mg (salt)/kg dose of clonidine HCl.

The third study examined the effect of naltrexone on 6 animals in a food deprivation (FD) consummatory test system. Food was absent for 24 hr before the testing period. On the test day, half of the rabbits received saline, and half received 2 mg (salt)/kg of naltrexone HCl, 10 minutes before being returned to home cages where they again had free access to food. On the second day of the test, the groups were crossed over.

In the fourth study, 3 sets of paired rabbits received 1 or 4 mg (base)/kg of Mr2266, 1 or 4 mg (base)/kg of Mr2267, or vehicle control, 10 min before receiving 0.1 mg/kg of clonidine HCl. Another 3 sets of paired rabbits received either 0.25 or 0.5 mg/kg of Mr2266, 0.25 or 0.5 mg/kg of Mr2267, or vehicle control before receiving 0.1 mg/kg of clonidine HCl.

Data were analyzed using a two-way analysis of variance. The least significance method was used to compare means within the analysis.

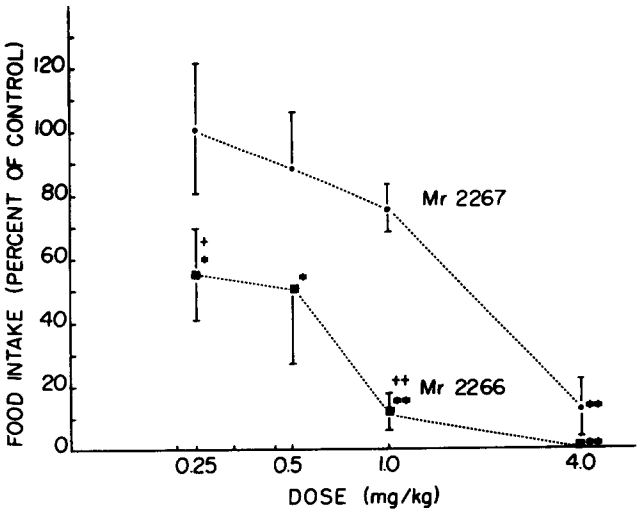

FIG. 1. Dose response comparing the effect of the benzomorphan isomers, Mr2266 and Mr2267, on clonidine-induced food intake in rabbits. Each value represents the mean food intake, expressed as percent of control, ±SEM for 6 rabbits. Mean control food intake in clonidine-treated rabbits receiving 0.25 or 0.5 mg/kg Mr2266/7 was 14.0 g and in rabbits receiving 1 or 4 mg/kg Mr2266/7 was 21.6 g. Statistical difference is denoted by: *$p<0.05$ or **$p<0.01$ compared with clonidine-treated animals in the absence of benzomorphan compounds, †$p<0.05$ or ††$p<0.01$ compared with a similar dose of Mr2267.

RESULTS

Table 1 shows that clonidine, in doses ranging from 0.025 to 0.1 mg/kg, increased feeding behavior over baseline (vehicle) in rabbits during a 1 hr observation period. However, at the highest dose of clonidine tested, 0.25 mg/kg, a return toward baseline feeding was observed.

Pretreatment of rabbits with 1 or 2 mg/kg naltrexone significantly reduced the ability of clonidine to increase food intake (Table 1). Both the number of rabbits eating and the amount eaten by individual rabbits responding to clonidine decreased. The effect of 1 mg/kg naltrexone was not significantly different from that of 2 mg/kg.

Naltrexone significantly reduced increased food intake following FD. The mean 1 hr compensatory food intake in six saline injected rabbits was 18.6±3.77 g compared with 6.7±1.27 g in naltrexone treated animals.

The effect of the benzomorphan compounds Mr2266 and Mr2267 on clonidine induced feeding is shown in Fig. 1. The (−)-isomer Mr2266, an opiate antagonist, produced a dose-related decrease in the quantity of food consumed. Doses of 0.25, 0.5 and 1 mg/kg decreased the mean amount of food eaten to 55%, 50% and 11%, respectively. In contrast, similar doses of Mr2267, the much less active stereoisomer of Mr2266, did not affect the clonidine-induced increase in food intake. Mr2267 significantly decreased food intake only at the highest dose used in the study, 4 mg/kg. Neither ben-

zomorphan nor naltrexone appeared to elicit overt behavioral effects.

DISCUSSION

Intramuscular injection of 0.025–0.1 mg/kg clonidine produced a significant increase in food intake over baseline levels. A higher dose (0.25 mg/kg) diminished the clonidine response, an occurrence which can perhaps be explained by a sedating effect. The present study confirms previous work [12] that the optimal hyperphagic dose of clonidine in rabbits is approximately 0.1 mg/kg.

