Pharmacology Biochemistry & Behavior, Vol 29, pp 541–545 © Pergamon Press plc, 1988 Printed in the U S A 0091-3057/88 $3 00 + 00

Nocturnal Depletion of Hypothalamic Dynorphin in Anorexic Walker-256 Tumor-Bearing Rats

T. D. STEELE, H. U. BRYANT,
P. V MALVEN* AND G. K W YIM[1]

*Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences and *Department of Animal Sciences, School of Agriculture Purdue University, West Lafayette, IN 47907*

Received 29 September 1986

STEELE, T D , H U BRYANT, P V MALVEN AND G K W YIM *Nocturnal depletion of hypothalamic dynorphin in anorexic Walker-256 tumor-bearing rats* PHARMACOL BIOCHEM BEHAV **29**(3)541–545, 1988 —Rats implanted with the Walker-256 (W-256) tumor exhibit marked anorexia that is most apparent at night In this model, the hypothalamic kappa opioid system was examined for deficits that might contribute to this tumor-induced anorexia In anorexic tumor-bearing rats (TBR), nocturnal levels of ir-DYN-8 were significantly reduced in the hypothalamus, but ir-DYN-17 levels were not Accumulation of ^{3}H-etorphine or ^{3}H-ethylketocyclazocine, a putative ligand for the kappa receptor subtype, was not increased in the hypothalamus of the TBR, as might have been expected if there were less endogenous dynorphin to occupy the opioid receptors in this region *In vitro* binding assays with ^{3}H-ethylketocyclazocine indicated that dynorphin depletion in the TBR was not sufficient to increase the numbers of kappa opioid receptors in the hypothalamus Also, the sensitivity of kappa opioid receptors involved in feeding was not altered in the TBR as indicated by an intact feeding response to ketocyclazocine In summary, the marginal deficits of hypothalamic dynorphin in W-256 tumor-bearing rats that coincide with the phase of tumor-induced anorexia may contribute to the reduction in food intake

Hypothalamic dynorphin Nocturnal depletion Anorexic

THE anorexia frequently associated with many forms of malignancies can be one of the primary debilitating features of the disease [30] This anorexia subsequently contributes to cancer cachexia, a syndrome which as many as two-thirds of cancer patients suffer from prior to death [23] Pharmacological treatments of the anorexic condition may improve the physiological well-being of the patient during the course of the disease

Numerous studies have sought to define inadequacies in the appetite control system which underlie the reduced appetitive drive and food intake observed during tumor growth in a variety of animal models [6, 17, 23, 27] Since the regulation of feeding is controlled by a variety of both central and peripheral components, a broad spectrum of possible defects exists Rats bearing the Walker-256 (W-256) carcinosarcoma have been utilized extensively for the study of potential defects in appetite regulation that may contribute to tumor-induced anorexia Alterations in plasma and brain amino acids [13,14] and central neurotransmitter levels [24,25] have been investigated as potential factors in the anorexia of the W-256 tumor-bearing rat (W-256 TBR) In addition, levels of hormones of the pituitary-adrenal axis are altered in these animals [35]

Much evidence has implicated the endogenous opioids as potential neuromediators in the control of both normal and pathological feeding states [5, 12, 19, 33] The kappa opioid receptor and its endogenous ligand dynorphin-A (DYN) [3] appear to have a profound influence on appetite regulation in rats [18, 20, 21] and in squirrel monkeys [9] The W-256 TBR displays a feeding profile similar to that of animals treated with the opiate antagonist naloxone [33], as well as that of normal rats forced to drink 2% NaCl [2] Spontaneous nocturnal and 2-deoxyglucose-induced hyperphagias are suppressed in response to both treatments [2,33] The NaCl drinking regimen depletes DYN and vasopressin levels of the neurohypophysis [11], but not in the hypothalamus or other brain areas generally associated with feeding [1] In the anorexic W-256 TBR, daytime levels of immunoreactive DYN 1–17 (ir-DYN-17) and DYN 1–8 (ir-DYN-8) are likewise depleted in the pituitary, but not in the hypothalamus [34] However, hypothalamic levels of DYN in rats follow a circadian rhythm, increasing at night when spontaneous food intake of the rat is greatest [26,28], and the anorexia of the W-256 TBR is most apparent [22,24] Thus, the present study was conducted to determine if nocturnal depletion of hypothalamic DYN accompanies the nocturnal anorexia of the W-256 TBR.

