

## Rapid Publication

### $\beta$ -Endorphin Inhibits Glucose Production in the Conscious Dog

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**A**bstract. The effect of human  $\beta$ -endorphin (h $\beta$ E) infusion (0.2 mg/h) on glucose homeostasis was studied in 10 conscious overnight fasted dogs in which endocrine pancreatic function was fixed at basal levels with somatostatin plus intraportal replacement of basal insulin and glucagon.

h $\beta$ E caused a fall in plasma glucose from  $107 \pm 5$  to  $76 \pm 6$  mg/dl by 3 h ( $P < 0.01$ ). This was due to a 25% fall in tracer-determined glucose production (Ra;  $P < 0.01$ ). A significantly larger fall in Ra was observed in four dogs in which hypoglycemia was prevented by use of an exogenous glucose infusion (45 vs. 25%,  $P < 0.05$ ). These changes occurred in the absence of changes in circulating levels of insulin, glucagon, epinephrine, norepinephrine, and cortisol.

We conclude that the naturally occurring opioid peptide,  $\beta$ -endorphin, inhibits glucose production by the liver in vivo. This appears to be a direct effect of the opioid on the liver, since the inhibition took place in the absence of changes in the other hormones measured. These results suggest that endorphins act on glucose homeostasis in a complex way, both by affecting other glucoregulatory hormones as demonstrated elsewhere, and by directly modulating hepatic glucose production as shown here.

#### Introduction

The role of opioids in glucoregulation is not completely clear. Several reports (1-3) have shown that opioids can cause hy-

perglycemia via centrally-mediated stimulation of circulating epinephrine. In addition, it has been reported that opioids can modulate glucagon and insulin secretion in vitro (4-6) and perhaps in vivo (7-10). Recently, we have shown that the opiate agonist morphine inhibits glucose production, and thus, results in hypoglycemia when infused at a relatively low dose into conscious overnight fasted dogs (11).

The aim of the present study was to determine whether the naturally occurring opioid peptide  $\beta$ -endorphin could affect glucose production in a manner similar to that of morphine.

#### Methods

**Animals.** Experiments were carried out on 17 overnight (18 h) fasted mongrel dogs (18-25 kg) of either sex, which had been fed a dog chow diet (Wayne Dog Food, Allied Mill, Inc., Chicago, IL) for 3 wk before their use. Silastic catheters were implanted under general anesthesia in a proximal splenic vein and a femoral artery, as previously described (12), at 17 d prior to the study. On the day of the study, the catheters were removed from the subcutaneous pockets through skin incisions made under local anesthesia (1% lidocaine, Astra Pharmaceutical Products, Inc., Worcester, MA). Each catheter was aspirated to remove its contents and then filled with saline until the experiment began. The arterial catheter was used to sample blood and the splenic vein was used to infuse insulin and glucagon at basal levels. Angiocaths (No. 18 gauge, Abbott Laboratories, North Chicago, IL) were inserted percutaneously into the right and left cephalic and saphenous veins for the infusion of  $\beta$ -endorphin, [ $3$ - $^3$ H]glucose, and somatostatin (SRIF).<sup>1</sup> After completion of preexperimental preparation, the conscious dogs were placed in a Pavlov harness and allowed to rest  $\sim 1$  h prior to the beginning of the experiment.

**Experimental design.** Each experiment consisted of an 80-min equilibration period (-120 to -40 min), a 40-min basal period (-40 to 0 min), and a 3-h (0 to 180 min) experimental period. A primed ( $120 \times 10^6$  dpm), continuous ( $8.1 \times 10^5$  dpm/min) infusion of [ $3$ - $^3$ H]glucose was started at -120 min and continued throughout the study for determination of glucose kinetics. At -90 min, all dogs received an infusion of SRIF (0.8  $\mu$ g/kg per min) followed 2 min later by intraportal replacement of basal glucagon (0.65 ng/kg per min) and insulin (175-250

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1. Abbreviations used in this paper: h $\beta$ E, human  $\beta$ -endorphin; SRIF, somatostatin.