The present study extends the list of hyperphagic agents or conditions which can be attenuated by opiate antagonists [7]. It also demonstrates that opiate antagonists attenuate feeding specifically induced through a noradrenoreceptor mechanism. Further studies are needed to resolve the question of whether the ability of opiate antagonists to reverse the effect of clonidine results from a dynamic opposition on the organism or a specific pharmacologic antagonism. Also, in view of recent evidence that clonidine may preferentially enhance either carbohydrate [2,3] or protein [6] ingestion in rats, studies should be conducted in rabbits to delineate the effect of clonidine and opiate antagonists on specific nutrient consumption.

The effect of naltrexone was determined on the compensatory food increase resulting from a 24 hr food deprivation, since it would be difficult to show a reduction in ingestive behavior in a 1 hr test of sated rabbits. Naltrexone inhibited deprivation induced feeding, suggesting a direct opiate antagonism effect on a normal physiological response. The result raises the interesting possibility that clonidine exerts its effect through the same mechanism which stimulates feeding in previously food deprived rabbits.

Although antagonism by naloxone, naltrexone and other narcotic antagonists has been used as a criterion to implicate endogenous opiates in physiological or pharmacological processes, the specificity of these agents has been questioned [9]. Actions independent of opiate systems could account for modulation of food seeking behavior [7]. In the present study, reduction of clonidine-induced eating occurred in response to the (−)-isomer of Mr2266, which is known to act as a stereospecific opiate antagonist [8,13]. This reduction occurred over the entire dosage range used in the experiments. In contrast, the (+)-isomer of this compound, which is not a potent antagonist, did not reduce food intake, except at the highest dose used in the study. The results suggest that the reduction of clonidine-induced food intake by opiate antagonists is due to a specific interaction with opiate systems.

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REFERENCES

1. Berthelsen, S. and W. A. Pettinger. A functional basis for classification of α-adrenergic receptors. *Life Sci* **21**: 585–606, 1977.
2. Fahrbach, S. E., J. R. Tretter, P. F. Aravich, J. McCabe and S. F. Liebowitz. Increased carbohydrate preference in the rat after injection of 2-deoxy-D-glucose and clonidine. *Soc Neurosci Abstr* **6**: 784, 1980.
3. Jhanwar-Uniyal, M., J. McCabe, B. E. Levin and S. F. Liebowitz. Clonidine effects on hypothalamic noradrenergic mechanisms: investigations of catecholamine turnover and eating behavior. *Soc Neurosci Abstr* **9**: 467, 1983.
4. Katz, N. L., A. O'Donnell, P. J. Hedrick and D. P. Waller. Antagonism by yohimbine of clonidine-enhanced feeding behavior in rabbits. *Fed Proc* **42**: 1159, 1983.
5. Marino, L. A., M. D. DeBellis and S. F. Leibowitz. α_2-Adrenergic receptors in the paraventricular nucleus mediate feeding induced by norepinephrine and clonidine. *Soc Neurosci Abstr* **9**: 467, 1983.
6. Mauron, C., J. J. Wurtman and R. J. Wurtman. Clonidine increases food and protein consumption in rats. *Life Sci* **27**: 781–791, 1980.
7. Morley, E., A. S. Levine, G. K. Yim and M. T. Lowy. Opioid modulation of appetite. *Neurosci Biobehav Rev* **7**: 281–305, 1983.
8. Sanger, D. J., P. S. McCarthy and G. Metcalf. The effects of opiate antagonists on food intake are stereospecific. *Neuropharmacology* **20**: 45–47, 1981.
9. Sawynok, J., C. Pinsky and F. S. LaBella. On the specificity of naloxone as on opiate antagonist. *Life Sci* **25**: 1621–1632, 1979.
10. Schlemmer, R. F., Jr., J. K. Elder, R. C. Casper and J. M. Davis. Clonidine-induced hyperphagia in monkeys: Evidence for α_2-adrenergic receptor mediation. *Psychopharmacology (Berlin)* **73**: 99–100, 1981.
11. Shearman, G. T. and H. Lal. Psychopharmacology of clonidine. In: *Neuropharmacology: Clinical Applications*, edited by W. B. Essman and L. Valzelli. New York: Spectrum Publications, 1982, pp. 221–255.
12. Waller, D. P., N. L. Katz and A. O'Donnell. Effect of clonidine on the feeding behavior of rabbits. *Pharmacologist* **24**: 163, 1982.
13. Waterfield, A. and H. W. Kosterlitz. Stereospecific increase by narcotic antagonists of evoked acetylcholine output in guinea-pig ileum. *Life Sci* **16**: 1787–1792, 1975.