Since the initial portion of this study indicated that noc-

[1]Requests for reprints should be addressed to G K W Yim, Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907

turnal levels of ir-DYN were reduced in the hypothalamus of the W-256 TBR, the kappa opioid receptor system was further examined for possible compensatory changes as have been observed following depletion of opioids by monosodium glutamate-induced lesions of the arcuate nucleus [29]. Specifically, hypothalamic uptake of exogenously administered radiolabelled opioid agonists, used to estimate *in vivo* binding, was measured to ascertain if the DYN depletion was severe enough to reduce the degree of opioid receptor occupation by the endogenous ligand *In vitro* binding of ^{3}H-ethylketocyclazocine to hypothalamic membranes was also examined to determine if the depletion of DYN resulted in up-regulation of hypothalamic opioid receptors Finally, the biological responsiveness of the kappa-mediated feeding system was assessed by monitoring the feeding response of the W-256 TBR to ketocyclazocine In view of the difficulty in stimulating food intake when ketocyclazocine is given at night [21], daytime ketocyclazocine-induced feeding was measured

METHOD

General

Male, Sprague-Dawley rats obtained from Harlan Sprague-Dawley Inc. (Madison, WI, Building 205) weighing 100–150 g were housed in individual cages (25×21×20 cm) with a wire mesh floor in a temperature-controlled room (24–26°C) on a 12/12 hr light cycle (light onset at 0800) During a 3–5 day acclimation period, the animals had free access to food (Wayne Lab-Blox) placed on the floor of the cage, and water was available ad lib

Tumor Implantation

The Walker-256 carcinosarcoma, a non-metastasizing tumor, was obtained from the E G and G Mason Research Institute (Worcester, MA) Using sterile techniques, and a 13-gauge trocar, the tumor fragments were implanted into the right hind limb of ether anesthetized host rats Control animals were anesthetized and received sham trocar injections in an identical manner

Feeding Studies

Measurements of nocturnal food intake were begun at least one day prior to tumor implantation. Measurements were made by placing a pre-weighed amount of food on the cage floor immediately prior to the beginning of the dark cycle and subtracting the amount remaining, including spillage collected on paper towels, at light onset Experiments were conducted when nocturnal feeding in W-256 TBR had decreased by about 50% compared to non-TBR, usually on day 8 of tumor growth when tumor volume was approximately 35 mm^3.

Dynorphin Radioimmunoassay

Six W-256 TBR and six sham-implanted animals were sacrificed by decapitation approximately 4 hr into the dark cycle on day 8 after tumor implantation Daytime measurements of hypothalamic ir-DYN-17 and ir-DYN-8 were obtained from two non-TBR Brains were dissected on a chilled, glass plate according to the method of Glowinski and Iversen [7]. After dissection, the tissue was placed in parafilm, frozen on dry ice and stored at −70°C until time of extraction

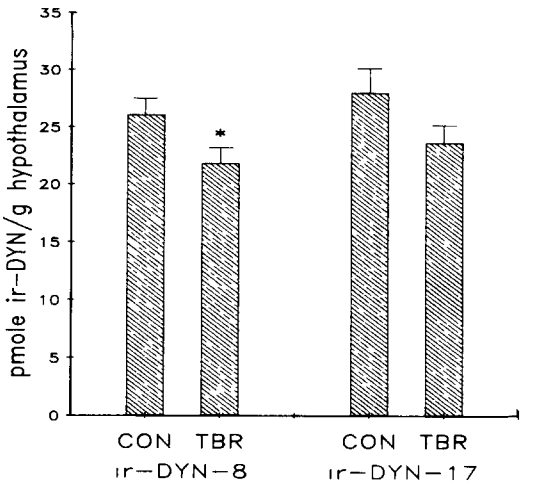

FIG 1 Hypothalamic tissue concentrations of immunoreactive dynorphin-A 1–8 (ir-DYN-8) and 1–17 (ir-DYN-17) for animals sacrificed during the nocturnal phase of the lighting cycle Bars represent means (±S E M) from six controls (CON) and six W-256 tumor-bearing rats (TBR) *$p<0$ 05 for CON vs TBR

