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The protective role of the opioid antagonist LY255582 in the management of high fat diet-induced obesity in adult male albino rats

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Objectives. The involvement of the opioid system in energy balance has been known for several decades but many questions remain unanswered. Therefore, this study was designed to investigate the effect of the non-selective opioid receptor antagonist (LY255582) on high fat diet (HFD)-induced obesity.

Methods. Twenty-four adult male albino rats were divided into 4 groups: Control, HFD non-treated, HFD+LY255582 treated during the first 4 weeks and Obese-LY255582- treated groups during the following 4 weeks after the induction of obesity. LY255582 (0.31 mg/kg, s.c.) was administrated daily with HFD feeding. Blood samples were collected for measurement of lipid profile, glucose, insulin, and leptin. Body weight, body mass index (BMI), and food intake were also measured.

Results. Consumption of HFD resulted in a significant increase in body weight, body mass index (BMI), glucose, insulin, leptin levels, and induced a state of dyslipideamia. Opioid antagonist LY255582 administration with HFD decreased food intake, body weight and BMI, in addition to the improvement of HFD related metabolic abnormalities (dyslipidemia and insulin resistance) during the dynamic phase of obesity development than in animals with already developed dietary obesity.

Conclusion. The use of opioid antagonist may be a promising approach in treatment of HFD-induced obesity.

Key words: obesity, high fat diet, leptin, insulin, glucose, opioid receptors

Obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. One of the main factors that contribute to the development of obesity is high-fat consumption (Kim et al. 2011).

The endogenous opioid system consists of the endogenous opioid peptides and their corresponding receptors by which these peptides produce their effects. The primary central nervous system (CNS) opioids (β-endorphin, enkephalins, and dynorphins) are agonists for mu (μ), kappa (κ), and delta (δ)-opioid receptors. Stimulation of the central μ-opioid receptor by infusion of opioid peptides including β-endorphin or synthetic agonists has been found to increase body mass and food intake (DiFeliceantonio et al. 2012).

Opioid receptors exist not only in the nervous system, but also in peripheral organs, such as heart, lungs, liver, gastrointestinal, and reproductive tracts (Feng et al. 2012). In the gastro-intestinal tract (GIT), opioid receptors are present in the smooth muscle cells and at the terminals of sympathetic and sensory peripheral neurons. It has been shown that opioid receptors are synthesized in the dorsal root ganglion and transported centrally and peripherally to the nerve terminals (Duraffourd et al. 2012).

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Control of food intake is regulated by a complex system of central and peripheral signals, which interact to modulate the individual response to food intake. Peripheral regulation includes satiety signals via hormones secreted from GIT and adiposity signals, while central control includes many factors, which are neuropeptidergic, monoaminergic and endocannabinoid system (Bermudez-Silva et al. 2012).

The brain opioid system has a role in regulating food intake and in mediating the rewarding impact of palatable food intake and the modulation of opioid receptors can interfere with the control of food intake. Within the hypothalamus, both μ and κ receptors stimulated agouti-related peptide (AgRP) and neuropeptide Y (NPY) induced food intake. Orexin-induced feeding behavior was also stimulated by opioids. In addition, endogenous opioids regulate the mesolimbic dopamine pathway, and activation of opioid receptors in this area stimulates food consumption, whereas their blockade suppresses feeding behavior. Thus, the endogenous opioid system likely interacts with both homeostatic and hedonic signals to control energy balance (Czyzyk et al. 2012).

On the other hand, the peripheral endogenous opioids mediate suppression of gastro-intestinal (GI) neural activity, inhibition of gastric emptying, delay of gastrointestinal transit and satiation via peripheral μ-opioid receptors (Khansari et al. 2013). From above mentioned data, the endogenous opioids, both centrally and peripherally, could affect food intake in different ways, which suggests an important role for endogenous opioid peptides in the control of body weight. Now the question is "Which of these mechanisms of opioid action was predominating, central mechanism mediating inhibition of satiety or peripheral mechanism mediate satiation?"

