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The Effects of Chronic Administration of Naltrexone on Appetite and Water Exchange in Rats[1]

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LANG, I. M., J. C. STRAHLENDORF, H. K. STRAHLENDORF, L. O. LUTHERER AND C. D. BARNES. *The effects of chronic administration of naltrexone on appetite and water exchange in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 909–913, 1982.—The effects of chronic administration of naltrexone (200 μg/kg/hr) on appetitive behaviors and renal water and electrolyte excretions were studied in rats. Naltrexone reduced food and water intake, the renal excretions of water and electrolytes, and osmolar clearance. No changes in plasma levels of electrolytes, plasma and urine Na^+-K^+ ratios, hematocrit ratio, plasma osmolality, the clearances of K^+ and Na^-, and the reabsorption of solute free water were found. The changes in appetite were compensated for by appropriate changes in renal excretions, resulting in no change in electrolyte balance or water exchange. These observations are discussed in relation to current theories of the role of endorphins in appetite control.

Naltrexone Feeding Drinking Renal water excretion Renal electrolyte excretion Water exchange

NUMEROUS studies have concluded that acute [2–8, 11, 13–15, 18, 23, 24, 27–29, 33] or chronic [2, 17, 20, 32] administration of opiate antagonists reduces food and water intake in rodents. Other experiments using rats indicated that opiates reduce urine flow [9, 16, 31] and that opiate antagonists caused diuresis [25]. These data suggest, and Margules [26] proposed, that endogenous opiates promote water conservation, but this hypothesis has not been tested directly. We examined, therefore, the effects of chronic administration of the opiate antagonist naltrexone on water exchange, by simultaneously recording appetitive behaviors and renal excretions.

A second objective of these experiments was to investigate the role of renal excretions in the reduction of drinking behavior caused by opiate antagonists. Comparison of the thresholds and magnitudes of the effects of opiate antagonists on appetitive behaviors reveals that drinking is suppressed to a greater extent than feeding [5, 7, 11, 15, 20, 32]. This greater sensitivity of drinking behavior is not explained by current theories [1, 11, 17] of the role of endorphins in appetite control. We examined the possibility that this difference in susceptibility between appetitive behaviors may be, in part, secondary to changes in renal excretions.

METHOD

Animals

Eighteen male Sprague-Dawley rats weighing between 131 and 180 g apiece (156 g average) were housed individually in metal metabolism cages in a temperature-controlled room (23–25°C) maintained on a 12-hr light-dark cycle (lights on at 0700). The animals had free access to tap water and ground stock laboratory diet (Purina Rat Chow). The water was supplied in bottles fitted with ball-point sipping tubes. Fecal pellets were collected on a wire screen, and urine was funneled below to a beaker and collected under light mineral oil to prevent evaporation.

Measurements

Body weight, water intake, food intake, wet fecal weight, urine volume (V), urine osmolality (U_{Osm}), and urine Na^+ (U_{Na}) and K^+ (U_K) concentrations were recorded daily. The fecal pellets were dried overnight in an oven and dry fecal weight determined. The difference between water intake and urine output, Δ water, was calculated, as well as the Na^+ ($U_{Na}V$), K^+ (U_KV), total osmolar excretions ($U_{Osm}V$), and the Na^+-K^+ ratio in urine ($U_{Na/K}$). Weights, volumes, and milliosmoles were coverted to the values per 100 g of body weight to compensate for individual variability. Hematocrit ratio, plasma osmolality (P_{Osm}), plasma Na^+ (P_{Na}) and K^+ (P_K) concentrations, and the Na^+-K^+ ratio in plasma ($P_{Na/K}$) were determined from venous blood. The clearances of Na^- (C_{Na}), K^+ (C_K), and total osmoles (C_{Osm}) were calculated using standard equations. The collected urine was hyperosmotic, and solute-free water reabsorption ($T_M{}^cH_2O$) was calculated

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as the difference between C_{Osm} and urine flow. In some animals, blood-derived parameters were not determined, because of insufficient volume of blood withdrawn. Osmolalities were determined using a Wescor 5100 B vapor pressure osmometer. Sodium and potassium concentrations were determined, using a Beckman KLiNa Flame Photometer.