For the extraction, the tissue was weighed, transferred to polypropylene tubes, homogenized in acidified methanol (1 1 mixture of absolute methanol and 0 1 N HCl) with a Brinkmann Polytron, and then centrifuged at 1500×g for 15 min at 4°C The resulting supernatant was used for the DYN assays

Assays for ir-DYN-17 and ir-DYN-8 have been previously described and validated [1] Tissue concentrations of ir-DYN-17 and ir-DYN-8 were expressed as picomoles/g wet weight Statistical comparisons were performed with a one-way analysis of variance (ANOVA)

In Vivo *Uptake Assay*

Two groups, consisting of eight W-256 TBR and eight controls, were used to determine the *in vivo* uptake of ^{3}H-etorphine, a non-specific opiate receptor ligand [32], and ^{3}H-ethylketocyclazocine, a putative kappa receptor agonist [16] Studies were conducted approximately 4 hr into the dark cycle on day 8 of tumor growth for both groups Rats were injected IV (tail vein) with 100 μl of either ^{3}H-etorphine (2 μCi, S A =46 Ci/mmol, Amersham) or ^{3}H-ethylketocyclazocine (5 μCi, S A =22 5 Ci/mmol, New England Nuclear) The rats were sacrified by decapitation 20 min later, and samples of hypothalamic and cerebellar tissue were taken Uptake in cerebellar tissue was subtracted from hypothalamic uptake to correct for non-specific uptake [4,10] Tissues were weighed, placed in plastic scintillation vials, and 500 μl of tissue solubilizer (Soluene) was added to each sample The samples were incubated in a shaking water bath at 37°C until decomposition occurred at which time 10 ml of ACS scintillant was added Samples were counted in a Packard Tricarb Model 4530 scintillation counter Corrected hypothalamic uptake for each rat was calculated as tissue dpm/mg tissue wet weight Comparison between W-256 TBR and controls was by Student's *t*-test

In Vitro *Binding Assay*

Animals were sacrificed by decapitation 4 hr into the dark cycle on day 8 of tumor growth, and the hypothalamus was

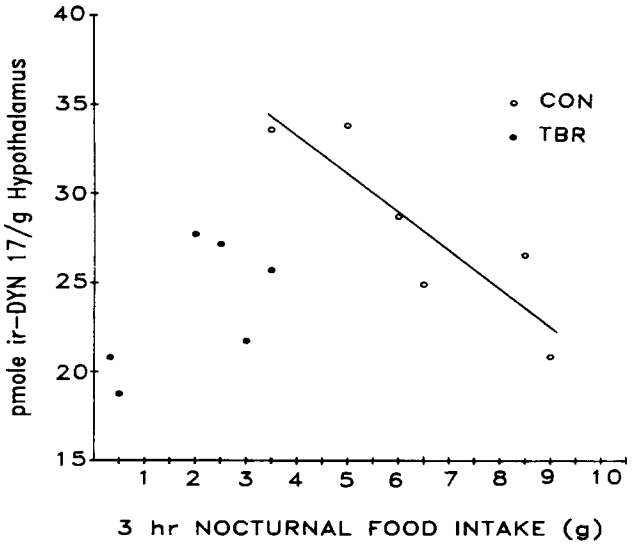

FIG 2 Relation between nocturnal ir-DYN-17 levels and 3 hr food intake in sham-implanted animals (CON, open circles, r=−0 88, $p<0$ 02) and in W-256 tumor-bearing rats (TBR, closed circles, $p>0$ 05)

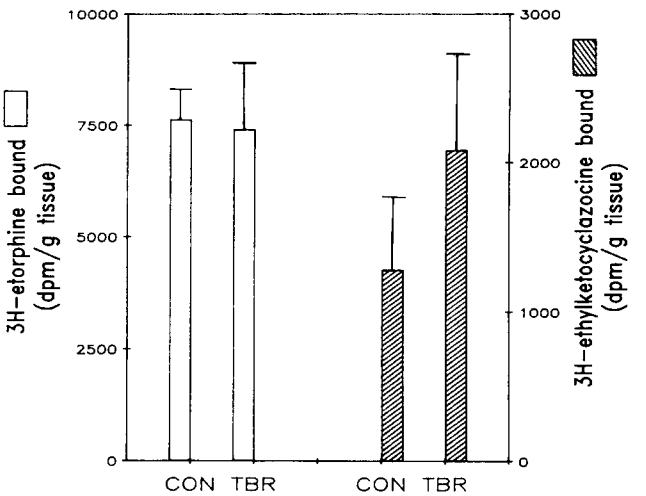

FIG 3 Corrected *in vivo* uptake of tritium in the hypothalamus of sham-implanted (CON) and W-256 tumor-bearing rats (TBR), 20 min after IV ^{3}H-etorphine (open bars) or ^{3}H-ethylketocyclazocine (hatched bars) Bars represent mean±S E of dpm/g tissue for 8 animals

