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Effect of Naloxon on Counter Insulin Hormone Secretion in Insulin-Induced Hypoglycemia

Yeong Shil Ju, M.D., Sung Woon Kim, M.D., In Myung Yang, M.D.
Jin Woo Kim, M.D., Young Seol Kim, M.D. and Young Kil Choi, M.D.

Department of Internal Medicine, Kyung Hee University, School of Medicine, Seoul, Korea

To investigate the normal physiologic role of endogenous opiates in glucose homeostasis and as a preliminary study for clarifying the association of endogenous opites with pathophysilogy of NIDDM, we obseved the changes in the secretion of counter-insulin hormones in response to insulin-induced hypoglycemia with or without naloxone.

The results were as follows:

1) Blood glucose was decreased significantly more rapidly with naloxone infusion than after insulin alone, which seems to play a role in the early responses of ACTH and GH.

2) Not only was the more rapid response of ACTH and GH, but also the prolonged secretion of ACTH and cortisol were observed after administration of insulin and naloxone.

We concluded that endogenous opiates may be involved in the feedback regulation of secretion of ACTH and GH during hypoglycemia either at hypophysis or hypothalamus, and involved in glucose homeostasis via a certain direct mechanism other than regulation of counter hormone secretion.

Key Words: *Endogenous opioid peptide, NIDDM, Glucose metabolism, Insulin-induced hypoglycemia counter-insulin hormones.*

INTRODUCTION

Since the discovery of opioid and its receptors in the brain and pituitary[1~4], progress has been made in the investigation of the role of opioid substances in the regulation of secretion of pituitary hormones. Also it attracts increasing interest that endogenous opioid peptide may be involved in the regulation of blood glucose and suggested a role in pathophysiology of NIDDM. There is no conclusion whether opiate can alter the secretion of counter-insulin hormones in response to induced hypoglycemia until now.

El-Tayeb et al[5] observed the response of counter-insulin hormones in insulin-induced hypoglycemia in dogs and found that blockade of endogenous opioid peptide with naloxon led to earlier and greater release of counter-insulin hormones, we observed the changes in the secretion of hormones in response to insulin-induced hypoglycemia in man with or without naloxon administration.

Address reprint requests: Yeong Shil Ju, M.D., Department of Internal Medicine, Kyoung Hee University Hospital of I. M., #1, Hoekidong, Dongdemoon ku, School 131 Korea

SUBJECTS AND METHODS

The subjects were five healthy men aging 24 to 31 year and wighing 64 to 70 kg.

On the first day. after an overnight fast the antecubital vein was cannulated with 19 gange butterfly needle at resting state. Saline was infused through the cannula. And 30 minute later, 0.1 u/kg of regular insulin (Actrapid®) was administered through the cannula at 0 minute. Blood sample was collected every 10 minutes after insulin administration for serum glucose, ACTH, GH, Cortisol, Glucagon and β-endorphin level.

On the second day. 0.5 mg/kg of naloxon was administered 15 minute before insulin initially and then infused at constant rate of 0.5 mg/min.

The serum glucose level was measured by the glucose oxidase method. Serum and plasma samples for hormones were separated and stored at −70℃ until assay.

ACTH was assayed by RIA with the kit from Immunonuclear corporation, GH with Radioassay System laboratory, Glucagon with Dinabott and β-endorphin with Immunonuclear corporation.

Datas were expressed as mean±standard deviation and statistical analysis was made with student's paired t-test. Total integrated response of each hormone was evaluated with area under curve (AUC) by trapezoidal rule.

RESULTS

1. Effect of Naloxon on Glucose Respose (Fig. 1)

Blood glucose was significantly decreased 20 minutes after insulin administration as compared with the baseline value without naloxon administration (88.0±8.5 vs. 50.6±18.4 mg/dl, $p<0.05$), while decreased significantly 10 minute after insulin with naloxon administration (93.4±9.7 vs. 67.8±8.5 mg/dl, $p<0.025$).