Surgery and Procedure

The animals were randomly divided into two groups of equal numbers. For the first two days, preimplantation values of all daily recorded parameters were determined. On the third day, the animals were anesthetized with ether and implanted subcutaneously (at the back of the neck) with either naltrexone-filled (80 mg/ml in 0.9% saline) Alzet Model 2002 miniosmotic pumps (naltrexone group) or a similarly sized piece of sterilized Tygon (S-50-HL) tubing (control group). The control group provided sufficient control for the surgical procedure and the tissue response to a foreign object. Considering the very low flow rates from the pumps, 0.56 μl/hr, we did not think it was necessary to provide control for this flow. The pumps released naltrexone at a rate of approximately 200 μg/kg/hr (based on body weight on the day of implantation) for more than 16 days. This dose was chosen because it was twice the lowest dose found to reduce drinking in acute experiments [3–5, 11, 14]. We wanted to ensure both an effect on drinking within five days of implantation and have minimal effects on feeding. The animals were allowed to recover from surgery for two days, after which recordings were resumed for five days. On the morning of the sixth day, blood was drawn from a tail vein and daily recordings terminated.

Data Analysis

All daily recorded measures were evaluated with a two-factor (treatment and time) analysis of variance (ANOVA) with repeated-measures on time. Separate analyses were performed before and after implantation of the miniosmotic pumps. The degrees of freedom for the tests of the repeated-measures factor (time) were reduced using the Greenhouse-Geiser adjustment in order to obtain conservative p values. Simple effects were examined using Scheffé's test. Differences in parameters determined from plasma measurements (Table 1) were tested with Student's t-test. All analyses were calculated using BMDP computer programs (BMDP programs were developed at the UCLA Health Sciences Computer Facility and were sponsered by NIH Special Resources Grant RR-3). A p value of less than 0.05 was considered the minimum level of significance.

RESULTS AND DISCUSSION

Before implantation, no differences in food or water intake, urine volume, renal electrolyte or total osmolar excretion, and body weight were found between control and naltrexone groups (Figs. 1–3). After implantation, differences in both appetitive behaviors and renal excretions were found.

Feeding, $F(1,16)=6.63$, $p<0.02$, and drinking, $F(1,16)=11.33$, $p<0.004$, were reduced in the naltrexone treated animals (Figs. 1 and 2). It is widely accepted that the effects of an opiate antagonist are mediated through opiate receptors. The opiate antagonist studied most frequently as an appetite suppressant is naloxone, but naltrexone was determined to be a more potent appetite suppressant [4,14].

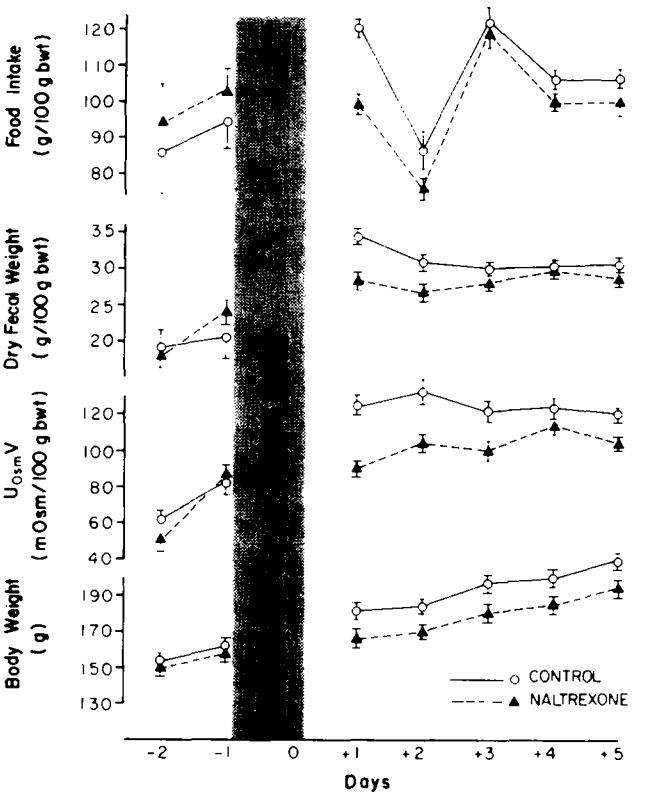

FIG. 1. The daily measures of solute intake and excretion and body weight before and after implantation. Points are means, and vertical bars are standard errors of the mean.

Although opiate antagonists have been shown by others to reduce feeding and drinking, in most of these reports, the drugs were injected less than 30 min before the appetitive behaviors were tested. Because stress can stimulate an endorphin-mediated feeding response [23,28], studies involving stress shortly before an appetite test cannot be used to substantiate endorphins having a role in ad lib feeding or drinking. Our animals were neither injected repeatedly with drugs nor stressed unduly otherwise during the postimplantation period; thus, our results confirmed earlier reports [2, 17, 20] that endogenous opioids participated in the regulation of ad lib daily feeding and drinking.