$\mu\text{U/kg}$  per min). Fine adjustments were made in the insulin infusion rate to maintain euglycemia. After this was achieved, the infusion rates of all hormones remained fixed for the remainder of the experiment. 40 minutes later, the basal sampling period was begun. During the 3-h experimental period, seven control dogs received a saline infusion and 10 dogs received synthetic human  $\beta$ -endorphin (h $\beta$ E, Beckman Instruments, Inc., Palo Alto, CA) at a rate of 0.2 mg/h. In four dogs receiving h $\beta$ E, euglycemia was maintained with a variable rate infusion of 10% dextrose in normal saline.

**Processing of blood samples.** Blood samples were drawn every 10 min in the basal period and every 15 min thereafter. The collection, immediate processing, and assaying of blood samples have been previously described (12). Plasma glucose was measured by the glucose oxidase method on a Beckman Model II glucose analyzer (Beckman Instruments, Inc.). Plasma immunoreactive glucagon was assayed using 30 K antibody obtained from the University of Texas, Southwestern Medical School. Immunoreactive insulin was measured by the Sephadex-bound antibody procedure (Pharmacia Fine Chemicals, Piscataway, NJ). Circulating catecholamines were assayed by radioimmunoassay with a Cat-A-Kit purchased from the Upjohn Co. (Kalamazoo, MI). Plasma cortisol levels were measured with a radioimmunoassay kit from Micromedics Systems, Inc. (Horsham, PA). Plasma  $\beta$ -endorphin was measured by a radioimmunoassay using  $^{125}\text{I}$ -labeled tracer and rabbit anti-h $\beta$ E antiserum (13).

Tracer-determined rates of glucose production and utilization (milligrams per minute per kilogram) and clearance (milligrams per minute per kilogram) were calculated as described previously (14).

Statistical analyses were performed using the Students *t* test, the paired *t* test, and analyses of variance where appropriate. All data are expressed as the mean  $\pm$  standard error of the mean.

## Results

The effect of h $\beta$ E infusion on glucose kinetics is shown in Fig. 1. Plasma glucose concentration fell from a stable basal value of  $107 \pm 5$  to  $76 \pm 6$  mg/dl by 3 h ( $P < 0.001$ ). This effect was due to a rapid and sustained 25% fall ( $P < 0.05$ ) in glucose production. Glucose utilization also decreased but the decline was slow and not significant. Glucose clearance, which initially did not change, increased by 20% ( $P < 0.05$ ) during the last hour of h $\beta$ E infusion.

Table I demonstrates that these glucose-lowering effects of h $\beta$ E cannot be attributed to changes in major glucoregulatory hormones. Insulin and glucagon remained clamped at basal levels ( $10 \pm 1$   $\mu\text{U/ml}$  and  $72 \pm 8$  pg/ml, respectively) throughout h $\beta$ E infusion. Human  $\beta$ E caused modest but not statistically significant rises in both epinephrine and norepinephrine. Plasma levels of h $\beta$ E appeared to reach steady state after 2 h at  $\sim 32 \pm 2$  ng/ml. Basal levels of endogenous canine  $\beta$ -endorphin were not measured in these studies, but were found to be  $8.7 \pm 4.7$  pg/ml (mean  $\pm$  1 SD) in a conscious, catheterized dog whose blood was sampled half-hourly for 24 h (D. N. Orth and M. E. Peterson, unpublished observations).

