Striatal opioid peptide gene expression differentially tracks short-term satiety but does not vary with negative energy balance in a manner opposite to hypothalamic NPY

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Submitted 5 December 2005; accepted in final form 29 June 2006

Will MJ, Vanderheyden WM, Kelley AE. Striatal opioid peptide gene expression differentially tracks short-term satiety but does not vary with negative energy balance in a manner opposite to hypothalamic NPY. Am J Physiol Regul Integr Comp Physiol 292: R217–R226, 2007; First published August 17, 2006; doi:10.1152/ajpregu.00852.2005.—It has long been known that central opioid systems play an important role in certain aspects of appetite and food intake, particularly with regard to the hedonic or rewarding impact of calorically dense food, such as fat and sugar. Ventral striatal enkephalin may be a key component of this system, as infusions of μ-opiate agonists into this region strongly increase feeding, whereas infusions of opiate antagonists decrease food intake. While pharmacological analysis has consistently supported such a role, direct measurement of enkephalin gene expression in relation to differing food motivational conditions has not been examined. In this study, the effects of a restricted laboratory chow diet (resulting in negative energy balance) as well as recent consumption of chow (short-term satiety) on striatal preproenkephalin (PPE) and prodynorphin (PD) mRNA expression were measured in rats, using both Northern blot analysis and in situ hybridization methods. As a comparison, hypothalamic (arcuate nucleus) neuropeptide Y (NPY) was also measured in these conditions. PPE expression was broadly downregulated throughout the striatum in animals that had recently consumed a meal, whereas it was unaffected by negative energy balance. Expression of an additional striatal peptide gene, PD, did not follow this pattern, although diet restriction caused a decrease in accumbens core dynorphin mRNA. Conversely, as expected, arcuate nucleus NPY mRNA expression was markedly upregulated by negative energy balance, but was unchanged by recent food consumption. This double dissociation between striatal and hypothalamic peptide systems suggests a specific role for striatal PPE in relatively short-term food motivational states, but not in long-term metabolic responses to diet restriction.

preproenkephalin; motivation; satiety prodynorphin; rat

Currently there is much concern about the worldwide epidemic of obesity. In the past several decades, there has been a steep rise in the prevalence of overweight and obesity, as well as the medical complications that accompany these conditions, such as type 1 diabetes and cardiovascular illnesses (24). Although there is much debate about the relative contributions of various factors, such as changes in exercise patterns, genetic predisposition, and overeating, it is clear that the widespread availability of calorically dense foodstuffs, such as those high in fat and sugar, play a critical role in the obesity epidemic (7).

Because of this epidemic, in recent years there has been a great deal of attention devoted to further understanding the neural pathways and molecular signaling that govern appetite and ingestive behavior (52, 54, 71). Much research has focused on the integration of hypothalamic neuronal processing with signals from the periphery. For example, it is now well established that neurons in the arcuate nucleus are sensitive to circulating factors, such as leptin and ghrelin, and that through their output to the other hypothalamic regions, such as the lateral hypothalamus and periventricular nucleus, promote or restrain feeding (16). The output of these systems, reaching brain stem, neuroendocrine, and limbic networks coordinates the complex behavioral and autonomic patterns that accompany feeding or satiety.

While hypothalamic and brain stem controllers are critical to many aspects of feeding behavior, it is important to consider how corticostriatal networks contribute to the endpoints of ingestion or restraint of ingestion. Emerging research on cortical and basal ganglia function has led to the development of models of higher order, executive control of voluntary motor behavior, including feeding. For example, processes, such as the computation of motor effort to be allocated to obtaining food, the devaluation of the incentive value of food by sensory-specific satiety, and the hedonic impact of preferred foods are now thought to be mediated by networks linking the amygdala, prefrontal cortex, and ventral striatum (1, 11, 29, 51, 53, 65). Human neuroimaging data show that activation in cortical and basal ganglia regions is a prominent aspect of brain activity patterns following exposure to highly preferred food or stimuli associated with food (15, 36, 37, 59, 65). These examples illustrate that critical aspects of the feeding motivational state are controlled by “higher order” corticolimbic substrates, which ultimately influence feeding-related behavioral sequences via their efferent control of effector sites within the hypothalamus and brain stem.