The role of opioids in the regulation of food intake is controversial: in general, opioid agonists enhance feeding and opioid antagonists decrease feeding but some studies showed that opioid agonist could decrease feeding depending on the dose and dosing regimen (Janssen et al. 2011). Therefore, our work aimed to study the effect of opioid receptor antagonist on HFD-induced obesity in adult male albino rats with its metabolic abnormalities during and after induction of obesity. In addition, an important question to this discussion is "Are there or there are not relations between leptin, insulin, and opioid receptors in relation to food intake and obesity?"

Materials and Methods

Animals. Twenty-four adult male albino rats (Sprague-Dawley strain) were used. Their weight ranged between 150-200 g at the beginning of this study. Rats were housed in stainless steel mesh cages offering individual housing and each rat had a tag number. They were housed at room temperature with natural light/dark cycles for one week for acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water ad libitum until the time of the experiment. All the procedures followed with the rats were in accordance with our institutional guidelines. The protocol was ethically approved by The Laboratory Animals Maintenance and Usage Committee of Faculty of Medicine in Minia University.

Rats were firstly divided into 3 main groups: 1) Control group (C): in which six rats were fed a commercially available standard diet and each rat received 0.5 ml of the vehicle subcutaneously (s.c.) once daily in the morning for 4 weeks. 2) High fat diet (HFD) non-treated group (HFD): in which 12 rats were fed HFD and each rat received 0.5 ml of the vehicle s.c. once daily in the morning for 4 weeks. 3) HFD+LY255582-treated group (HFD+LY group): in which six rats were fed a HFD with concurrent morning subcutaneous injection of LY255582 (opioid antagonist, 0.31 mg/kg body weight) for 4 weeks (Shaw et al. 1991).

After 4 weeks, we found that all rats fed with HFD without treatment had a Lee index higher than 0.3 and were considered obese (Campos et al. 2008). We sacrificed only six rats from the HFD non-treated group with all rats of control and HFD+LY groups after fasting overnight for blood samples collection. In the next 4 weeks after the induction of obesity, retro-orbital blood samples were taken from the remaining 6 obese rats from HFD non-treated group to be considered as initial pretreated samples for the fourth group that was called Obese – LY255582-treated group (O-LY group). The obese rats of this group were continued on the HFD for further 4 weeks with concurrent daily subcutaneous injection of LY (0.31 mg/kg body weight). At the end, the rats of this fourth group were fasted for overnight and then decapitated. After collection of blood from jugular vein, the samples were left to clot at room temperature, and then centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge). The serum layer was then withdrawn into identified Eppendorf tubes and stored at -20°C until the time of assay.

Drug protocol. LY255582 (powder, Sigma- USA) is a centrally active non-selective opioid receptor antagonist and it was dissolved in water acidified with 1% lactic acid (vehicle).

Diet protocol. The composition of standard diet (g/kg diet) was according to the formula of Davidson et al. (2012) that contained (Fat 5% [corn oil 5%], carbohydrates 65% [corn starch 15% and sucrose 50%], proteins 20.3% [casein 20% and DL-Methionine 3%], fiber 5%, salt mixture 3.7%, and vitamin mixture 1%) and provided 3.0 kcal/g of diet. On the other hand, the HFD contained 20 g of fat/100 g of diet (19 g of butter oil and 1 g of soybean oil to provide essential fatty acids) and provided 4.1 kcal/g of diet (Woods et al. 2003).

Standard diet was purchased from El-Gomhoria Company, Cairo, Egypt, while high fat, diet was prepared manually and preserved at 4°C until used. The daily food intake was measured for each group. Individual body weight of rats in each group was assessed once a week.

Body mass index (BMI) and Lee index. Body length (nose-to-anus length) was determined in all rats at the beginning and every week up to the end of the experiment. The body weight and body length were used to determine BMI and Lee index.

BMI was calculated according to the following formula:

Body mass index (BMI) = body weight (g)/ length^2 (cm^2).