Fig. 2 shows the same studies conducted in four dogs where plasma glucose was maintained near basal levels by administration of exogenous glucose. As shown, there was a 45% drop

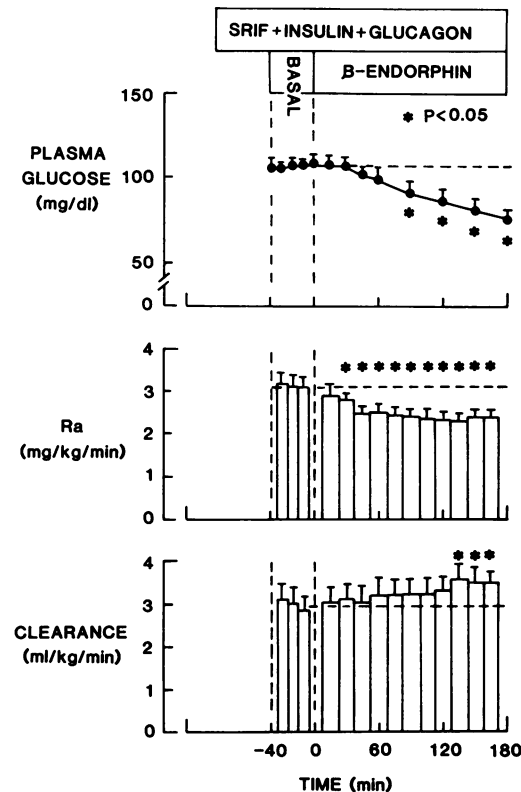


Figure 1. The effect of h $\beta$ E (0.2 mg/h) on plasma glucose, glucose production, and glucose clearance in six dogs receiving SRIF plus intraportal replacement of basal insulin and glucagon.

in glucose production. This was considerably more ( $P < 0.05$ ) than the 25% fall seen in the dogs which received h $\beta$ E without glucose. In contrast, there was no increase in glucose clearance in this group. The small rise in catecholamines noted previously was prevented by the maintenance of euglycemia; insulin, glucagon, and cortisol remained unchanged (Table I).

Fig. 3 shows that SRIF plus intraportal replacement of basal insulin and glucagon did not change any of the glucose parameters in seven control dogs which did not receive h $\beta$ E.

## Discussion

Recently, we reported that a low-dose infusion of morphine sulfate inhibited glucose production and caused hypoglycemia in conscious dogs. This effect was independent of pancreatic glucoregulatory hormones which were fixed at basal levels by infusion of SRIF with intraportal replacement of insulin and glucagon (11). The present study demonstrates that the endogenous opioid peptide,  $\beta$ -endorphin, has similar effects.

Human  $\beta$ -endorphin at 0.2 mg/h infused into conscious dogs with the endocrine pancreas clamped at basal levels caused

Table I. Effect of  $\beta$ -Endorphin Infusion on Plasma Insulin, Glucagon, Epinephrine, Norepinephrine Cortisol, and  $\beta$ -Endorphin Levels in 10 Conscious Overnight Fasted Dogs Receiving SRIF with Intraportal Replacement of Basal Insulin and Glucagon\*

	Time						
	Basal	+30	+60	+90	+120	+150	+180
<b>Insulin (<math>\mu</math>U/ml)</b>							
Group I	10 $\pm$ 1	10 $\pm$ 1	10 $\pm$ 1	10 $\pm$ 1	10 $\pm$ 1	10 $\pm$ 1	10 $\pm$ 1
Group II	11 $\pm$ 1	11 $\pm$ 2	10 $\pm$ 2	10 $\pm$ 2	10 $\pm$ 2	10 $\pm$ 2	11 $\pm$ 2
<b>Glucagon (pg/ml)</b>							
Group I	72 $\pm$ 8	74 $\pm$ 6	72 $\pm$ 6	73 $\pm$ 6	70 $\pm$ 7	71 $\pm$ 6	69 $\pm$ 6
Group II	91 $\pm$ 18	85 $\pm$ 20	86 $\pm$ 23	86 $\pm$ 23	88 $\pm$ 21	96 $\pm$ 26	96 $\pm$ 27
<b>Epinephrine (pg/ml)</b>							
Group I	66 $\pm$ 6	107 $\pm$ 10	92 $\pm$ 17	—	82 $\pm$ 5	—	71 $\pm$ 20
Group II	41 $\pm$ 9	39 $\pm$ 11	42 $\pm$ 8	—	33 $\pm$ 12	—	45 $\pm$ 16
<b>Norepinephrine (pg/ml)</b>							
Group I	109 $\pm$ 5	147 $\pm$ 32	130 $\pm$ 19	—	118 $\pm$ 8	—	105 $\pm$ 11
Group II	88 $\pm$ 6	82 $\pm$ 4	100 $\pm$ 14	—	91 $\pm$ 13	—	103 $\pm$ 18
<b>Cortisol (g/dl)</b>							
Group I	1.7 $\pm$ 0.5	1.6 $\pm$ 0.4	2.2 $\pm$ 0.5	—	2.7 $\pm$ 1.0	—	2.5 $\pm$ 1.2
Group II	1.3 $\pm$ 0.2	1.5 $\pm$ 0.6	1.8 $\pm$ 0.7	—	2.0 $\pm$ 0.5	—	1.2 $\pm$ 0.3
<b><math>\beta</math>-endorphin (ng/ml)</b>							
Group I and Group II	—	23 $\pm$ 2	28 $\pm$ 2	—	32 $\pm$ 2	—	32 $\pm$ 2