rapidly dissected according to the methods of Glowinski and Iversen [7] Hypothalamic samples from eight W-256 TBR and eight control animals were pooled, weighed, and homogenized in 30 volumes of 50 mM Tris-HCl buffer (pH=7 4) with a Brinkmann Polytron at setting 6 for 20 seconds The homogenate was centrifuged at 49,000×g for 20 min at 4°C in a Beckman L5-65 ultracentrifuge The pellet was resuspended in 30 volumes of buffer and incubated in a 37°C water bath for 30 min to remove any endogenously bound ligand The sample was centrifuged as before and resuspended in 30 volumes of buffer

For binding analysis, a 500 μl aliquot of homogenate was preincubated for 10 min at room temperature with 100 μl of either unlabelled ethylketocyclazocine (supplied by D Leander, Eli Lilly & Co , Indianapolis, IN) or Tris-HCl A 20 min incubation with ^{3}H-ethylketocyclazocine was begun by adding 100 μl of radioligand with final concentrations ranging from 0 1 to 10 nM The incubation was terminated by filtration through Whatman GF-B filters, followed by a wash with 4 ml of Tris buffer Filters were placed in plastic scintillation vials to which 500 μl of Soluene was added and incubated overnight Sample counting was done under the same conditions as for the *in vivo* uptake assay An aliquot of each tissue was assayed for protein content by the method of Lowry [15]

Assay tubes were prepared in triplicate and the bound dpm for each tube was subjected to Scatchard analysis using a microcomputer program Data are reported as picomoles/mg protein

Daytime Ketocyclazocine-Induced Feeding

Ketocyclazocine, a gift of the Sterling-Winthrop Institute, was dissolved in an equimolar amount of HCl Twelve W-256 TBR and twelve non-tumor animals were divided into two groups, one of which received ketocyclazocine (3 mg/kg, SC) and the other received an acidic saline solution (pH=4 9) as a control Two-hour daytime food intake was monitored as described for the nocturnal feeding studies Statistical analysis was performed with a one-way ANOVA and a post-hoc Newman-Keuls test

RESULTS

The levels of ir-DYN-8 and ir-DYN-17 in the hypothalamus of animals sacrificed approximately four hours into the nocturnal phase of the lighting cycle are shown in Fig 1 In W-256 TBR, nocturnal levels of ir-DYN-8 were 15% lower than those of sham-implanted animals ($p<0.05$) Mean nocturnal levels of ir-DYN-17 were also decreased by 15% in W-256 TBR, but were not significantly different from control

The results of the regression analysis shown in Fig 2 reveals that nocturnal hypothalamic ir-DYN-17 levels of control rats were inversely correlated (r=−0 88, $p<0.05$) to nocturnal food intake that was measured during the 3 hours just prior to sacrifice In the W-256 TBR, no such correlation was observed (r=+0 64, $p>0$ 10)

As shown in Fig 3, corrected *in vivo* uptake of ^{3}H-etorphine and ^{3}H-ethylketocyclazocine in the hypothalamus did not differ in W-256 TBR and non-tumor-bearing rats The *in vitro* binding analysis with ^{3}H-ethylketocyclazocine also failed to detect significant differences in the number of kappa receptors present in the hypothalamus of the two groups [B_{max}±S E.M. (pmole/mg protein) W-256 TBR=0 198±0 082, non-TBR=0 136±0 026]

Administration of the kappa agonist ketocyclazocine markedly increased ($p<0$ 05) two-hour daytime food intake in both the sham-implanted animals and the W-256 TBR (Fig 4) The degree of enhancement was similar in the two groups