BMI is used for comparison between rats as considered by Novelli et al. (2007) a significant increase in BMI in comparison to a control is a marker of obesity and obesity is usually taken as any significant increase in body weight or energy content relative to control animals. This was also according to Li et al. (2008).

Lee index was calculated for each rat according to the following formula:

Cube root of body weight (g) X 10 / nasoanal length (mm).

All rats fed with HFD had a Lee index higher than 0.3 and were considered obese (Campos et al. 2008).

Biochemical analysis. Total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL-c), high density lipoprotein (HDL-c) and glucose were determined by enzymatic colorimetric methods, using kits purchased from Bio-diagnostic, EGYPT. Serum insulin and leptin were determined by enzyme-linked immunosorbent assay (Glory).

Statistical analysis. Statistical analysis was performed using Graph pad Prism 5 software and significant difference between groups was done by one-way ANOVA followed by Tukey-Kramer post hoc test for multiple comparisons with a value of $p \leq 0.05$ considered statistically significant.

Results

Effect of opioid antagonist during the induction of obesity. The results of the present study revealed that HFD showed significant higher values of the final body weight and BMI as compared with the control group and with its initial values (Table 1). This was accompanied with significantly higher food intake in the first and second weeks in the HFD group as compared with control group. There was also a significant lower amount in the final food intake in HFD group as compared with control group and with its initial value (Table 2). In addition, there were significantly higher levels in the total cholesterol, TGs, LDL-c, serum glucose, insulin and leptin with a significant lower level of HDL-c as compared with control group (Table 3).

Injection of LY lowered significantly the body weight from the second week up to the end of the study as

Table 1
Body weight and BMI changes during the induction weeks of obesity in the different studied groups.

Parameter	Control		HFD		HFD+LY	
	Body weight (g)	BMI	Body weight (g)	BMI	Body weight (g)	BMI
Initial	169.4±4.4	0.54±0.01	168±3.7	0.53±0.01	169.6±2.6	0.55±0.02
After 1 week	189.8±4.8	-	192.4±4.1	-	179.2±3.9	-
After 2 weeks	212.4±5.7	-	233.6±6.0^{a}	-	180.4±4.0^{ab}	-
After 3 weeks	218.4±6.8	-	240.2±6.3^{a}	-	180.0±4.4^{ab}	-
Final week	220.2±2.8^{c}	0.57±0.01	245±3.7^{ac}	0.66±0.01^{ac}	184.4±5.3^{abc}	0.49±0.004^{abc}

Data are expressed as mean ± S.E.M. a – significant from control group; b – significant from high fat diet (HFD) group; c – final value significant from its corresponding initial value, $p<0.05$. BMI – Body mass index; HFD+LY – HFD+LY255582-treated group

Table 2
Changes in food intake during the induction weeks of obesity in the different studied groups

Food intake (g/d)	Control	HFD	HFT+LY
After 1week	20.5±0.9	24.5±1.4[a]	20.8±1.0
After 2 weeks	18.1±1.0	24.3±1.3[a]	19.4±0.9[b]
After 3 weeks	19.1±0.3	20.5±0.7	18.1±0.5[b]
After 4 weeks (Final week)	19.4±0.23	18.0±0.5[ac]	16.5±0.2[abc]

Data are expressed as mean ± S.E.M. of 6 rats in each group. a - significant from control group; b – significant from high fat diet (HFD); c – final value significant from its corresponding initial value; p<0.05. HFD+LY – HFD+LY255582-treated group.

compared with control and HFD groups. The final body weight in the HFD+LY group was significantly higher than its initial value. As regard BMI, there was a significantly lower value in the final as compared with control, HFD groups and with its initial value (Table 1). In addition, there was a significantly lower amount of the final food intake as compared with control, HFD groups and with its initial value (Table 2). There were also significantly lower values in the total cholesterol, TGs, LDL-c with a significantly higher levels in HDL-c but no significant change in the serum glucose, insulin, and leptin levels from the control group (Table 3).