The ANOVA on food, $F(2.3,37.5)=48.8$, $p<0.001$, and water, $F(3,48)=14.4$, $p<0.001$, intake revealed significant main effects for time. The values obtained during the first (water intake) or second (food intake) day within both treatment groups differed (all $p<0.05$) from the remaining days. These within factor effects were probably residual effects of surgery caused by deprivation and postoperative pain. It is unlikely that the differences observed between groups were caused by the residual effects of surgery, because these differences persisted for seven days after surgery. Furthermore, in a similar study [20], we had found that chronic administration of low doses of naltrexone reduced drinking for up to sixty days after implantation.

As others had, we found that drinking was suppressed to a greater extent than feeding (Figs. 1 and 2). The average

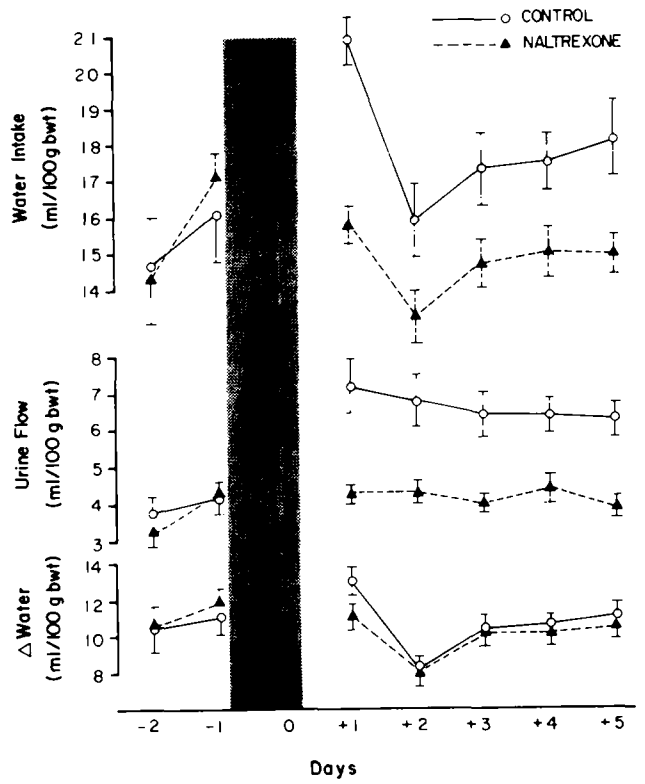

FIG. 2. The daily measures of water exchange before and after implantation. Points are means, and vertical bars are standard errors of the mean.

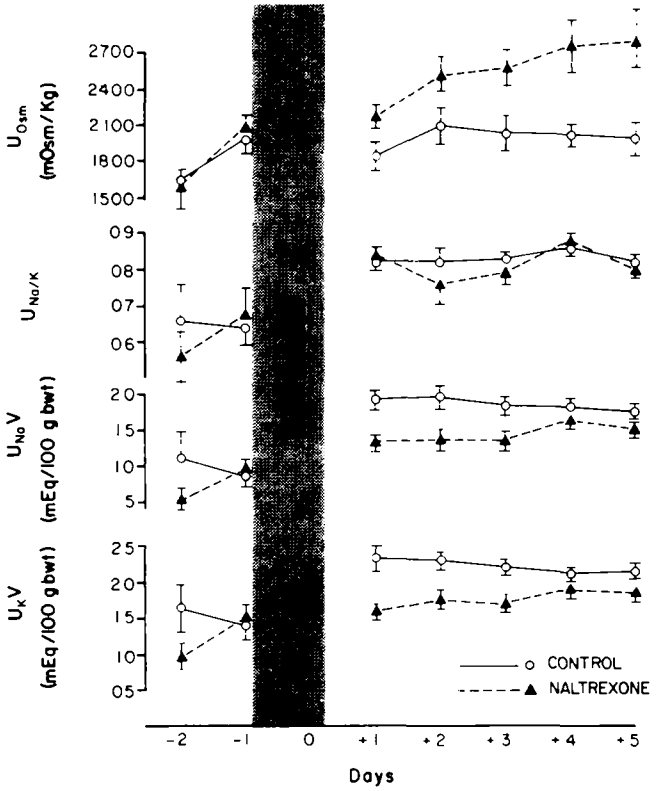

FIG. 3. The daily measures of renal excretions before and after implantation. Points are means, and vertical bars are standard errors of the mean.