DISCUSSION

In the present study of the anorexic W-256 TBR, modest reductions in hypothalamic levels of ir-DYN-17 and ir-DYN-8 were observed during the *nocturnal* phase of the light cycle This contrasts with our previous findings that *daytime*

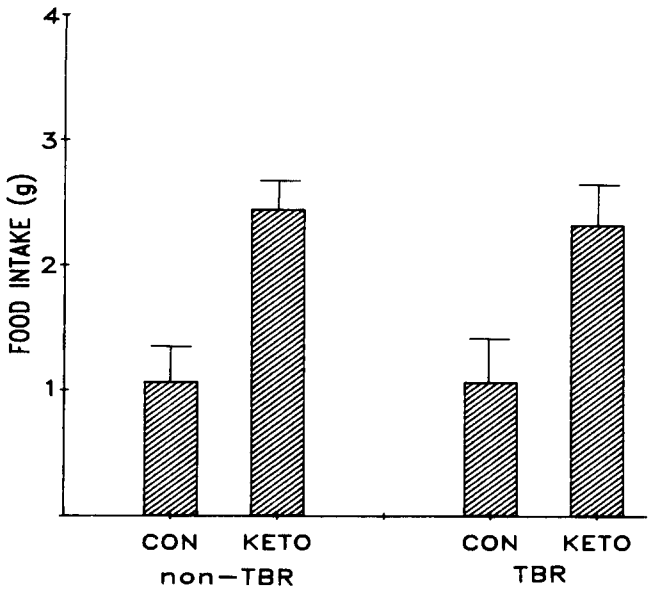

FIG 4 Daytime feeding response to ketocyclazocine (3 mg/kg, SC, KETO) in sham-implanted (non-TBR) and W-256 tumor-bearing rats (TBR) Control animals (CON) were injected with an acidic saline solution Bars represent mean±S E 2 hr food intake for 6 animals

hypothalamic levels of these peptides in W-256 TBR and non-tumor-bearing rats did not differ [34] These divergent findings can be reconciled by the association of elevated nocturnal DYN levels in the hypothalamus with the maximal nocturnal feeding period of rats [26,28] The observed nocturnal depletion concurs with our anticipation in this study that depletion of DYN in the W-256 TBR would be more apparent at night, the time when the W-256 TBR is anorexic [19, 22, 30]

The nocturnal hypothalamic levels of ir-DYN-17 and ir-DYN-8 reported here for non-tumor-bearing animals were higher than our previously reported levels obtained during the daytime [34] Measurements of ir-DYN-8 and ir-DYN-17 in the hypothalamus of two non-TBR sacrificed during the daytime in the present study were similar to these previously reported daytime levels (approximately 16 pmole/mg tissue) Although the nocturnal levels of hypothalamic ir-DYN-17 and ir-DYN-8 levels in the W-256 TBR were also higher than daytime levels, the elevation of DYN in the W-256 TBR is not as marked as in the normal rat Thus, if DYN levels indeed follow a circadian rhythm [27,29], the apparently modest reduction in nocturnal DYN may potentially reflect considerable "blunting" of the circadian elevation, and hence could contribute significantly to the nocturnal anorexia of the W-256 TBR

Determining the relative importance of nocturnal DYN depletion in this tumor-induced anorexia is hindered by the inability to assess turnover rates of this peptide [1] This problem allows for only simple model speculation that a decrease in DYN levels, rather than release, leads to decreased food intake The relationship between ir-DYN-17 levels and 3 hr food intake just prior to sacrifice in the W-256 TBR was in the direction consistent with this expectation (i e , positive), but not significant ($r=+0\ 64$, $p<0\ 17$) Surprisingly, the relation was reversed in non-tumor animals animals that consumed the most food had the lowest ir-DYN-17 levels (Fig 2) Given the well-documented stimulatory effect of kappa agonists on food intake [18, 20, 21], one interpretation of these data is that animals consuming the most food early in the night depleted a larger portion of their nocturnal DYN, thereby lowering their hypothalamic ir-DYN-17 levels toward daytime levels A more detailed time-course analysis of the relationship between DYN levels and food intake in the W-256 TBR is required for a clearer understanding of the role of DYN depletion in this nocturnal anorexia