Glucose nadir was observed 30 minutes after insulin administration without naloxon while 20 minutes after with naloxon and significantly lower level (43.2±26.3 vs. 28.2±7.5 $p<0.005$) was observed with naloxon administration. These data showed that the earlier and more severe hypoglycemia was induced through naloxon administration.

2. Effect of Naloxon on the response of ACTH to Insulin-Induced Hypoglycemia (Fig. 2)

Without naloxon administration, plasma ACTH levels began to increase 20 minute after insulin administration, when serum glucose fell significantly, but was statistically not significant (32.5±32.8 vs. 103.8±50.9 pg/ml $p<0.1$), while with naloxon administration, plasma ACTH level rose 10 minute after insulin administration, the time was compatible with insulin-induced significant hypoglycemia (21.8±29.5 vs. 167.3±36.0 pg/ml $p<0.025$). The earlier ACTH response to hypoglycemia was noted after naloxon infusion.

Without naloxon infusion, increased ACTH returned to baseline level 10 minute after glucose nadir, while with naloxon infusion, ACTH secretion was continuously increased to reach its peak level until 20 minute after glucose nadir. These data showed the augmented and prolonged secretion of ACTH after naloxon administration.

The total integrated response of ACTH with or wihout naloxon infusion was not statistically significant (3650.1±2042.6 vs. 7207.0±2421.1 pg/ml. min $p>0.1$).

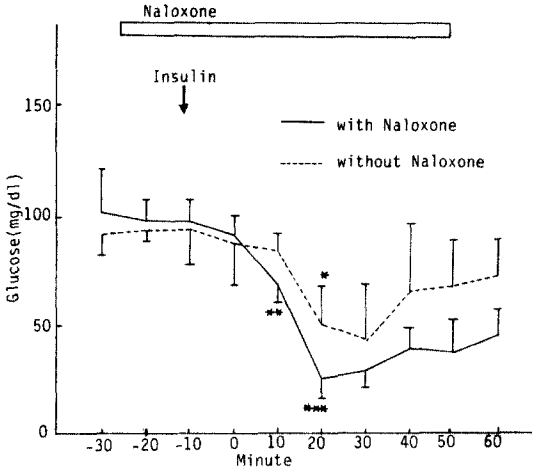

Fig. 1. *Glucose response to insulin and naloxone.*
** P < 0.05, ** P < 0.025*
**** P < 0.005 compare to the mean of 30 min period before insulin administration.*

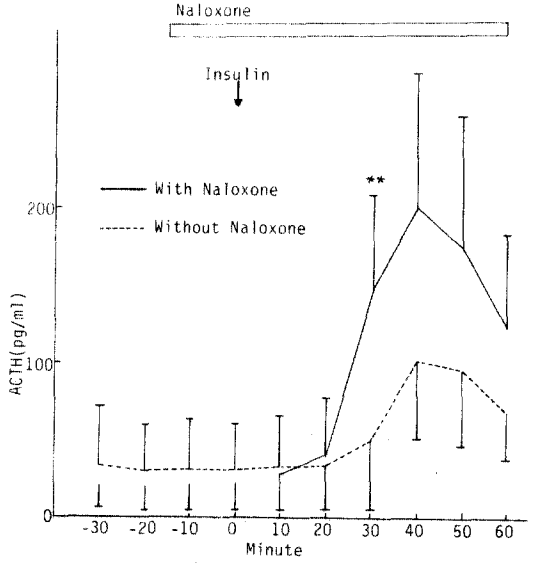

Fig. 2. *Effect of naloxon on the response of ACTH to insulin-induced hypoglycemia.*
*** P < 0.035 compared to the mean of 30 min period before insulin administration.*

3. Effect of Naloxon on the Response of GH to Insulin-Induced Hypoglycemia (Fig. 3)

The response time of GH was observed to be parallel to that of the serum glucose level which fall significantly 10 minute and 20 minutes after insulin administration respectively, increasing 20 minutes after the time of significant glucose fall.