(mean±SE) reduction in appetite over the 5-day postimplantation period was 17.5±1.8% for drinking and 8.9±3.0% for feeding. The ingesta of the naltrexone-treated group was therefore more osmotically active, and this was reflected by the increased osmolality, $F(1,16)=7.11$, $p<0.017$, of the urine (Fig. 3).

The renal excretion of solutes was reduced in the naltrexone-treated group. Total osmolar excretion, $F(1,16)=17.43$, $p<0.001$, (Fig. 1), Na^+, $F(1,16)=16.37$, $p<0.001$, and K^+, $F(1,16)=24.3$, $p<0.001$, excretions (Fig. 3), and C_{Osm}, $t(14)=2.52$, $p<0.025$, (Table 1) were decreased. These results probably reflected the decreased solute, i.e., food intake. On the other hand, P_{Na}, P_K, P_{Osm}, $P_{Na/K}$, and $U_{Na/K}$ did not differ between groups (Table 1). These data suggested that the electrolyte balance of the rats was not affected by naltrexone. It is doubtful, then, that changes in electrolyte balance contributed to the observed changes in water intake. Furthermore, this lack of effect on electrolyte balance implied an absence of effect on systems controlling renal electrolyte excretion, e.g., the renin-angiotensin system, a possibility substantiated by experiments using acutely prepared rats. In 1980, Knepel *et al.* [19] found that subcutaneous administration of 1 mg/kg of naloxone had no effect on renin secretion but significantly increased stimulus-evoked ADH secretion.

The low dose of naltrexone used in these experiments did not affect renal water excretion directly. Although the urine flow, $F(1,16)=13.58$, $p<0.002$, of the naltrexone-treated group was significantly reduced (Fig. 2), the magnitude of this reduction was equal to the magnitude of the reduction in water intake, resulting in no change in Δ water (Fig. 2). This indicated that the naltrexone-treated animals excreted volumes of water in the urine equal to the water intake. We found also no difference in $T_M{}^cH_2O$ between groups (Table

TABLE 1

THE EFFECTS OF CHRONIC ADMINISTRATION OF NALTREXONE ON PLASMA CONCENTRATIONS AND RENAL CLEARANCES OF WATER AND ELECTROLYTES

Parameter	Group	
	Control (N)	Naltrexone (N)
P_{Osm} (mOsm / Kg)	324 ± 6 (9)	324 ± 8 (8)
P_{Na} (mEq / l)	150.1 ± 3.2 (8)	147.4 ± 4.3 (7)
P_K (mEq / l)	6.4 ± 0.2 (8)	6.1 ± 0.3 (7)
$P_{Na/K}$	23.8 ± 0.8 (8)	24.5 ± 1.3 (7)
C_{Na} (ml/100 g bwt/24 hr)	12.0 ± 0.3 (8)	10.7 ± 0.8 (7)
C_K (ml/100 g bwt/24 hr)	343.8 ± 13.7 (8)	321.3 ± 24.9 (7)
C_{Osm} (ml/100 g bwt/24 hr)	37.6 ± 1.1 (8)	33.0 ± 1.5 (8)*
$T_m{}^cH_2O$ (ml/100 g bwt/24 hr)	31.0 ± 1.2 (8)	29.0 ± 1.4 (8)
Hematocrit Ratio	50.0 ± 2.0 (6)	49.8 ± 1.8 (5)

Values are measn ± SE. N, number of values per group. *$p<0.05$ for a difference from the control group.

1). The $T_M^cH_2O$ is a measure of renal water reabsorption relative to the C_{osm}. This measure was particularly pertinent to our experiments, because C_{osm} differed after food intake decreased. Thus, renal water excretions in relation to water intake (Δ water) or osmolar clearance (C_{osm}) were not affected by the chronic administration of low doses of naltrexone.

In the rat, renal water excretion may amount to only 30% of the total water losses [10]. We did not record the nonrenal water losses; thus, we cannot infer an effect on water balance from the effects on renal water excretion. The effects of opiate antagonists on nonrenal, largely evaporative [10] water loss are unknown.

Although water balance was not measured directly, we found indirect evidence that naltrexone had no effect: changes in extracellular (or plasma) osmolality and electrolyte concentration that would be expected to occur with alterations in water balance were not found. No differences in P_{osm}, P_{Na}, or P_K were found between groups (Table 1). The sodium concentration in blood has in particular been used as an index of the activity of water in body fluids [21]. Changes in body fluid osmolality indicate changes in fluid balance, except when the fluid lost or gained is isosmotic [12]. With isomotic fluid shifts, changes in hematocrit ratio should occur, but we found no difference between groups (Table 1).