Effect of opioid antagonist administration in obese rats. The results of the present study revealed that injection of LY to obese rats with the continuation of HFD caused a significant decrease in the food intake in the third and fourth weeks as compared with its initial value. This was accompanied with a significantly higher final body weight, but the final BMI showed a significant decrease as compared with its corresponding initial values (Table 4). In addition, there was a significant decrease in the serum glucose level without any significant change in the total cholesterol, TGs, LDL-c, HDL-c, insulin and leptin levels from its corresponding initial measures (Table 5).

Discussion

Obesity is a worldwide health threat challenge (de-Lartigue et al. 2014). Therefore, it is necessary to explore actively approaches for this problem. In this study, we are focusing on the effect of an opioid antagonist against HFD induced obesity.

In the present study, the result clearly demonstrated that the final body weight of the control group significantly increased as compared with its initial body weight, in spite of the fact that BMI and food intake were not changed. This could be explained by a natural body growth with no excess fat deposition since the BMI did not significantly change. This agrees with the results reported by Ble-Castillo et al. (2012).

One of the main factors that contribute to the development of obesity is high-fat consumption. Rat models are therefore useful tools for inducing obesity as they will readily gain weight when fed HFD (Lee 2013). In the present study, obesity was induced in male albino rats by using a HFD formula for 4 weeks. The weight gained by rats fed HFD formula, was significantly more than that gained by those fed the normal diet after 4 weeks from the start of experiment as proved by significantly higher final BMI and body weight from the second week up to the end of the study. Obesity increase in rats has

Table 3
Serum levels of lipid profile, glucose, insulin and leptin after the induction weeks of obesity in the different studied groups

Parameter		Control	HFD	HFD+LY
Lipid profile	**TC (mg/dl)**	69.2±1.2	89.3±2.0[a]	60.5±2.6[ab]
	TGs (mg/dl)	44.5±1.7	74.8±3.2[a]	34.8±2.3[ab]
	HDL-c (mg/dl)	25.2±1.9	17.1±1.1[a]	32.5±2.2[ab]
	LDL-c (mg/dl)	34.9±1.8	57.4±2.7[a]	20.7±1.7[ab]
Serum glucose (mg/dl)		58.9±2.4	98.9±4.1[a]	60.9±2.9[b]
Serum insulin (mIU/l)		3.1±0.1	4.6±0.2[a]	3.2±0.2[b]
Serum leptin (ng/ml)		3.9±0.3	8.5±0.2[a]	5.1±0.5[b]

Data are expressed as mean ± S.E.M. of 6 rats in each group. a - significant from control group; b – significant from high fat diet (HFD); p<0.05. HFD+LY – HFD+LY255582-treated group.

Table 4
Effect of opioid antagonist on the body weight, food intake and BMI in obese rats

Parameter	Body weight (g)	HFD-LY Food intake (g/d)	BMI
Initial week	205.0±9.4	18.0±0.5	0.60±0.001
After 1 week	216.6±8.4	17.4±1.2	-
After 2 weeks	214.2±7.8	16.9±0.5	-
After 3 weeks	218.4±8.6	15.1±0.3[a]	-
Final week	233.0±4.4[a]	14.2±0.4[a]	0.52±0.03[a]

Data are expressed as mean ± S.E.M. of 6 rats in each group. a – significant from its corresponding initial value; p<0.05. HFD-LY – HFD+LY255582-treated group; BMI – Body mass index.

Table 5
Serum levels of lipid profile, glucose, insulin and leptin in obese treated groups

Parameter		HFD-LY	
		Initial	Final
Lipid profile	**TC (mg/dl)**	89.3±2.0	82.3±2.5
	TGs (mg/dl)	77.8±2.2	74.6±0.9
	HDL-c (mg/dl)	16.1±1.0	18.6±0.7
	LDL-c (mg/dl)	54.4±2.5	48.8±2.1
Serum glucose (mg/dl)		92.9±5.0	73.5±3.3[a]
Serum insulin (mIU/l)		4.0±0.2	3.7±0.2
Serum leptin (ng/ml)		7.5±0.3	8.0±0.2

Data are expressed as mean ± S.E.M. of 6 rats in each group. a - significant from its corresponding initial value; b – significant from HFD-LY group, p<0.05. HFD-LY – HFD group treated with LY25558.

been also achieved by different formulas of high fat diets used (Jia et al. 2013).