\* Group I dogs ( $n = 6$ ) did not receive exogenous glucose to maintain euglycemia. Group II dogs ( $n = 4$ ) received exogenous glucose to maintain euglycemia.

a 29% decline in plasma glucose over 3 h. The data indicate that the fall in glucose was due primarily to inhibition of tracer-determined glucose production. In addition, there was a 20% increase in glucose clearance during the last hour of h $\beta$ E infusion in these dogs which raised the possibility that the peptide might affect glucose utilization. When hypoglycemia was prevented in four additional dogs, however, the apparent effect of h $\beta$ E on clearance was essentially abolished. The maintenance of euglycemia during endorphin infusion significantly enhanced the inhibitory effect of the opioid on glucose production. This indicated that the fall in plasma glucose blunted h $\beta$ E's effect, possibly in part through the small rise in circulating catecholamines. These effects of h $\beta$ E on plasma glucose and glucose kinetics are markedly similar to those we reported for morphine (11).

Inhibition of glucose production by the h $\beta$ E was independent of changes in circulating insulin, glucagon, and cortisol. The data also indicate that endorphin action was not mediated by changes in catecholamine levels. The small increase in catecholamines observed in the first group of dogs was probably brought about indirectly by the fall in plasma glucose since it

failed to occur when the fall was prevented. This increase, if anything, would also tend to oppose rather than mediate the effect of endorphin on glucose production. Of interest here is the observation that the initial inhibition of glucose production by morphine (11) was associated with a small but significant decrease in circulating epinephrine. This suggested that a decrease in basal catecholamines might contribute to the hypoglycemia. The present study indicates that opioid-induced hypoglycemia occurs even when catecholamines levels are unchanged.

Since the liver is essentially the sole source of endogenous glucose production in the 18-h fasted dog (15), our results would suggest that  $\beta$ -endorphin exerts its hypoglycemic effect by inhibiting hepatic glucose production. Furthermore, glycogen stores are still fairly replete in the overnight fasted dog (14); therefore,  $\beta$ -endorphin might act by inhibiting glycogenolysis and/or gluconeogenesis. The relative contributions of these possibilities cannot be assessed from the present data.

The effect of endorphin could involve direct interaction of opioid with hepatocytes or it could be centrally mediated via autonomic innervation to the liver. Our results suggest that it is a peripheral effect most likely occurring at the liver since  $\beta$ -