In summary, we found no evidence that chronic administration of a low dose of naltrexone affected water or electrolyte balance although both food and water intake were reduced significantly. Thus, the reduction in water intake caused by opiate antagonists cannot be accounted for by changes in renal water or electrolyte excretions.

Theories proposed to account for the effects of opiate antagonists on appetitive behaviors do not satisfactorily explain physiological findings. The proposal by Belluzzi and Stein [1] and Frenk and Rogers [11] that opiates participate in the modulation of central reward processes does not explain the differential sensitivity of drinking behavior to opiate antagonists. One would expect that drinking and feeding behaviors would be surpressed equally by opiate antagonists only if a common reward process were inhibited. Similarly, the theory of altered emotional tone posited by Jalowiec *et al.* [17] does not account for this appetitive difference: evidence has not been presented that the emotional states of comfort or distress affect one type of appetitive behavior preferentially. Finally, Margules's [26] theory that endorphin and endoloxone mediate an autonomic nervous system response controlling metabolism and behavior in anticipation of famine or feast seems incomplete. This theory predicts that opiate antagonists would cause a loss of body water resulting from reduced water intake and diuresis. Using low doses of naltrexone, we found no primary change in renal water excretion or water balance. Opiate antagonists can cause diuresis [25] and decreased ADH secretion [22,25], but the dose of antagonist used in these studies was over tenfold greater than we or others [3–8, 11, 14, 15, 17, 20, 24, 29] used to suppress water intake. This differential sensitivity to opiate antagonists between drinking behavior and renal water excretion is not fully explained by the feast or famine theory.

We propose that the greater sensitivity of water intake (as compared with food intake or water excretion) to opiate antagonists suggests a unique role for endorphins in the regulatory processes of drinking behavior. Furthermore, these regulatory processes are probably centrally located. We found no evidence for opiate antagonist suppression of drinking as a secondary consequence of altered renal excretions. Similarly Rockwood *et al.* [30] excluded the possibility of effects secondary to alterations in gut water absorption by demonstrating the suppression of drinking in rats with open gastric fistulas. Further study is needed to identify the particular central opioid mechanisms responsible for the high sensitivity of drinking behavior to opiate receptor inhibition.

REFERENCES

1. Belluzzi, J. D. and L. Stein. Enkephalin may mediate euphoria and drive-reduction reward. *Nature* **266**: 556–558, 1977.
2. Brands, B., J. A. Thornhill, M. Hirst and C. W. Gowdey. Suppression of food intake and body weight gain by naloxone in rats. *Life Sci.* **24**: 1773–1778, 1979.
3. Brown, D. R. and S. G. Holtzman. Suppression of drinking by naloxone in the rat: a further characterization. *Eur. J. Pharmac.* **69**: 331–340, 1981.
4. Brown, D. R. and S. G. Holtzman. Evidence that opiate receptors mediate suppression of hypertonic saline-induced drinking in the mouse by narcotic antagonists. *Life Sci.* **26**: 1543–1550, 1980.
5. Brown, D. R. and S. G. Holtzman. Suppression of deprivation-induced food and water intake in rats and mice by naloxone. *Pharmac. Biochem. Behav.* **11**: 567–573, 1979.
6. Brown, D. R., M. S. Blank and S. G. Holtzman. Suppression by naloxone of water intake induced by deprivation and hypertonic saline in intact and hypophysectomized rats. *Life Sci.* **26**: 1535–1542, 1980.
7. Cooper, S. J. Naloxone: effects of food and water consumption in the non-deprived and deprived rat. *Psychopharmacology* **71**: 1–6, 1980.
8. Czech, D. A. and E. A. Stein. Naloxone depresses osmoregulatory drinking in rats. *Pharmac. Biochem. Behav.* **12**: 987–989, 1980.
9. DeBodo, R. C. The antidiuretic action of morphine and its mechanism. *J. Pharmac. exp. Ther.* **82**: 74–85, 1944.
10. Dicker, S. F. and J. Nunn. The role of the antidiuretic hormone during water deprivation in rats. *J. Physiol.* **136**: 235–248, 1957.
11. Frenk, H. and G. H. Rogers. The suppressant effects of naloxone on food and water intake in the rat. *Behav. Neural Biol.* **26**: 23–40, 1979.
12. Gutman, Y. and M. Krausz. Regulation of food and water intake in rats as related to plasma osmolarity and volume. *Physiol. Behav.* **4**: 311–313, 1968.
13. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and d-amphetamine. *J. Pharmac. exp. Ther.* **189**: 51–60, 1974.
14. Holtzman, S. G. Effects of narcotic antagonists on fluid intake in the rat. *Life Sci.* **16**: 1465–1470, 1975.
15. Holtzman, S. G. Suppression of appetitive behavior in the rat by naloxone: lack of effect of prior morphine dependence. *Life Sci.* **24**: 219–226, 1979.
16. Inturrisi, C. E. and J. M. Fujimoto. Studies on the antidiuretic actions of morphine in the rat. *Eur. J. Pharmac.* **2**: 301–307 1968.
17. Jalowiec, J. E., J. Panksepp, A. J. Zolovick, N. Najam and B. H. Herman. Opioid modulation of ingestive behavior. *Pharmac. Biochem. Behav.* **15**: 477–484, 1981.
18. King, B. M., F. X. Castellanos, A. J. Kastin, M. C. Berzas, M. D. Mauk, G. A. Olson and R. D. Olson. Naloxone-induced suppression of food intake in normal and hypothalamic obese rats. *Pharmac. Biochem. Behav.* **11**: 729–732, 1979.
19. Knepel, W., D. Nutto, H. Anhut and G. Hertting. Naloxone promotes stimulus-evoked vasopressin release in vivo. *Eur. J. Pharmac.* **65**: 449–450, 1980.