In this regard, a considerable amount of recent research has focused on the role of opioid peptides and opiate receptors within the striatum. It has long been postulated that central opioids play a major role in food intake, and more specifically in palatability and the hedonic impact of energy-dense foods (12, 13, 34, 38, 70). The ventral and dorsal striatum are rich in the peptides enkephalin and dynorphin, which are localized in separate populations of medium-sized spiny neurons and contain high levels of μ-, δ-, and κ-opiate receptors. Much research suggests that release of enkephalin and stimulation of μ-opiate receptors within the ventral striatum is associated...
Methods

Subjects. Adult male Sprague-Dawley rats (total n = 28, Harlan Sprague Dawley, Indianapolis, IN) weighing 300–400 g were maintained in a temperature- and humidity-controlled animal colony on a 12:12-h light-dark cycle (lights off at 1900). All subjects were naive and were allowed a minimum of a week adaptation followed by 2 days of daily handling before the beginning of the experiment. Subjects had either free access to normal laboratory chow (24% protein, 4% fat; ad libitum group) or were gradually brought to 85% of their original body weight (food-restricted group), as described below. Drinking water was available ad libitum for both groups throughout the experiment. Animals that were given food fed at their usual time, whether food restricted or not. The rats were then killed at 2030, 2.5 h after food was given and lights went out. This was accomplished by isoflurane anesthesia and decapitation. The time point was chosen on the basis of pilot data in our laboratory, indicating a relatively slow response of PPE to stimulus events (in contrast to, for example, immediate early genes, which can respond very quickly to stimulus events). This design, schematically presented in Fig. 1A, allowed the assessment of two factors on gene expression: 1) chronic food restriction (negative energy balance) and whether animals had recently consumed a meal or not (acute motivational condition), and 2) their potential interaction. In experiment 2, this exact same procedure was repeated (n = 8/group), but in this case tissue was prepared for in situ hybridization as described below (see Fig. 1C). All experimental procedures were in accord with protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Probe generation. For Northern blot analysis, DNA templates were generated by PCR (see primer list below) from total brain RNA using standard techniques. They were then labeled with 35P using the Megaprime DNA labeling system (Amersham Biosciences, England). For in situ hybridization, rat-specific 32P-labeled cRNA probes were transcribed from a DNA clone tagged with a T7 primer at the 3’ end (PerkinElmer Life Sciences). Templates for in vitro transcription were generated by PCR from total brain RNA. Complementary DNA for PPE, PD, and NPY used for in situ hybridization was amplified from this library using standard PCR procedures. The following primers were used to generate PCR products for the gene indicated: PPE, forward primer 5’-ACCTTGTCAGACAGACAGGGC-3’, reverse primer 5’-CTTGGTGTCGGATGACTGGC-3’; PD, forward primer 5’-CTTGGATTCTTTCTCTGTGG-3’, reverse primer 5’-CTTGGTGTCCACCT-3’; NPY, forward primer 5’TGGACTGAC- CCTGCGTCTATC, reverse primer 5’-ACAAACGACAAACAGGGG- AAATG-3’. A T7 polymerase recognition sequence (GGCGGAT- GAATTTATACGACTCACTATAGGGAGGCGG) was added to the 5’ end of each reverse primer for use in generating the 35S-labeled antisense probe.

Northern blot analysis. Animals were killed as described above, and brains were rapidly removed and cooled. The brain was then dissected on a cold dissecting dish, and 3-mm punches were taken from the nucleus accumbens (tissue from each hemisphere was pooled, and no distinction was made between core and shell subregions; see Fig. 1B). Tissue was stored at −80°C. RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) using the manufacturer’s suggested protocol. Briefly, tissue punches from brain were homogenized in Trizol reagent using a Tissue Tearor (Biospec Products, Bartlesville, OK) and phase separated using chloroform. Total mRNA was precipitated using isopropanol and stored in 70% ethanol before being reconstituted in diethylpyrocarbonate-treated H2O. The concentration of RNA was determined using the RiboGreen (Molecular Probes, Eugene, OR) method, and RNA quality was assessed using agarose electrophoresis. One microgram of total RNA from each rat was electrophoresed on a 1.2% agarose/formaldehyde/1% MOPS gel. The gels were transferred to GeneScreen Plus (PerkinElmer Life Sciences, Boston, MA) using PosiBlotter (Stratagene, La Jolla, CA). 32P-labeled cDNA probes (2×106 cpm/ml) were hybridized to the membrane in Hybisol I (Intergen, Norcross, GA). Northern blots were washed 2×30 min in 2× saline-sodium citrate (SSC), 0.1% SDS, at 42°C and then at a final stringency of 0.5× SSC, 0.1% SDS, at 60°C for 30 min. Membranes were exposed to a phosphorimagerr screen for 1 day. Screens were scanned on a Typhoon phosphorimager (Molecular Dynamics, Sunnyvale, CA). Quantification was performed using ImageQuant software (Molecular Dynamics).

