Chapter 15
Adaptive and Mal-Adaptive Signaling in Cells of the Cardiovascular System: Effect of Obesity-Associated Peptides on Human Blood Platelet Activation

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Abstract Non-communicable, chronic diseases are responsible for ~60 % of all deaths in developing and developed countries. Currently, these diseases account for ~75 % of health care spending in Canada. Although a majority of Canadians express the opinion that health care systems should emphasize prevention strategies, and state supporting funding of prevention programs, the reality is that participation rates in prevention programs are low. Indeed, in North America, 1 in 3 adults are obese. Most disturbingly, 1 in 5 girls and boys between the ages of 6 and 19 is obese and has two or more risk factors for heart disease, including high blood pressure, high cholesterol, diabetes, current smoking and physical inactivity. Research has unequivocally linked obesity, the metabolic syndrome, and other components of "modern life", such as physical inactivity, as factors that increase the burden of chronic disease. The risk of cardiovascular morbidity is significantly augmented by obesity. A number of peptides, including orexins, obestatin, and neuropeptide Y (NPY), play a pivotal role in the regulation of energy expenditure and also affect other systems and cells. Our studies have elucidated some of the mechanisms through which the endothelium and blood platelets integrate these myriad physiopathological stimuli and take advantage of the findings to highlight novel potential therapies to promote adaptive endothelial functions and to reduce the chronic disease-associated mal-adaptive actions of the endothelium.

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15.1 Introduction

Obesity is an unquestionable risk factor for cardiovascular disease (CVD). While several underlying mechanisms linking obesity to CVD have been described, there is no single unifying concept to fully explain this linkage. Increased adipose mass, especially abdominal adiposity, has been shown to represent a strong predictor of CVD and this factor has been proposed to relate to the impact of obesity-related peptide hormones on cells of the cardiovascular system. Of potential importance, abdominal adipocytes have been reported to synthesize and deliver to the blood stream vasoactive peptide hormones, including leptin and adiponectin, and evidence has accumulated correlating differences in levels of these peptides and CVD. Recently we reported that some of these, leptin [1], adiponectin or ghrelin [2] could influence platelet and vascular endothelial cell functions. Other peptide hormones that control appetite and metabolism are also known, including orexins, obestatin, and neuropeptide Y (NPY) [3]. Herein we report on the potential that these important peptides also have on platelet activity to test for their possible involvement in linking obesity to CVD.

Orexins (A and B), formerly known as hypocretins 1 and 2, are hypothalamic neuropeptides that act through actions on two homologous G protein-coupled receptors (GPCRs); orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R) [4–6]. Orexin A, the more potent of the orexins, is excitatory in the central nervous system, stimulating sympathetic activity, arousal, spontaneous physical activity, thermogenesis and food intake [6, 7]. Encoded by the *ghrelin* gene, the 23 amino acid peptide; obestatin; suppresses appetite by opposing the effects of ghrelin on food intake; a dual system of appetite control that may explain why ghrelin-null mice have normal appetites. Obestatin is primarily secreted by the gastrointestinal mucosa, and acts within the gut as well as at the hypothalamus and the pituitary [8], Initially thought to act by activating GPR39, an orphan GPCRGPR39 [8], further studies will be required to identify this peptide hormone's actual receptor protein [9]. Neuropeptide Y is an orexogenic peptide secreted by the hypothalamus which has both central and peripheral effects. Six NPY receptors, designated Y1 through Y6, are known [10, 11]. Neuropeptide Y is present in platelets and has been associated with increased angiogenesis and formation of atherosclerotic-like lesions [10, 12].

15.2 Methods

15.2.1 Preparation of Platelet Rich Plasma and Aggregation Studies

Platelet aggregations were determined as previously described [1]. Briefly, blood was obtained from healthy volunteers who were drug-free for a minimum of one week. Blood was anti-coagulated with heparin (15 U/ml) and platelet rich plasma

(PRP) was obtained by centrifugation (284 g, 15 min at room temperature). Platelet poor plasma (PPP) was obtained by PRP centrifugation (2,750 g, 5 min at room temperature). Aliquots of PRP (500 µl, 2×10^8 platelets/ml) were incubated in siliconized glass disposable cuvettes with peptide hormones (orexin A, obestatin, NPY (Phoenix Pharmaceuticals, Belmont, CA), or vehicle for 5 min at 37 °C and then aggregated (3 min) by addition of ADP (2 µM) in a dual chamber optical aggregometer (490-2D, CHRONO-LOG Corporation, Haverton, PA).

15.2.2 Statistical Analysis

Data are presented as means ± S.E.M. from a minimum of three independent experiments within which individual platelet samples were tested in triplicate or quadruplicate. Statistical differences between conditions were determined using the Student's t-test with $P < 0.05$ considered significant.

15.3 Results

15.3.1 Effect of Orexin A on Platelet Aggregation

Orexin A (100–500 ng/ml) significantly inhibited ADP-induced aggregation of human platelets. Thus, while ADP (2 µM) aggregated platelets by 62 ± 2 %, in the presence of the lower concentration of orexin A tested (100 ng/ml), this effect of ADP was reduced such that platelet aggregation was 55 ± 2 % ($n > 3$ experiments; $P < 0.05$). The higher dose of orexin A tested did not further inhibit ADP-induced platelet aggregation and in fact was equivalent at inhibiting platelet aggregation.