20. Lang, I. M., J. C. Strahlendorf, H. K. Strahlendorf and C. D. Barnes. Effects of chronic administration of naltrexone on appetitive behaviors of rats. In: *Progress in Clinical and Biological Research, Vol. 68, The Role of Peptides and Amino Acids as Neurotransmitters*, edited by J. B. Lombardini and A. D. Kenny. New York: Alan R. Liss, Inc., 1981, pp. 197–207.
21. Leaf, A. The clinical and physiologic significance of the serum sodium concentration. *New Engl. J. Med.* **267:** 24–30, 1962.
22. Lightman, S. L. and M. L. Forsling. Evidence for endogenous opioid control of vasopressin release in man. *J. clin. Endocr. Metab.* **50:** 569–571, 1980.
23. Lowy, M. T., R. P. Maickel and G. K. W. Yim. Naloxone reduction of stress-induced feeding. *Life Sci.* **26:** 2113–2118, 1980.
24. Maickel, R. P., M. C. Braude and J. E. Zabick. The effects of various narcotic agonists and antagonists on deprivation-induced fluid consumption. *Neuropharmacology* **16:** 863–866, 1977.
25. Miller, M. Inhibition of ADH release in the rat by narcotic antagonists. *Neuroendocrinology* **19:** 241–251, 1975.
26. Margules, D. L. Beta-endorphin and endoloxone: Hormones of the autonomic nervous system for the conservation or expenditure of bodily resources and energy in the anticipation of famine or feast. *Neurosci. behav. Rev.* **3:** 155–162, 1979.
27. Margules, D. L., B. Moisset, M. J. Lewis, H. Shibuya and C. B. Pert. Beta-endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa). *Science* **202:** 988–991, 1978.
28. Morley, J. E. and A. S. Levin. Stress-induced eating is mediated through endogenous opiates. *Science* **209:** 1259–1261, 1980.
29. Ostrowski, N. L., T. L. Foley, M. D. Lind and L. D. Reid. Naloxone reduces fluid intake: effect of water and food deprivation. *Pharmac. Biochem. Behav.* **12:** 431–435, 1979.
30. Rockwood, G. A., S. M. Siviy and L. D. Reid. Naloxone reduces fluid intake in rats with open gastric fistulas. *Pharmac. Biochem. Behav.* **15:** 319–321, 1981.
31. Schneider, H. and E. K. Blackmore. The effect of nalorphine on the antidiuretic action of morphine in rat and man. *Br. J. Pharmac.* **10:** 45–50, 1955.
32. Schulz, R. S., M. Wuster and A. Herz. Interaction of amphetamine and naloxone in feeding behavior in guinea pigs. *Eur. J. Pharmac.* **63:** 313–319, 1980.
33. Siviy, S. M., F. Bermudez-Rattoni, G. A. Rockwood, C. M. Dargie and L. D. Reid. Intracerebral administration of naloxone and drinking in water-deprived rats. *Pharmac. Biochem. Behav.* **15:** 257–262, 1981.