The results of the subsequent studies did not indicate that the magnitude of the DYN depletion was severe enough to induce significant compensatory changes in the kappa opioid receptor The unchanged uptake of ^{3}H-etorphine, a non-specific, high affinity opioid receptor ligand [32], in the hypothalamus of the W-256 TBR indicates that opiate receptor occupation by endogenous opioids was not reduced Even with the use of a radioligand with a higher selectivity for the kappa receptor, ^{3}H-ethylketocyclazocine, significantly enhanced accumulation of radiolabel could not be demonstrated in the hypothalamus of the W-256 TBR The *in vitro* study of ^{3}H-ethylketocyclazocine binding to hypothalamic kappa receptors likewise failed to provide evidence for receptor up-regulation, since B_{max} was similar in control and the W-256 TBR The intact ketocyclazocine-induced feeding response that was observed in the W-256 TBR in the daytime feeding study indicates that the kappa opioid receptor itself in these animals is not defective

In conclusion, the apparently "blunted" nocturnal elevation of a potent appetite stimulant such as DYN may play a role in the selective reductions in nocturnal feeding in the W-256 TBR In this regard, a coincident depression of nocturnal feeding and depletion of hypothalamic DYN has been previously observed in arcuate nucleus-lesioned rats [28] It is most likely that diminished function of hypothalamic dynorphin systems represents only one of a multitude of factors, including alterations in brain catecholamine [13,25] and serotonergic activity [14,24], that underlie the nocturnal anorexia of the W-256 tumor-bearing rat

ACKNOWLEDGEMENTS

We thank Dr A Goldstein for supplying the anti-DYN-17 antibody used in these experiments, and K M Johnson and S A Haglof for their technical assistance This study was supported in part by the American Cancer Society, grant 194B and the Pardee Foundation Henry Bryant received additional support as an AFPE H A B Dunning Memorial Fellow

REFERENCES

1 Bryant, H U , G K W Yim and P V Malven CNS tissue levels of dynorphin-A immunoreactivity and the anorexia associated with sodium chloride imbibition in the rat *Peptides* **6**: 59–65, 1985

2 Bryant, H U , M T Lowy, P V Malven, T D Steele and G K W Yim Effects of 2% sodium chloride imbibition on various opiate related hyperphagic conditions *Pharmacol Biochem Behav* **23**: 391–395, 1985

3 Chavkin, C , I F James and A Goldstein Dynorphin is a specific endogenous ligand of the kappa-opiate receptor *Science* **215:** 413–414, 1982

4 Dum, J , C Gramsch and A Herz Activation of hypothalamic beta-endorphin pools by reward induced by highly palatable food *Pharmacol Biochem Behav* **18:** 443–447, 1983

5 Ferguson-Segall, M , J J Flynn, J Walker and D L Margules Increased immunoreactive dynorphin and leu-enkephalin in posterior pituitary of obese mice (ob/ob) and supersensitivity to drugs that act at kappa-receptors *Life Sci* **31:** 2233–2236, 1982

6 Garattini, S , A Bizzi, M G Donelli, A Guaitini, R Samanin and F Spreafico Anorexia and cancer in animals and man *Cancer Treat Rev* **7.** 115–140, 1980

7 Glowinski, J and L L Iversen Regional studies of catecholamines in rat brain—I The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-DOPA in various regions of the brain *J Neurochem* **13:** 655–669, 1966

8 Goldstein, A and V E Ghazarossian Immunoreactive dynorphin in pituitary and brain *Proc Natl Acad Sci USA* **77·** 6207–6210, 1980

9 Herman, B H and S G Holtzman Repeated administration of naltrexone and diprenorphine decreases food intake and body weight in squirrel monkeys *Life Sci* **34:** 1–12, 1984

10 Hollt, V and A Herz In vivo receptor occupation by opiates and correlation to the pharmacological effect *Fed Proc* **37:** 158–161, 1978

11 Hollt, V , I Haarman, B R Seizinger and A Herz Levels of dynorphin (1–13) immunoreactivity in rat neurointermediate pituitaries are concomitantly altered with those of leucine-enkephalin and vasopressin in response to various endocrine manipulations *Neuroendocrinology* **33:** 333–339, 1981

12 Jalowiec, J E , J Panksepp, N N Zolovick and B H Herman Opioid modulation of ingestive behavior *Pharmacol Biochem Behav* **15:** 477–484, 1981

