Neuropeptide Y Receptor-Expressing Neurons Play a Critical Role in Feeding Behavior and Metabolic Function

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INTRODUCTION

In 2002, an estimated 6.3% of the U.S. population (about 18.2 million persons) had diabetes (Centers 2003). Before we can understand treatment and prevention of obesity, metabolic syndrome and type 2 diabetes in humans we must understand the neuronal circuitry and biochemical mechanisms that regulate these processes. The arcuate nucleus of the hypothalamus (Arc) plays an important role in the regulation of appetite and metabolism. The orexigenic peptide, neuropeptide Y (NPY), is an integral part of the neural circuitry in the Arc. Previous work in our laboratory has shown that Arc microinjections of NPY-saporin (NPY-sap), a targeted neurotoxin that selectively destroys NPY receptor-expressing neurons, significantly increased food intake and led to rapid-onset obesity (Bugarith 2005). The goal of the present experiment is to further understand the underlying mechanisms associated with this phenomenon.

METHODS

Rats weighing approximately 340 g were housed individually in suspended wire mesh cages and maintained under a 12-h light/12-h dark cycle. The goal of our first experiment was to determine whether the rapid onset obesity after ARC injections of NPY-sap is determined entirely by increased food intake or whether the weight gain is due in part to changes in metabolism. Three groups of rats were prepared. A control group was microinjected with the control substance, blank-saporin (B-sap) into the ARC and the other two groups were microinjected at the same site with NPY-sap. The B-sap and one NPY-sap group were given ad libitum food access, while the food intake of the second NPY-sap group was limited to the amount eaten by the B-sap controls. Body weights and feeding behavior was measured twice weekly. At the conclusion of the experiment, rats were euthanized and immediately scanned with a Dual Energy X-ray Absortiometry (DEXA) to measure body the proportion of fat in the body tissues. In the second experiment, we examined the diurnal distribution of feeding behavior in rats injected into the Arc with NPY-sap or B-sap. Rats were given free (ad libitum) access to food at all times and intake was measured during the 12 hr light and 12 hr dark phases of the circadian light cycle. At the conclusion of each experiment, we examined the hypothalamus using immunohistochemistry to characterize the lesion produced by the NPY-sap microinjections. Tissues were stained to reveal NPY Y1 receptors.

REFERENCES

Bugarith, K., Li, A., Dinh, T., Speth, R., & Ritter, S. (2005). Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. Endocrinology, 206(3), 1179-91.

Centers for Disease Control and Prevention. National Diabetes Fact Sheet: General Information and National Estimates on Diabetes in the United States, 2003. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2003. Accessed athttp://www.cdc.gov/diabetes/pubs/factsheet.htm on 30 November 2003.

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1 Increased body weight after NPY-sap injections into the Arc is due to increased food intake.

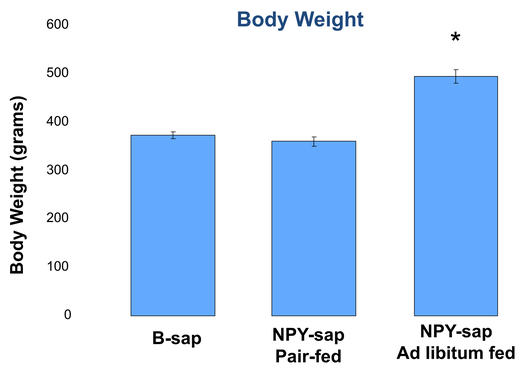

Fig. 1. Effect of pair feeding on body weight gain in rats injected into the Arc with NPY-sap (n=3 per group). When food intake of NPY-sap injected rats was restricted to the level of B-sap controls, their body weights remained at the level of the controls. Ad libitum fed NPY-sap injected rats became obese. Means ± SEMs are shown. *p < .05 vs controls.