The time of significant increase of GH level was observed also to be parallel with the decrease of blood glucose level increasing significantly 10 minute after glucose nadir (2.4±.15 vs 25.1±24 ng/ml $p < 0.0025$). Serum GH increased significantly 10 minutes after glucose nadir with naloxon infusion (2.0±1.8 vs 32.6±36.7 ng/ml $p < 0.005$).

There is no statistically significant difference in total integrated response of GH with or without naloxon infusion (1342.2±375.9 vs 2054.8±1286.0 ng/ml. min $p > 0.1$).

4. Effect of Naloxon on the Response of Corisol to Insulin-Induced Hypoglycemia (Fig. 4)

The response time of cortisol was parallel to the decrease of blood glucose levels increasing significantly as compared to the base line values 20 minute after the time when the significant decrease of glucose level was observed with naloxone and to increase significantly 20 minute after the significant decrease of blood glucose level with naloxon too.

The mode of response of cortisol was similar to that of ACTH, revealing a decrease after reaching its peak value 50 minutes after insulin administration with naloxone and prologed, continous increase 60 minute after insulin administration with naloxon.

There is no statistically significant difference in the total integrated response of cortisol with or wihout naloxon administration (1677.8±597.8 vs 1963.0±591.7 ug/dl. min $p > 0.1$).

5. Effect of Naloxone on the Response of Glucagon to Insulin-Induced Hypoglycemia (Fig. 5)

The glucagon response was not significantly different in time, the mode of response and total integrated response of glucagon secretion between the two values with or without naloxon administration.

DISCUSSION

Endogenous opioid peptides may be involved in blood sugar regulation in diabetes mellitus. The relationship was repeatedly suggested by same reports[6~8] by discovering the localization of

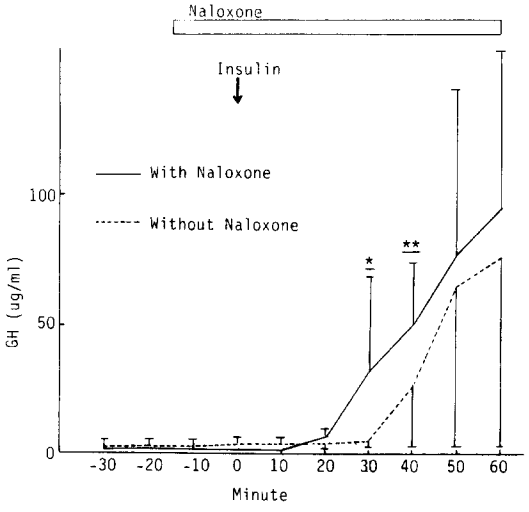

Fig. 3. *Effect of naloxon on the response of GH to insulin-induced hypoglycemia.*
** $P < 0.05$, ** $P < 0.025$ compare to the mean of 30 min period before insulin administration.*

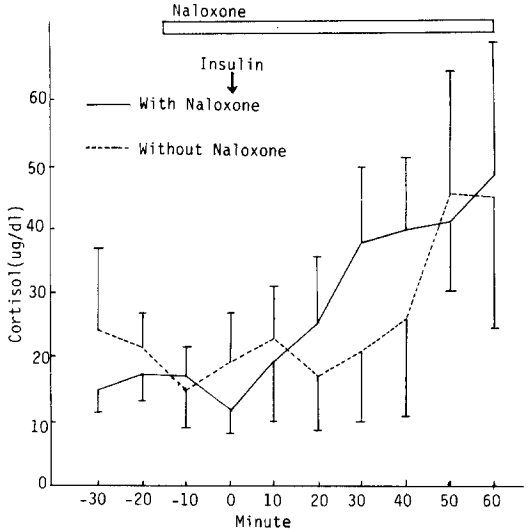

Fig. 4. *Effect of naloxon on the response of cortisol to insulin-induced hypoglycemia.*
*** $P < 0.025$ compare to the mean of 30 min period before insulin administration.*