In situ hybridization. For these rats, upon death the brains were removed and flash frozen in 2-methylbutane and then transferred to dry ice and stored at −80°C. For sectioning, coronal sections (20 μm) were taken from fresh frozen tissue using a Leica CM3050 cryostat. Sections were mounted on slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA) and dried on a slide warmer and then stored at −80°C. Slides were postfixed in 4% paraformaldehyde (pH 7.2 in 0.1 M NaPO4) for 1.5 h at room temperature. Slides were washed 3×5 min in 2× SSC. Slides were then placed in buffer (0.1 M Tris-HCl, 0.05 M Na2EDTA, pH 8–8.2) containing protease K (0.2 mg/ml) for 10 min at 37°C and washed for 2 min in ddH2O to stop the action of the protease K. Slides were then washed in 0.01 M triethanolamine containing 0.25% vol/vol acetic anhydride for 10 min at room temperature under constant stirring. Slides were washed in 2× SSC for 5 more minutes and finally dehydrated using a graded ethanol series. Sections were hybridized overnight at 55°C in a solution containing 10% dextran sulfate, 3× SSC, 0.5 M NaPO4, 0.5% formamidine, 1× Denhart’s, and 200 μg/ml RNA, pH 7.5, 0.05 M DTT and 0.1 ng/μl 35S]-labeled antisense cRNA probe. Following hybridization, sections were washed in 500 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, and digested for 1 h at 37°C in the same solution containing 20 μg pancreatic RNase A. Slides were then washed in 1× SSC, 0.2 M DTT (5 min), 0.5× SSC, 0.2 M DTT (5 min), and 0.1 M SSC, 0.2 M DTT (1 h, 70°C), dehydrated in a graded ethanol series, and dried. For autoradiography, sections were exposed to a phosphorimager screen for 4 days and scanned with a Molecular Dynamics Typhoon phosphorimager.
Optical density data values were generated using ImageQuant software (Molecular Dynamics, Amersham Biosciences). Values were corrected for background activity using regions of the tissue (white matter) that did not hybridize to the probe in question. All sections were subjected to the same thresholding for analysis. Striatal regions examined for PPE and PD expression are shown in Fig. 1.

Statistical analysis. The Northern blot data (optical density readings) were analyzed by two-factor between-subjects ANOVA with diet restriction and food consumption being the two factors. For the in situ experiment, all data were subjected to a three-factor ANOVA with diet restriction and food consumption the between-subjects factors and region the within-subjects factor.

RESULTS

Experiment 1: Northern blot analysis of ventral striatal PPE and hypothalamic NPY. As an initial attempt to measure potential changes in gene expression in targeted regions before conducting a more widespread examination with in situ hybridization, PPE mRNA within the nucleus accumbens and...
In other words, the food-deprived and ad libitum groups, both levels of PPE expression, regardless of deprivation condition. The consumption of food is associated with a down-regulation of accumbens PPE, while this factor does not affect NPY expression, but in different ways. Fig. 2 shows the results from experiment 1 using Northern blot analysis. We found that the two groups of animals that consumed food had relatively lower levels of PPE expression in relation to those that did not eat. Overall, ANOVA indicated a significant effect of deprivation condition \(F(1,8) = 10.08, P < 0.0131\). It can be observed from the figure that PPE was relatively less down-regulated by food consumption in the food-restricted group (although it still tended to decrease). Subanalyses by ANOVA on each region indicated that the only regions that did not show an effect of food consumption on PPE were the medial striatum and dorsolateral striatum, suggesting that the greatest effects were observed in ventromedial and ventrolateral regions of striatum (including nucleus accumbens).

