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Effects of Sucrose on Opioid Peptide Gene Expression in the Rat Brain

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Abstract. Opioid peptide neurotransmitters stimulate feeding and are involved in mediating the rewarding aspects of feeding, as well as in energy regulation in the brain. The effects of sucrose diets on opioid peptide gene expression were measured in the arcuate nucleus (ARC) and the paraventricular nucleus (PVN) of the rat. Rats were fed a cornstarch-based diet or a low (16.7%), medium (33.4%), or high (50%) sucrose containing diet for 7 days. Analyses of the ARC and PVN demonstrated that sucrose in the diet had no effect on mRNA levels of opioid peptides. The lack of an opioid response in the ARC and PVN suggests that opioids in the ARC and PVN are involved in energy regulation rather than in mediating hedonic aspects of feeding.

Keywords: feeding behaviour, opioids, sucrose diet, paraventricular nucleus, arcuate nucleus

1. Introduction

A mounting body of evidence suggests that opioids are involved in palatability and mediate the rewarding aspects of feeding behaviour, for example, administration of the opioid antagonist naloxone (NLX) reduces intake of saccharin and sucrose solutions more effectively than intake of water or quinine solutions [1] [2]. Under a sham-feeding paradigm, NLX administration also markedly reduced the intake of a 10% sucrose solution in deprived and non-deprived rats [3]. In addition, Levine et al. reported that NLX decreased intake of a sweet chow diet more effectively than a normal chow diet, even when rats were chronically food deprived [4]. Conversely, ingestion of saccharin solutions was stimulated by morphine [5] and selective μ- and δ- receptor agonists [6] [7]. Also, Yirmiya et al. found that opioid receptor deficient mice (CXBK) had a lower saccharin preference than normal control mice [8]. Although evidence from pharmacological and behavioural studies support the contention that opioids mediate palatability in feeding behavior, little evidence exists as to whether the palatability of food directly stimulates the synthesis of endogenous opioid peptides in specific nuclei of the brain.

The present study evaluated mRNA levels of proOpiomelanocortin (POMC), proDynorphin (proDYN), and proEnkephalin (proENK) in the in the ARC and PVN following access to different levels of sucrose in the diet for 7 days. Previously, we found that caloric-over consumption of a highly palatable fat/sucrose diet increased proDYN mRNA levels in the ARC and dynorphin peptide levels in the PVN [9], therefore we also examined whether sucrose stimulated the opiodergic neurons of the ARC-PVN pathway.

2. Materials and Methods

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Male Sprague-Dawley rats (Harlan, Madison, Wisconsin), weighing 225-250 g, were housed individually in stainless steel hanging cages, with the temperature in the vivaria controlled at 22°C, under a 12 h light/12 h dark cycle with lights on at 0700 h. Prior to experimental manipulations, subjects were given ad libitum access to water and standard laboratory diet (Rodent Chow, Teklad, IN).

Table 1. Composition of experimental diets

	Diet (% by weight)			
	CHO diet	16.7% sucrose	33.4% sucrose	50% sucrose
Cornstarch	70	53.33	36.67	20
Sucrose	-	16.67	33.33	50
Casein	20	20	20	20
D,L-Methionine	0.3	0.3	0.3	0.3
Vitamin Mix	1	1	1	1
Mineral Mix	3.5	3.5	3.5	3.5
Choline-Cl	0.2	0.2	0.2	0.2
Cellulose	5	5	5	5
Metabolizable energy (kcal/g)	3.652	3.652	3.652	3.652
CHO	77.77	77.77	77.77	77.77
Protein	22.23	22.23	22.23	22.23
Fat	-	-	-	-

Vitamin and mineral mixtures were American Institute of Nutrition vitamin mixture 76 and mineral mixture 76, respectively. Fiber was Celufil (US Biochemical, Cleveland, OH). Metabolizable energy (ME) was based on Atwater values of 16.8, 37.7 and 16.8 kJ/g for carbohydrate (CHO), fat and protein, respectively.

Forty-two subjects were randomly allotted to treatment by weight at the beginning of the study. Four groups of subjects were employed. Subjects were fed either a cornstarch-based diet, or a diet with 16.7%, 33.4% or 50% sucrose, substituted at the expense of cornstarch, for 7 days. Experimental diets were nutritionally complete (Table 1).

At the time of sacrifice, brains were rapidly excised, chilled in ice-cold saline and sliced using a Stoelting tissue slicer. Brains were sectioned at 0, -2 and -5.5 mm relative to the anterior commissure, corresponding to the brain atlas of Paxinos and Watson [10]. The PVN was removed from the 0 to -2 mm section and the entire ARC was removed from the -2 to -5.5 mm sections. Brain tissue samples were frozen in liquid nitrogen and stored at -70°C until analyzed.

mRNA analysis; Analysis of mRNA levels in the ARC and PVN was performed as previously described [11]. In brief, total RNA was extracted by the guanidine thiocyanate-phenolchloroform method of Chomczynski [12]. Tissue samples were homogenized in guanidine thiocyanate with added ß-mercaptoethanol and rehomogenized in water-saturated phenol. Homogenates were mixed with 10% sarcosyl, 2 M sodium acetate, and chloroform. After centrifugation at 14,000 x g for 15 min, the aqueous phase was precipitated with isopropanol overnight, resuspended in guanidine thiocyanate:ß-mercaptoethanol:sarcosyl buffer for 2-h, and precipitated with isopropanol. The RNA pellet was washed twice with 75% ethanol and stored at -70°C in 100% ethanol until analysis. The RNA pellet was reconstituted in RNA storage buffer, 0.5% sodium dodecyl sulfate (SDS), and sterile water, and the amount of total RNA was determined from absorbency at 260 nm. The ratio of A260/A280 was used to estimate the quality of extracted RNA.