This weight gain could be explained by the fact that HFDs are characterized by a high palatability that is often considered to increase the energy intake and promote hyperphagia (Guyenet and Schwartz 2012) as proved in our study by a significant higher amount of initial food intake as compared with control group. Other factors that may contribute to obesity induced by HFD include the overconsumption of high caloric diet and poorly satiating properties of the HFDs (Talukdar et al. 2012).

Another possible mechanism of increased body weight is the increased lipogenesis and decreased lipolysis, which was evidenced in the present work by a significant increase in BMI. These findings are consistent with Kumar et al. (2014). In addition, HFD caused an attenuation of the vagal afferent function, which express receptors for many of the regulatory peptides and molecules released from the intestinal wall, pancreas, and adipocytes that influence GI function, glucose homeostasis, and regulate food intake and body weight. The mechanism(s) leading to this attenuation is not clear but it may be due to an altered balance in expression of anorexigenic and orexigenic peptides and receptors, leading to dysregulation of intestinal feedback control of GI function and food intake (de Lartigue et al. 2011).

Finally, a preference for certain foods, including fats, are a complex behavior regulated by: a) homeostatic mechanisms, which serve to maintain energy balance, and b) reward-related mechanisms, which process the hedonic properties of food independently of energy status (Haghighi et al. 2013) and this was mediated by the brain opioid systems. This reward effect was supported by Kraft et al. (2013) who have been found that obesity caused by increasing the preference for a HFD was attributed to the higher expression in the hypothalamus of μ-opioid receptors.

In the final week of the present study, the result clearly demonstrated that the body weight of HFD group still significantly increased in spite of the decrease in the final food intake as compared with control group and its initial values. This could be attributed to the peripheral inhibitory effects of fat on the gastrointestinal motility and stimulation of anorexic GI hormones including cholecystokinin, peptide YY and glucagon-like peptide-1, as well as the suppression of ghrelin which is the only GI orexigenic hormone (Little and Feinle-Bisset 2011).

In addition, HFD could decrease food intake through central mechanisms mediated by an increase in the leptin level as reported in our study and others (Belin de Chanteme`le et al. 2011). It enters the brain arcuate nucleus (ARC) where it induces/represses a network of important neuropeptide regulators of energy intake and expenditure (Moran and Ladenheim 2011). However, leptin failed to decrease body weight in spite of its central inhibitory effect on food intake, as leptin alone could not fight adiposity in the presence of continuous HFD consumption with higher caloric value as compared with the standard diet (Borer 2014).

Finally, HFD intake is associated with excessive circulating free fatty acids and glucose, aggravating insulin resistance and increasing lipolysis and insulin secretion (Marques et al. 2010). Hyperinsulinemia synergistically acts centrally with hyperleptinemia (Leptin-insulin lipo-

stat) to decrease food intake as these two hormones reach the hypothalamus and activate specific "catabolic" neuroendocrine circuits, which inhibit food intake (Paspala et al. 2012). This indicated that the central mechanism for insulin is still working in spite of the development of peripheral insulin resistance marked by the associated hyperglycemia. Hyperglycemia could also induce satiety by a peripheral action through stimulating afferent vagal fibers (Punjabi et al. 2011) and additionally induced a state of dyslipidemia via stimulation of de novo hepatic lipogenesis (Zhukova et al. 2014) as observed in our study.