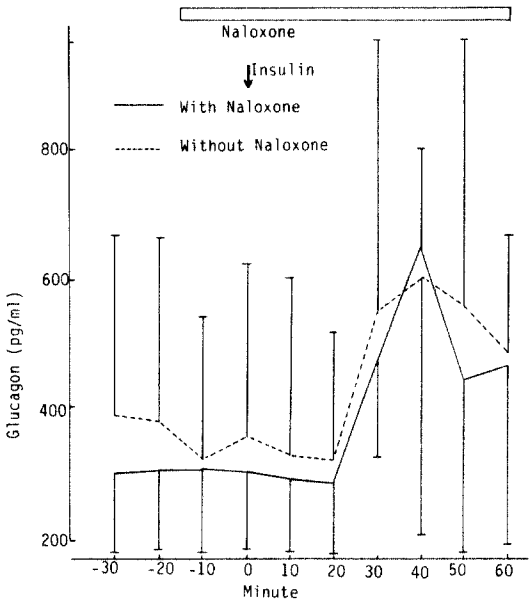

Fig. 5. *Effect of naloxone on the response of glucagon to insulin-indeced hypoglycemia.*

β-endorphin immunohistochemically only in normal healthy human pancreas but not rat in diabetic patients[9]. These findings emphasized the relationship between the opioid peptides and NIDDM.

It is widely accepted that stress can induce insulin resistance by increasing the secretion of counter-insulin hormones and may serve a role as a precepitating factor for development of NIDDM. The role of endogenous opioid peptide in modulation of counter-insulin hormone secretion in the pitutiary of normal or NIDDM to search for the relationship of endogenous opiod peptides and pathophysiology of NIDDM.

We observed the changes of blood ACTH. and GH to insulin-induced hypoglycemia after naloxone administration. The ACTH and GH response were earlier than without naloxon. These results were compatible with the report of E1-Tayeb et al[5]. They speculated that opioid peptide may change the setpoint of the secretion of counter-insulin hormones in response to hypoglycemia in the central nervous system. But considering the ealier hypoglycemic response with naloxon administration in this study, one cannot be excluded that the earlier hypoglycemia may result in the secretion of ACTH and GH earlier. We also noted the response of ACTH and GH were tend to be prolonged and angmented after naloxone. These findings suggested that naloxone blocks the effect of endogenous opioid peptides competitively which may tonically inhibit the secretion of anterior pituitary hormones. Therfore, we can speculate that these findings suggests that endogenous opioid peptides may be involved in the feedback regulation of the secretion of ACTH and GH either at the level of pitutary or hypothalamus.

ACTH and β-endorphin is known to originate from one large common precursor[16], and β-endorphin is involved in the hypothalamic-pitutary-adrenal axis. Morphin inhibits the secretion of ACTH in the face of stress in animal studies[17]. The coupled feedback mechanism that regulates the secretion of ACTH and GH simultaneously was proposed, to say, blocking of endogenous opioid peptide by naloxone competitively increases the secretion of endorphin as well as ACTH. So our results of the prolonged and augmented response of ACTH and GH could be explained.

The response of cortisol was similar with that of ACTH ie, early and prolonged release to hypoglycemia with naloxon infusion. If endogenous opioid peptide is deppressed in NIDDM, hyperglycemia may be induced by augmented release of counter-insulin hormons in stressful condition. The exact mechanism of the earlier and severe hypoglycemia with naloxone administration is not conclusive in this study, but several possibilities can be considered.