For dynorphin mRNA expression, the pattern was very different. The overall ANOVA indicated no significant effect of deprivation condition \(P < 0.13\), food consumption \(0.81\), or interactions \(P < 0.18\). Survey of Fig. 4 indicates that most striatal regions did not show consistent differences between conditions. However, as the overall ANOVA may have obscured real effects within regions, we conducted subanalyses of individual regions. The only analysis to reveal significance was the accumbens core \(F(1,8) = 10.08, P < 0.0131\). It can be noted from Fig. 4C that food restriction diminished dynorphin expression in this area, regardless of whether the rats had recently eaten or not.

In summary, striatal enkephalin gene expression was up-regulated in rats that were denied food and down-regulated in rats that had consumed food within the past several hours. Enkephalin gene expression was not affected by the chronic deprivation condition. Dynorphin gene expression did not change in any consistent direction, although it was lowered in the accumbens core by food restriction.

**Fig. 2. Northern blot analysis of nucleus accumbens preproenkephalin (PPE) mRNA (A) and arcuate nucleus neuropeptide Y (NPY; B) mRNA in different motivational conditions.** The consumption of food is associated with a down-regulation of accumbens PPE, while this factor does not affect NPY expression, but in different ways. Fig. 2 shows the results from experiment 1 using Northern blot analysis. We found that the two groups of animals that consumed food had relatively lower levels of PPE expression, regardless of deprivation condition. In other words, the food-deprived and ad libitum groups, both of which recently consumed food (the food-restricted animals had consumed all 18 g in the cage; the ad libitum group consumed an average of 14.4 ± 2.3 g/cage before death) had lower levels of accumbens PPE compared with their counterparts that did not have food present [effect of food consumption, \(F(1,8) = 9.13, P < 0.016\)]. Deprivation condition did not alter PPE expression \([F(1,8) = 1.09, P = 0.33]\). NPY expression in the hypothalamus followed a different pattern. Whether food was consumed or not had no effect on NPY mRNA levels, whereas the rats on the restricted diet had markedly higher levels of NPY, compared with nonrestricted rats. The ANOVA indicated an overall effect of deprivation condition \([F(1,8) = 21.96, P < 0.002]\), but no effect of food consumption \((P < 0.44)\). Body weights in grams for the four experimental groups were, on the final evening of the experiments (before feeding): R+, 272 ± 2.6; NR+, 376 ± 4.0; R−, 273.3 ± 4.0; NR−, 388 ± 11.1.

**Experiment 2: In situ hybridization analysis of striatal peptide gene expression and NPY.** The results with in situ hybridization showed a similar pattern, but this experiment allowed a more extensive analysis of striatal subregions in these different motivational conditions. We found that the alteration in PPE expression was clearly apparent throughout widespread regions of the striatum. These results are presented in Fig. 3. Overall, it can be observed that the two groups that consumed food tended to have lower levels of PPE expression in relation to those that did not eat. Overall, ANOVA indicated a significant effect of food consumption \([F(1,12) = 15.09, P < 0.002]\). There was no main effect of deprivation condition \((P < 0.78)\); however, a significant interaction of the two factors was observed \([F(1,12) = 9.36, P < 0.009]\). This interaction was due to effects in two regions, the medial shell and accumbens core (see figure legend for details). In those regions, it can be observed from the figure that PPE was relatively less down-regulated by food consumption in the food-restricted group (although it still tended to decrease). Subanalyses by ANOVA on each region indicated that the only regions that did not show an effect of food consumption on PPE were the medial striatum and dorsolateral striatum, suggesting that the greatest effects were observed in ventromedial and ventrolateral regions of striatum (including nucleus accumbens).

NPY expression in the hypothalamus were quantified. The pattern of results indicated that changes in motivational state in relation to food affected both PPE expression and NPY expression, but in different ways. Fig. 2 shows the results from experiment 1 using Northern blot analysis. We found that the two groups of animals that consumed food had relatively lower levels of PPE expression, regardless of deprivation condition. In other words, the food-deprived and ad libitum groups, both of which recently consumed food (the food-restricted animals had consumed all 18 g in the cage; the ad libitum group consumed an average of 14.4 ± 2.3 g/cage before death) had lower levels of accumbens PPE compared with their counterparts that did not have food present [effect of food consumption, \(F(1,8) = 9.13, P < 0.016\)]. Deprivation condition did not alter PPE expression \([F(1,8) = 1.09, P = 0.33]\). NPY expression in the hypothalamus followed a different pattern. Whether food was consumed or not had no effect on NPY mRNA levels, whereas the rats on the restricted diet had markedly higher levels of NPY, compared with nonrestricted rats. The ANOVA indicated an overall effect of deprivation condition \([F(1,8) = 21.96, P < 0.002]\), but no effect of food consumption \((P < 0.44)\). Body weights in grams for the four experimental groups were, on the final evening of the experiments (before feeding): R+, 272 ± 2.6; NR+, 376 ± 4.0; R−, 273.3 ± 4.0; NR−, 388 ± 11.1.