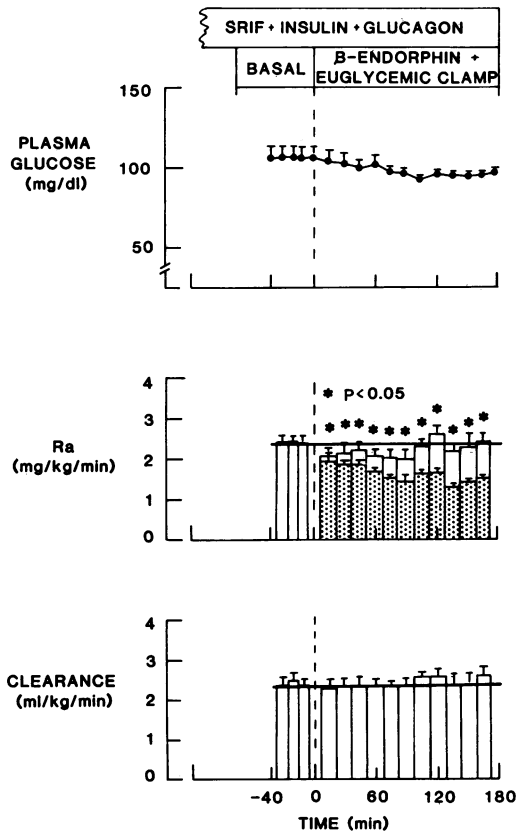


Figure 2. The effect of h $\beta$ E (0.2 mg/h) plus euglycemic clamp on plasma glucose, glucose production, and glucose clearance in four dogs receiving SRIF plus intraportal replacement of insulin and glucagon.

endorphin, in contrast to morphine, does not cross the blood-brain barrier in significant amounts when given intravenously (16).

The relationship of our findings to the overall importance of opioids in gluoregulation remains to be determined. Our results do not necessarily contradict those from previous studies. They indicate, however, that opioids may have both hyperglycemic and hypoglycemic effects. The nature of these effects could be dose-related (11) and might ultimately depend on the prevailing metabolic state. Opioid effects might involve modulation of pancreatic secretions (4–10) or a centrally mediated change in circulating catecholamines (1–3). As described in this report, endorphins can also exert direct alterations in glucose production which are independent of detectable change in major gluoregulatory hormones. Recognition of the multiple actions of opioids will be necessary in future studies in order to better understand the mechanism of their effects on glucose homeostasis.

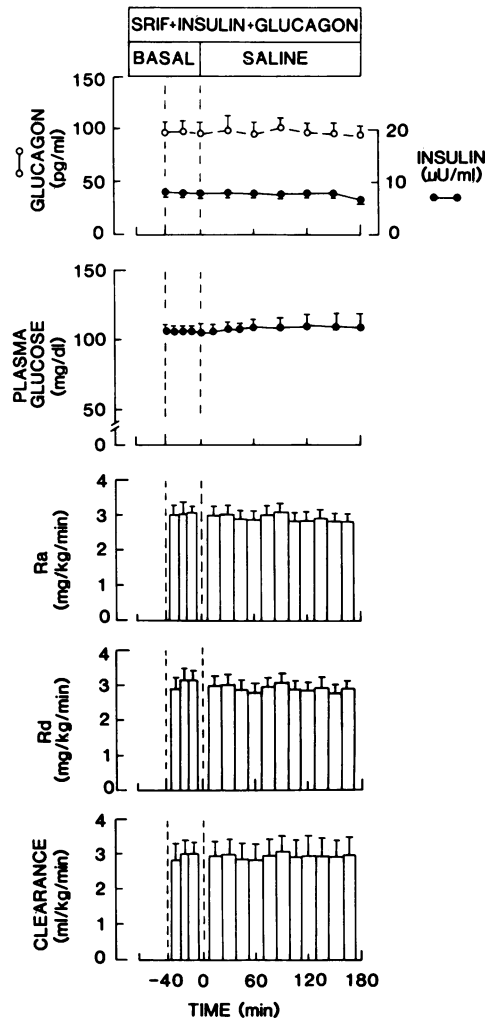


Figure 3. The effect of SRIF plus intraportal replacement of basal insulin and glucagon in seven overnight fasted dogs. Values for epinephrine, norepinephrine, and cortisol which remained unchanged from basal values of  $56 \pm 16$  pg/ml,  $68 \pm 14$  pg/ml, and  $2.6 \pm 0.7$   $\mu$ g/dl, respectively, in these dogs are not shown.