The first possibility is that nalxon is intervene in glucose metabolism indirectly through modulating counter-insulin hormone secretion. This suggestion is least likely, because ACTH, GH and cortisol release showed earlier and response as compare to the degree of hypoglycemia with naloxone administration. And the glucagon showed no significant difference with or without naloxone administration

Second, we can speculate the possibility as previousely suggested by Jeanrenaud et al[18] that opioid peptide may directly modulate the glucose metabolism or alter tissue sensitivity toinsulin. Paul et al[19] studied the effect of human β-endorphin on glucose homeostasis in conscious dogs. They observed significant fall in serum glucose with significant fall in glucose production and modest fall in glucose utilization. They concluded that β-endorphin inhibits glucose production by the liver, the essential source of glucose production in vivo and suggested that the effect is a peripheral effect most likely occurring at the liver since β-endorphin dose not cross the blood-brain-

barrier in significant amount when given intravenously.

On the other hand, Werther et al[20,21] examined the effect of opiates and opiate blockade on glucose fluxes induced by mild physiological hyperinsulinemia. They observed that glucose fell similarly in both the control and study groups while in control groups, insulin maminly lowered glucose production in contrast to naloxone and DMPE studies mainly rased glucose utilization. And they suggested that opiates increases the effect of insulin in peripheral tissues, enhancing glucose entry into muscle or other metabolically active sites, thereby allowing increased glucose availability for peripheral metabolism. There results showed to apparrent paradoxical finding that naloxon had similar effects to DMPE. Naloxon is able to mimic morphin or β-endorphin in stimulating insulin release in the isolated dog pancreas[22] and, pretreatment of rats with intraperitoneal naloxon led to a subsequent marked increase in brain opiate binding[23], suggesting a possible mechanism by which naloxone may induce opiate-like effects under some conitions.

Ipp, et al[22] initially reported about the effect of the opioid peptide on pancreatic hormone secretion that morphin and β-endorphin increases the secretion of insulin and glucagon in endocrine pancreas. Lacalization of β-endorphin in pancreatic iselet cell was demonstrated[9] and immunocytochemical identification of colocalization of β-endorphin and somatostatin in pancreatic D-cell[24~27] was confirmed. These studies suggested that opioid peptides influences the secretory function of pancreatic α-and β-cells through increasing insulin and glucagon[28] and induces hyperglycemia partialy by the increased release of glucagon[29]. But there is no cofirming result about how opiates influences the secretory function of endocrine pancreas.

In vitro studies of the effect of endogenous opioid peptide on pancreatic hormone secretion[22,30~33] has been done. They reported that pharmacologic doses of naloxon increases the secretion of glucagon and also insulin. E1-Tayeb et al[5] reported that glucagon increases significantly before glucose nadir with naloxon administration in insulin-induced hypoglycemia of dogs. They insisted that blocking of endogenous opioid peptide may sensitizes the counter-regulating response to hypoglycemia.

In this study, glucagon increased in response to fall in glucose level but no statisticaly significant difference was noted between with and wihout naloxon. It cannot be speculated that blocking of endogenous opioid peptide may increases the secretion of glucagon.

The precise explanation for the disagreement of results with ours cannot be made at present, we can consider the differences in the subjects and dosages of administered naloxon in the studies. Levin et al[21] observed the changes in pancreatic hormone secretion in response to appropriate dosages of naloxon which can block the physiologically secreted function in the previous studies. Their findings reconcile with our results in that naloxone cannot affect the secretion of insulin and glucagon in hypoglycemia and that opiate can intervene in glucose metabolism without influencing the pancreatic hormone secretion.

In summary we can speculate that endogenous opioid peptide may be involved in the feedback regulation of ACTH and GH either at pituitary or at hypothalamus and may intervene in glucose metabolism through certain direct manner wihout the aid of counter-insulin hormones. Further studies considering the dosage of opiate agonists or antagonists used and its relation to specific opiate receptor blockade are neccessary to clarify the association of endogenous opiates on the pathophysiology of NIDDM.

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