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In summary, striatal enkephalin gene expression was up-regulated in rats that were denied food and down-regulated in rats that had consumed food within the past several hours. Enkephalin gene expression was not affected by the chronic deprivation condition. Dynorphin gene expression did not change in any consistent direction, although it was lowered in the accumbens core by food restriction.
NPY expression was also measured in the arcuate nucleus using optical density analysis of NYP mRNA under these different conditions (Fig. 5). As observed in the figure, NPY was markedly upregulated by chronic food restriction $[F(1,20) = 16.46, P < 0.001]$, but was unchanged by recent food consumption ($P = 0.29$).

**DISCUSSION**

In this paper, we present novel observations concerning the activity of striatal peptide gene expression in relation to changing food motivational conditions. The experiments reveal, using several approaches that measure gene expression, that PPE mRNA in widespread regions of the striatum tends to be downregulated in animals that have recently consumed a meal, whereas these levels are relatively upregulated in rats that have been denied food during their normal feeding period. This pattern was observed regardless of whether the rats had been kept on a chronic food restriction diet or not. Dynorphin mRNA, which is localized in separate populations of medium spiny neurons from those containing enkephalin (17), did not appear to fluctuate with acute motivational conditions. In marked contrast to striatal PPE expression, arcuate nucleus NPY mRNA expression did not track recent food consumption but rather, as expected from previous findings (see below), was
found to be strongly upregulated by chronic negative energy balance. Taken together, this set of results provides direct evidence that the striatal enkephalin system participates in the more immediate aspects of food consumption and short-term satiety, a role that contrasts with, but perhaps complements, the well-established role of hypothalamic NPY and other peptides in energy balance.

Factors contributing to food-associated differences in PPE expression. It is important to first consider what factors could account for the differences in PPE expression observed here. The main finding is that animals that had recently consumed food had relatively lower levels of PPE mRNA expression throughout most striatal regions, whereas those that had not eaten in the past 2 h, regardless of level of hunger or general energy balance, had comparatively higher levels. Thus, long-term food deprivation, changes in energy balance, and overall body weight clearly cannot account for the PPE patterns. Other possibilities are differences in the amount of food consumed, as the restricted animals ate ~4 g more during the 2.5-h feeding period than the ad libitum-fed rats. However, once again, this cannot account for the PPE differences, as both the R+ and NR+ rats showed similar decreases in PPE levels. Finally, one might consider the motor effects of anticipatory arousal in the animals expecting to feed but where food does not become available, as it is well known that hungry animals are more active than satiated ones and that expectation of a meal is linked to anticipatory motor activity (8, 42). Although this possibility cannot be ruled out, one might expect that the

Fig. 4. A: optical density analysis of prodynorphin (PD) expression in various subregions of the striatum. In all areas measured, except for the accumbens core, there were no significant effects of food consumption or restricted diet. In the accumbens core, there was a significant decrease in PD levels in rats on the restricted diet. *P < 0.05, effect of diet condition. B: pseudocolor images of sections through three levels of striatum from representative rats in each treatment group. Red and yellow represent the most intense labeling. See Fig. 1 for regions analyzed and METHODS for details of image analysis.
Food-deprived rats would be considerably more active motorically, but PPE changes were not observed with regard to this factor. Nevertheless, the surge in PPE transcription and presumed synthesis of enkephalin may be an integral part of food-anticipatory motor responses. In this regard, it is interesting that an increase in Fos expression is found within the nucleus accumbens during food-entrained activity, although opioids were not specifically examined in that study (41).