Aliquots of total RNA were dissolved in 7.4% formaldehyde and 6x saline sodium citrate (SSC) (1x SSC = 0.15 M NaCl, 0.0015 M Na citrate), then denatured for 10 min at 68°C. Samples were blotted in duplicates of 2 µg total RNA onto nylon membranes (Zeta Probe, Bio-Rad, Richmond, CA) presoaked in 6x SSC. Control for RNA loading onto the slot-blots was verified using the ultraviolet shadowing technique, in which total unhybridized RNA was imaged. Membranes were prehybridized for 24-h at 42°C in 50% formamide, 5x SSC, 10x Denhardt's solution, 0.1% SDS, and denatured salmon sperm DNA in 50 mM Na phosphate, pH 6.5. The hybridization procedure was performed using radiolabeled cDNA probe for proDYN, proENK and POMC produced in our laboratory from transformed cells generously provided by Dr. James O. Douglass (Vollum Institute for Advanced Biomedical research, Oregon Health Sciences University, Portland, Oregon). The cDNA utilized were 1700-, 1070-, 923-, and 511-base pair nucleotide sequences coding for the genes of rat proDYN, rat proENK, and mouse POMC, respectively. Specificity of the opioid probes was verified

using Northern blot analysis. The hybridization cocktail was 50% formamide, 5x SSC, 2x Denhardt's solution, 0.2% SDS, denatured salmon sperm DNA, and yeast tRNA in 50 mM Na phosphate, pH 6.5, with 15 x 10^6 counts·min^{-1}·ml^{-1} of [^{32}P] deoxycytidine diphosphate random primer-labeled probe. After hybridization for 48 h at 42^{o}C, the nylon membrane was subjected to high- and low-salt washing, then exposed to X-ray film (XAR-2, Eastman Kodak Co., Rochester, NY) at -70^{o}C. All RNA from a single experiment was slotted onto a single filter, hybridized in a single vessel, and autoradiographed in a single cassette to assure comparability of group treatment. Hybridization was quantified in arbitrary optical density units by scanning densitometry (BioRad) and all comparisons were made relative to ad libitum control values.

***Statistics*;** Data were analyzed by one-way analysis of variance with post hoc testing by Fisher's protected least significant difference test. Data reflecting mRNA levels of opioid peptides are expressed as % of control and presented as the mean ± SEM.

3. Results

There were no significant differences in food intake and body weight between treatments (Table 1, P>0.05), and the consumption of sucrose did not alter gene expression for opioid peptides in the ARC and PVN (P>0.05) (Table 2).

Table 2. Effects of various sucrose contents in the diet on food intake and on opioid peptide mRNA levels in the ARC and PVN.

	CHO diet	16.7% sucrose	33.4% sucrose	50% sucrose
Food intake (g/d)	160.1 ± 3.3	158.9 ± 2.3	164.5 ± 2.7	162.3 ± 3.1
Initial BWT (g)	289.1 ± 2.1	279.3 ± 2.6	280.6 ± 1.9	280.9 ± 2.5
Final BWT (g)	311.3 ± 3.1	304.6 ± 2.8	312.3 ± 2.0	310.4 ± 2.9
Weight gain (g)	30.3 ± 2.0	25.3 ± 2.1	31.7 ± 1.5	29.6 ± 2.0
ARC proDYN $	100.0 ± 10.2	100.7 ± 10.8	100.9 ± 10.9	99.0 ± 10.1
ARC proENK	100.0 ± 25.5	99.1 ± 25.7	97.9 ± 24.9	104.3 ± 24.8
ARC POMC	100.0 ± 13.3	97.4 13.0	106.5 ± 19.9	95.7 ± 10.5
PVN proDYN $	100.0 ± 20.3	103.1 ± 16.2	96.2 ± 16.6	98.0 ± 12.6
PVN proENK	100.0 ± 18.3	96.5 ± 11.4	102.6 ± 16.0	95.7 ± 11.1

There was no significant diet effect on food intake, body weight (BWT) or on opioid peptide mRNA levels in the ARC and PVN. $ Opioid peptide mRNA levels in the ARC and PVN were expressed as % of CHO diet control.

4. Discussion

Consumption of sucrose did not affect opioid gene expression in the ARC or the PVN. Previously, we found that a palatable fat/sucrose diet increased proDYN gene expression in the ARC and dynorphin peptide levels in the PVN [8]. In the present study, consumption of sucrose did not significantly affect caloric intake, and did not affect opioids in the ARC-PVN pathway. Therefore, it is possible that opioids in the ARC and PVN may be more responsive to caloric or energy needs than palatability. In negative energy conditions such as food restriction and food deprivation [11], lactation [13], and streptozotocin-induced diabetes [14], POMC gene expression is decreased in the ARC. Endogenous opioids have been suggested to be involved in two aspects of feeding; one is hunger-related behavior and the other is mediation of reward or palatability. The three opioid genes may be involved differently in feeding in different brain areas, and may mediate palatable aspects of feeding, while the POMC related melanocortin system in the ARC to PVN pathway may regulate energy needs.

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6. References

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