13 Krause, R , J H James, C Humphrey and J E Fischer Plasma and brain amino acids in Walker 256 carcinosarcoma-bearing rats *Cancer Res* **39.** 3065–3069, 1979

14 Krause, R , J H James, V Ziparo and J E Fischer Brain tryptophan and the neoplastic anorexia-cachexia syndrome *Cancer* **44:** 1003–1008, 1979

15 Lowry, O H , N J Rosebrough, A L Farr and R J Randall Protein measurement with the Folin phenol reagent *J Biol Chem* **193:** 265–275, 1951

16 Martin, W R , C G Eades, J A Thompson, R E Huppler and P E Gilbert The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog *J Pharmacol Exp Ther* **197:** 517–532, 1976

17 Mordes, J P , C Longcope, J P Flatt, D B MacLean and A A Rossini The rat LTW(m) Leydig cell tumor Cancer anorexia due to estrogen *Endocrinology* **115:** 167–173, 1984

18 Morley, J E and A S Levine Dynorphin-(1–13) induces feeding in rats *Life Sci* **29:** 1901–1903, 1981

19 Morley, J E , A S Levine, G K W Yim and M T Lowy Opioid modulation of appetite *Neurosci Biobehav Rev* **7:** 281–305, 1983

20 Morley, J E , A S Levine, M Grace and J Kneip Dynorphin-(1–13), dopamine and feeding in rats *Pharmacol Biochem Behav* **16:** 701–705, 1982

21 Morley, J E , A S Levine, M Grace and J Kneip An investigation of the role of kappa opiate receptor agonists in the initiation of feeding *Life Sci* **31:** 2617–2626, 1982

22 Morrison, S D Diurnal distribution of motor activity and feeding during growth of tumors *Cancer Res* **34:** 1632–1635, 1974

23 Morrison, S D Control of food intake in cancer cachexia A challenge and a tool *Physiol Behav* **17:** 705–714, 1976

24 Nichols, M , R P Maickel and G K W Yim Increased central serotonergic activity associated with nocturnal anorexia induced by Walker 256 carcinoma *Life Sci* **32:** 1819–1825, 1983

25 Nichols, M B , R P Maickel and G K W Yim Brain catecholamine alterations accompanying development of anorexia in rats bearing the Walker 256 carcinoma *Life Sci* **36:** 2223–2231

26 Przewlocki, R , W Lason, A M Konecka, C Gramsch, A Herz and L O Reid The opioid peptide dynorphin, circadian rhythms and starvation *Science* **219:** 71–73, 1983

27 Radcliffe, J D The MT-W9A mammary carcinoma A promising new model for studying the anorexia-cachexia syndrome *Nutr Rep Int* **29:** 839–844, 1984

28 Reid, L O , A M Konecka, R Przewlocki, M H Millan, M J Millan and A Herz Endogenous opioids, circadian rhythms, nutrient deprivation, eating and drinking *Life Sci* **31:** 1829–1832, 1982

29 Simantov, R and S Amir Regulation of opiate receptors in mouse brain arcuate nuclear lesion induces receptor upregulation and supersensitivity to opiates *Brain Res* **262:** 168–171, 1983

30 Theologides, A Cancer Cachexia In *Nutrition and Cancer,* edited by M Winick New York Wiley, 1977, pp 75–79

31 Weber, E , C Evans and J D Barchas Predominance of the amino terminal octapeptide fragment of dynorphin in rat brain regions *Nature* **299:** 77–79, 1982

32 Wuster, M , R Shulz and A Herz Specificity of opioids towards the μ, δ and ϵ-opiate receptors *Neurosci Lett* **15:** 193–198, 1979

33 Yim, G K W , M T Lowy, J M Davis, D R Lamb and P V Malven Opiate involvement in glucoprivic feeding In *The Neural Basis of Feeding and Reward,* edited by B G Hoebel and D Novin Brunswick, ME Haer Institute, 1982, pp 485–498

34 Yim, G K W , H U Bryant and P V Malven Assessment of dynorphin-A depletion in the anorexia of Walker-256 tumor bearing rats *Physiol Behav* **35:** 117–120, 1985

35 Zepp, E A and M V Gray Hormones of the pituitary-adrenal axis in rats bearing the Walker-256 carcinoma *Cancer Lett* **18:** 149–155, 1983