It is important to consider whether variations in the day-night cycle per se might account for the changing patterns of PPE expression, as several studies have established diurnal fluctuations in enkephalin release (6, 14, 63) and in rewarding and/or feeding effects of opiates can be influenced by diurnal rhythms (5, 19, 27). However, rats were all killed at the same period, and the brains of both nonrestricted and the food-restricted rats (which might tend to be entrained by the daily meals) showed similar patterns of PPE expression. Thus, we conclude that recent consumption of food is responsible for the differences observed in PPE expression. Nevertheless, as ingestive behavior and diurnal or food-entrained rhythms are closely linked, it is likely that such rhythms do normally contribute to the general changes in PPE expression over the course of a day. Indeed, we have found that ventral striatal PPE levels tend to be lower during the daylight hours (subjective night for the rats), and to be somewhat higher in the evening hours, in agreement with what has previously been reported.

It is notable that alterations in PPE expression were observed in most regions of striatum examined (refer to Fig. 3). Most studies examining striatal opioids and feeding have focused on the nucleus accumbens (10, 30, 45, 49); mapping studies indicate that relatively more ventral aspects of striatum are most sensitive to opioids (3, 73). This is not surprising, given that more ventral aspects of striatum have traditionally been implicated in reward-related processes. However, it is clear from the regional distribution of the effects that both ventral and dorsal aspects of striatum show PPE alterations in relation to food motivational state. This widespread effect suggests that motivational state is able to induce dynamic enkephalin fluctuations that in turn exert a broad influence over striatal function, influencing sensory, motor, and cognitive processes. It is of interest to compare this regional pattern with a recent report by Pecina and Berridge (44). They found that only a very restricted region in the nucleus accumbens medial shell was sensitive to opioid-mediated enhancement of taste reactivity, while a more widespread area of medial accumbens (and as we have found, accumbens core and striatum) supported opioid-induced increase in ingestion per se. At first glance, that study may seem discrepant with the widespread nature of the upregulation seen here. However, it may be that a very restricted zone (which is intimately connected with the lateral hypothalamus) specializes in mediating hedonic perception of food, while the entire striatum, via its engagement of the indirect pathway (see below), modulates voluntary food-directed motor behavior.

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It is also important to note that while enkephalin gene expression was not influenced by food restriction, other striatal systems mediating foraging and learning are clearly influenced by deprivation states; for example, the work of Haberny and Carr (21) has demonstrated marked changes in plasticity-related molecular substrates response to food restriction. These systems, involving dopamine, glutamate, and intracellular signaling factors, such as CREB, undoubtedly interact with the neuropeptide systems in the coordination of goal-directed behavior.

**PD expression does not change globally with motivational state.** In contrast to fluctuating PPE levels, striatal PD mRNA levels did not track any aspect of motivation state, short- or long-term (with the exception of the accumbens core, see below). A salient feature of striatal organization is that all medium-sized spiny neurons, comprising the vast majority of cell types within the striatum and all of which contain GABA as their main neurotransmitter, can be divided into two main

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**Fig. 5.**

A: optical density analysis of neuropeptide Y (NPY) expression in different motivational conditions with the arcuate nucleus of hypothalamus. ***P < 0.01, effect of food restriction. B: photomicrographs of cross section at level of hypothalamus showing upregulation of NPY mRNA in restricted diet condition.
types with regard to neuropeptide content: those containing the opioid peptide enkephalin, and those containing substance P and dynorphin (18, 62, 69). While information processed by both subtypes eventually reaches major basal ganglia output structures, enkephalin-containing neurons project mainly to pallidal regions (the so-called indirect pathway) and the dynorphin/substance P neurons to the substantia nigra and entopeduncular nucleus (direct pathway). We find here evidence for substantial alterations in PPE, while PD does not appear to consistently fluctuate in relation to recent food consumption. This result suggests diverse motivational functions for neurons containing enkephalin compared with those containing dynorphin (although further work is needed to ascertain a potential role for substance P). Consistent with this hypothesis, stimulation of μ-opiate receptors (mimicking enkephalin release) is invariably rewarding in many behavioral paradigms, whereas stimulation of kappa receptors (the target for dynorphin) or increases in dynorphin levels are consistently associated with aversive motivational states (56, 58, 68). For example, increases in striatal dynorphin are observed in the brains of suicide subjects and in drug withdrawal states, and animal models of stress and depression (25, 39, 43, 57), and place aversion is observed in response to kappa stimulation of the nucleus accumbens (4). Moreover, there is a substantial literature showing differential effects of these two striatal opioid peptides on dopamine function, which may also relate to the proposed motivational differences. Overall, μ agonists activate dopamine release, whereas kappa agonists decrease it (60, 64, 67, 72). It is of interest that a fairly substantial reduction in accumbens core dynorphin was found in the brains of rats on restricted diets, also reported by Haberny and Carr (20). The functional significance of this change requires further study.