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### References

1. Vassalle, M. 1961. Role of catecholamine release in morphine hyperglycemia. *Am. J. Physiol.* 200:530–534.

2. Feldberg, W., and S. V. Shaligram. 1972. The hyperglycemic effect of morphine. *Br. J. Pharmacol.* 46:602-618.
3. Van Loon, G. R., and N. M. Appel. 1981.  $\beta$ -Endorphin-induced hyperglycemia is mediated by increased central sympathetic outflow to adrenal medulla. *Brain Res.* 204:236-241.
4. Ipp, E., R. Dobbs, and R. H. Unger. 1978. Morphine and  $\beta$ -endorphin influence the secretion of the endocrine pancreas. *Nature (Lond.)* 276:190-192.
5. Kanter, R. A., J. W. Ensink, and W. Y. Fujimoto. 1980. Disparate effects of enkephalin and morphine upon insulin and glucagon secretion by islet cell cultures. *Diabetes.* 29:84-86.
6. Green, I. C., D. Perrin, K. C. Pedley, R. D. G. Leslie, and D. A. Pyke. 1980. Effect of enkaphalins and morphine on insulin secretion from isolated rat islets. *Diabetologia.* 19:158-161.
7. Reid, R. L., and S. S. C. Yen. 1981.  $\beta$ -Endorphin stimulates the secretion of insulin and glucagon in humans. *J. Clin. Endocrinol. Metab.* 52:592-594.
8. Ipp, E., V. Schusdziarra, V. Harris, and R. H. Unger. 1980. Morphine-induced hyperglycemia: role of insulin and glucagon. *Endocrinology.* 107:461-463.
9. Feldman, M., R. S. Kiser, R. H. Unger, and C. H. Li. 1983.  $\beta$ -Endorphin and the endocrine pancreas. *N. Engl. J. Med.* 308:349-353.
10. Ipp, E., J. Dhorajiwala, W. Pugh, A. R. Moossa, and A. H. Rubenstein. 1982. Effects of an enkephalin analogue on pancreatic function and glucose homeostasis in normal and diabetic dogs. *Endocrinology.* 111:2110-2116.
11. Radosevich, P. M., J. R. McRae, P. E. Williams, and N. N. Abumrad. 1983. Effect of the opiate antagonists, morphine and naloxone, on glucose turnover independent of changes in insulin and glucagon. *Surg. Forum.* 34:56-58. (Abstr.)
12. Abumrad, N. N., L. S. Jefferson, S. L. Rannels, P. E. Williams, A. D. Cherrington, and W. W. Lacy. 1982. The role of insulin in the regulation of leucine kinetics in the conscious dog. *J. Clin. Invest.* 70:1031-1041.
13. Bertagna, X. Y., W. J. Stone, W. E. Nicholson, C. D. Mount, and D. N. Orth. 1981. Simultaneous assay of immunoreactive  $\beta$ -lipotropin,  $\gamma$ -lipotropin, and  $\beta$ -endorphin in plasma of normal human subjects, patients with ACTH/lipotropin hypersecretory syndromes, and patients undergoing chronic hemodialysis. *J. Clin. Invest.* 67:124-133.
14. Abumrad, N. N., A. D. Cherrington, P. E. Williams, W. W. Lacy, and D. Rabin. 1982. Absorption and disposition of a glucose load in the conscious dog. *Am. J. Physiol.* 242:E398-E406.
15. Cherrington, A. D., J. L. Chiasson, J. E. Liljenquist, W. W. Lacy, and C. R. Park. 1978. Control of hepatic glucose output by glucagon and insulin in the intact dog. *Biochem. Soc. Symp.* 43:31-41.
16. Houghten, R. A., R. W. Swann, and C. H. Li. 1980.  $\beta$ -Endorphin: stability, clearance behavior, and entry into the central nervous system after intravenous injection of the tritiated peptide in rats and rabbits. *Proc. Natl. Acad. Sci. USA.* 77:4588-4591.