2. Body fat, but not body weight, was increased in NPY-sap lesioned rats restricted to control levels of food intake.

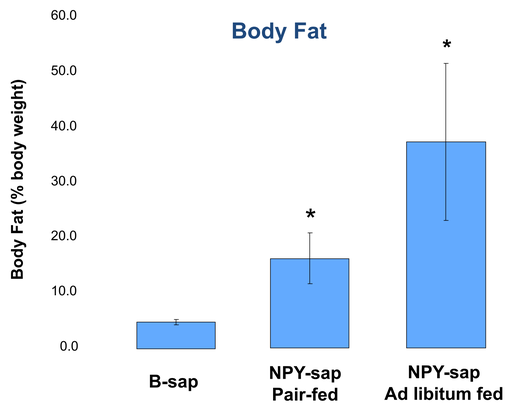

Fig. 2. Body fat as a percent of body weight in rats injected into the Arc with B-sap (control), or with NPY-sap. NPY-sap rats that w ere pair-fed to match the caloric intake of the control had significantly more fat than controls of the same body weight 5 weeks after surgery, but less fat than the NPY-sap rats that had ad libitum access to food. Body fat content was determined by DEXA scans. This suggests that NPY-sap lesions may alter nutrient disposition, in addition to appetite (n=3/grp, means ± SEM are shown, *p≤ 0.05 vs B-sap control)

3. NPY-sap injections into the Arc increased food intake both during the day and the night.

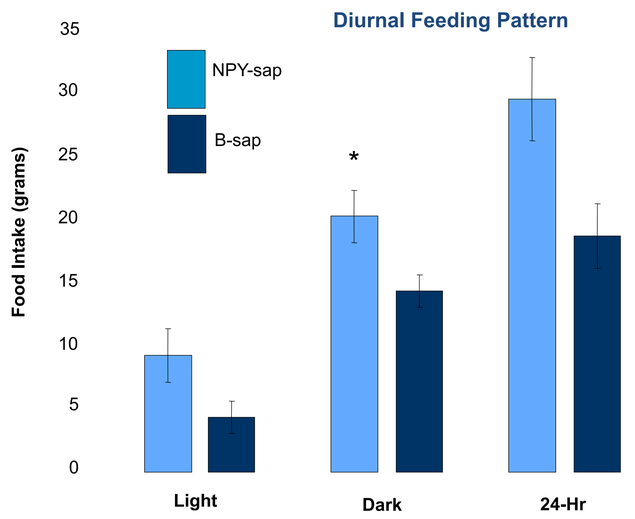

Fig. 3. Daytime, night time and total 24 hr food intake in rats injected into the Arc with B-sap or NPY-sap (n=8 and 6, respectively). Means ± SEM are shown. *p < 0.05, B-sap vs NPY-sap for the same condition.

4. Loss of NPY-Y1 receptor immunoreactivity in the Arc indicates that NPY-sap destroyed neurons with NPY receptors.

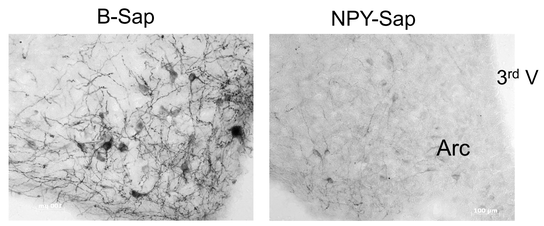

Fig. 4. Anatomically equivalent sections from the arcuate nucleus of the hypothalamus (Arc) from control rats injected into the Arc with B-sap (left) or NPY-sap (right). Sections were stained using an antibody against the NPY receptor subtype, NPY-Y1. Black coloration indicates the presence of Y1 receptors. NPY-Y1 immunoreactivity was greatly reduced after NPY-sap injection, indicating the loss of neurons that express this receptor subtype. 3rd V = third cerebroventricle

SUMMARY

Our data confirmed that rats microinjected with NPY- sap into the Arc show an increase in body weight and feeding compared to B-sap groups.

Our data revealed body adiposity was increased in animals injected with NPY-sap, even when body weight gain was restricted by limiting access to food. We also found that the diurnal pattern of feeding was altered. Lesioned rats increased their food intake both during the day and at night, whereas controls normally eat very little during the day.

We found that the lesion caused a loss of NPY-Y1 receptor immunoreactivity in the Arc, indicating that neurons known to express NPY receptors were destroyed at the injection site.