NPY expression in arcuate nucleus changes with negative energy balance. The finding that NPY mRNA is markedly upregulated in association with negative energy balance replicates many well-established reports in the literature and supports recent notions of arcuate nucleus integration of peripheral and central signals governing feeding (26, 35, 46, 50, 55). Through the processing of various complex signals, such as leptin and ghrelin, NPY-containing neurons promote food search and feeding via connections to other brain regions, such as lateral hypothalamus and paraventricular nucleus (26, 61). What is most salient with regard to the present study is that NPY gene expression did not change in response to recent consumption of a meal, even in the food-deprived rats. A related study that supports this notion is provided by Kim et al. (31), who showed that consumption of a palatable diet alone did not alter NPY mRNA, whereas restricted access to that diet (60% of ad libitum) increased its expression. Nevertheless, a caveat is in order; diurnal fluctuations of arcuate nucleus NPY clearly contribute to the signal to initiate feeding (2), and as we did not take multiple time points of NPY gene expression following ingestion, it is possible that NPY changes may have occurred before or beyond the time point measured here. At least one study has shown increases in arcuate NPY following consumption of a high-carbohydrate meal (66). Moreover, gene expression changes (or lack of changes) may not necessarily reflect peptide levels or release; in one study, food-restricted animals that had recently consumed a meal did indeed show lowered peptide levels 2 h following consumption.

Potential mechanisms. Given the pattern of striatal peptide gene expression observed in these experiments, it is of interest to speculate as to why such variations are observed and what other neural systems may be controlling these changes. Much evidence suggests that opioids serve to maintain or prolong feeding (32, 33); these authors found that opiate antagonists shorten meal length once food consumption is initiated, although the actual appetitive responses (food-seeking) are not affected. Thus, opioids may act to prolong feeding beyond fulfillment of acute energy needs, to increase energy reserves. In the current experiments, it may be that PPE gene expression is augmented as the animal awakes and prepares to feed, and consequently, increased enkephalin release serves to prolong eating once consumption begins. The food-related increase in synaptic enkephalin causes a compensatory downregulation of PPE activity, associated with short-term satiety. In contrast, in animals that have not fed and that are hungry, PPE gene expression levels (and perhaps vesicular pools of enkephalin) remain comparatively high. Moreover, these changes may also reflect, as noted earlier, dynamic shifts in incentive motivation and anticipatory arousal in relation to whether the animals are food-seeking or not. Further studies with peptide measurements and preferably microdialysis in behaving animals could test these hypotheses.

Where might such a mechanism be initiated? If striatal PPE is elevated when animals are hungry and seeking food, it would follow that brain systems mediating metabolism and arousal must communicate with striatal neurons. The only known direct input to the striatum from the hypothalamus consists of hypocretin/orexin and melanin-concentrating hormone-containing fibers arising from the lateral hypothalamus to the accumbens shell. This pathway may potentially play a role. However, the nature of the effects observed here, involving widespread regions of ventral and dorsal striatum, suggest a more broad influence. Based on observations that striatal cholinergic neurons may play a key role in food motivation (22, 23, 40, 47, 48), we have recently proposed a model of hypothalamic-thalamic-striatal interactions in which hypothalamic signals modulate striatal enkephalin gene expression via cholinergic interneurons of the striatum (29). While space does not allow a detailed description of that model here, we have hypothesized that information flow through a hypothalamic-midline thalamic-striatal axis impinges on the large aspiny cholinergic interneurons of the striatum, which via their large, overlapping axonal fields, act as a network to modulate enkephalin-containing striatal output neurons. This system may have evolved to coordinate feeding and arousal, and, via engagement of the striatal μ-opioid system, to prolong the feeding central motivational state beyond the fulfillment of acute energy needs. While much further experimentation is needed to test these hypotheses, the present data suggest shifting patterns of striatal enkephalin gene activity, depending on whether an animal is food-seeking or food-sated.

ACKNOWLEDGMENTS

We thank Charles Landry for consultation on this project.

GRANTS

This study was supported by National Institute on Drug Abuse Grant DA-09311 and the Food Research Institute of the University of Wisconsin.
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