15.3.2 Effect of Obestatin and NPY on Platelet Aggregation

In contrast to the ability of orexin A to inhibit ADP-induced aggregation of human platelets, neither obestatin (100–1,000 ng/ml) nor NPY (100–1,000 ng/ml) impacted ADP-induced platelet aggregation. Indeed, while ADP (2 µM) aggregated human platelets by 64 ± 2 %, values in the presence of obestatin (100, 500 or 1,000 ng/ml) were 62 ± 2 %, 64 ± 2 % or 65 ± 2 %, respectively ($n > 3$; $P > 0.05$). Similarly, compared to aggregation induced by ADP, aggregation of human platelets in the presence of NPY (100, 500 or 1,000 ng/ml) were unaffected (~2 ± 2 % at all doses tested, $n > 3$ experiments; $P > 0.05$).

15.4 Discussion

Several mechanisms have been proposed to link obesity and CVD. Among these, an interaction between the endocrine and cardiovascular systems is popular. In this context, a significant number of peptide hormones are known to influence satiety and energy expenditure. In addition, these hormones also impact functions of cells of the cardiovascular system. In two previous reports, we described the effect of three hormones; leptin, adiponectin, and ghrelin on platelet function [1, 2]. In these earlier studies we reported that leptin, but not adiponectin or ghrelin potentiated ADP-induced platelet aggregation. Indeed, we identified an effect of leptin on cAMP hydrolysis as a likely basis for its effects. Herein, we report that while orexin A could potentiate ADP-induced aggregation of human platelets that obestatin and NPY did not. Since orexin A was known to stimulate the sympathetic nervous system, we had predicted that this peptide hormone would enhance platelet activation. In marked contrast, our data indicated that orexin A inhibited ADP-induced platelet aggregation. Although of potential physiological or pathological importance, the molecular basis for this discordant observation will require further work. Similar to our report that ghrelin did not influence platelet function [2], obestatin also was without effects in our studies. Despite the fact that NPY is known to be expressed in platelets, and that it can compromise certain functions of cells of the cardiovascular system in experiments [11, 12], our studies showed that NPY did not augment platelet activity. This observation is consistent with the likely conclusion that NPY does not act as an autocrine factor for platelets, but rather is released from these cells to act on other systems of the cardiovascular system. Taken together, our data indicate that only orexin A might have a protective effect against cardiovascular morbidity through its inhibitory effect on platelet activity. Further studies are recommended to explore the mechanism through which orexin A shows this inhibitory effect.

15.5 Conclusion

In combination with our previous work in which we report the effects of leptin on platelets and human VECs, we believe that these findings further reinforce the idea that obesity-related peptides directly impact functions of cells of the cardiovascular system. Indeed, we conclude that a direct link is highly likely between the effects of these peptide hormones on control of satiety, hunger, weight gain and cardiovascular disease-associated increases in morbidity and mortality.

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References

1. Elbatarny HS, Maurice DH (2005) Leptin-mediated activation of human platelets: involvement of a leptin receptor and hosphodiesterase 3A containing cellular signaling complex. Am J Physiol Endocrinol Metabol 289:E695–E702
2. Elbatarny HS, Netherton SJ, Ovens JD et al (2007) Adiponectin, ghrelin, and leptin differentially influence human platelet and human vascular endothelial cell functions: implication in obesity-associated cardiovascular diseases. Eur J Pharmacol 558:7–13
3. Pischon T (2009) Use of obesity biomarkers in cardiovascular epidemiology. Dis Markers 26:247–263
4. de Lecea L, Kilduff TS, Peyron C et al (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 95:322–327
5. Sakurai T, Amemiya A, Ishii M et al (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92:573–585
6. Spinazzi R, Andreis PG, Rossi GP et al (2006) Orexins in the regulation of the hypothalamic-pituitary-adrenal axis. Pharmacol Rev 58:46–57
7. Teske JA, Levine AS, Kuskowski M et al (2006) Elevated hypothalamic orexin signaling, sensitivity to orexin A, and spontaneous physical activity in obesity-resistant rats. Am J Physiol Regul Integr Comp Physiol 291:R889–R899
8. Zhang JV, Ren PG, Avsian-Kretchmer O et al (2005) Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 310:996–999
9. Lauwers E, Landuyt B, Arckens L et al (2006) Obestatin does not activate orphan G protein-coupled receptor GPR39. Biochem Biophys Res Commun 351:21–25
10. Parker E, Van Heek M, Stamford A (2002) Neuropeptide Y receptors as targets for anti-obesity drug development: perspective and current status. Eur J Pharmacol 440:173–187
11. Kamiji MM, Inui A (2007) Neuropeptide Y receptor selective ligands in the treatment of obesity. Endocrine Rev 28:664–684
12. Kuo LE, Abe K, Zukowska Z (2007) Stress, NPY and vascular remodeling: implications for stress-related diseases. Peptides 28:435–440