In the present study, the rats that consumed the HFD with injection of LY showed significantly lower amount of final food intake with correlated decrease in final body weight and BMI as compared with control and obese-non treated groups. This could be attributed to inhibition of μ-opioid receptor signaling as reported by Kraft et al. (2013). Additionally, our results demonstrated that the final body weight significantly increased as compared with its initial value and this could be explained by a natural body growth with no further fat deposition since the final BMI significantly decreased as compared with its initial value. This effect could be secondary to improved glucose tolerance and insulin sensitivity as observed in the following results. From the above-mentioned data, opioids appear to exert their effect on food intake predominantly within the central nervous system, although peripheral effects on taste and gastrointestinal motility play a minor role (Janssen et al. 2011).

In the present study, the decreased blood glucose level with LY administration either during induction or after induction of obesity could be explained by the increase in insulin sensitivity caused by reduction in body adiposity (Banin et al. 2014). Furthermore, the LY hypoglycemic effect observed in this study could be mediated through blocking or reversal of the opioid hyperglycemic effects. Such hyperglycemic effects of opioids are in accordance with Mysels and Sullivan (2010) who have reported that opioid administration caused impairment in key enzymes related to glucose metabolism; the glycolytic activity of hexokinase and phosphofructokinase-1 activity was diminished, leading to less breakdown of plasma glucose, while, the gluconeogenic activity of glucose-6-phosphatase and fructose-1,6-biphosphatase was increased, leading to increased production of plasma glucose and a metabolic state similar to non-insulin-dependent diabetes. In addition, opioids were found to induce adrenal excitation through α2-adrenoceptors and caused the subsequent changes in the liver function (Vahidi et al. 2012).

The data of the present study clearly demonstrated that administration of opioid antagonist LY during induction of obesity caused a significant decrease in insulin level. This could be achieved directly by blocking parasympathetic stimulation of insulin release (Hosseini 2011), or secondary to the decreased blood glucose level, the main direct stimulant of beta cells (Bandaru et al. 2011). Additionally, LY decreased insulin level indirectly by reduction in the body fat, with a consequent increased insulin sensitivity in accordance with Paspala et al. (2012). Because serum leptin correlated positively and strongly with BMI, and since LY administration decreased the BMI in the present work, so leptin level significantly decreased.

The relationship between insulin resistance, hyperglycemia and dyslipidemia is mutual and is a cause and effect response (Bardini et al. 2012; Lia et al. 2013). Insulin decreases adipose tissue lipolysis and improves plasma lipid profile (Jocken et al. 2014). In the present work, LY decreased blood glucose level and improved insulin sensitivity, hence the observed correction of the dyslipidemic effect produced by the HFD.

In the present study, continuous administration of the opiate receptor antagonist LY with HFD to obese rats caused a significant decrease of food intake in the third and fourth weeks as compared with its initial value, with a significantly decreased final BMI, however body weight did not significantly change except in the final week that was increased probably by natural body growth. The serum lipid profile, insulin or leptin did not significantly change but glucose level decreased. The central delayed effect of LY on food intake could be attributed to the elevation of brain levels of the opioid agonists, β-endorphin and [Met5] encephalin, in obese rats according to Marczak et al. (2009). Hence, the longer time it takes to compete with these opioids before exerting recognizable effects, or to the decreased response induced by habituation and tolerance frequently observed with these drugs (Morgan and Christie 2011). One of the mechanisms that contribute to this effect is down regulation of receptors on prolonged use of its ligand. The failure to correct the dyslipidemic state produced by HFD or to correct the hormonal imbalance of both leptin and insulin strongly supports failure of the peripheral metabolic mechanism of the opiate antagonist in obese rats and supports the tolerance concept.

In conclusion and according to our results, after 4 weeks of LY administration with HFD, the opioid antagonist LY produced a consistent protective effect in rats during the dynamic phase of obesity development than in animals

with preexisting dietary obesity. Further studies are needed to identify if there was any effect of LY255582 treatment on the circadian rhythm, hormonal status (like corticosterone) and behavioral status in rats with regard to the food intake and distinguish the impact of the partial starvation during the LY255582 treatment on the metabolism. Additionally, future prolonged studies more than 4 weeks, using opioid antagonist in obese rats either alone or in combination with other anti-obesity measures, are required to establish an ideal model of management.

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