Impaired Insulin Secretion in Human Diabetes Mellitus

The Effect of Naloxone-Induced Opiate Receptor Blockade

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SUMMARY

Human diabetes mellitus is characterized by impaired insulin response to intravenous glucose. In search of possible factors which impair insulin release, we have investigated the effect of naloxone, a specific opiate receptor blocker, on insulin responses to glucose in subjects with non-insulin-dependent diabetes, as well as in normal subjects. Naloxone was given as a priming dose of 0.4 mg followed by a constant infusion of either 0.4 mg (N = 7), 2 mg (N = 7), or 4 mg (N = 8) for 90 min. Acute insulin response to glucose (mean change 3–10 min insulin), second phase insulin secretion (change 10–60 min), as well as glucose disappearance rates (%/min) were significantly increased in the diabetics receiving the two higher doses of naloxone (2 and 4 mg, respectively). None of these effects were seen in diabetics receiving saline or in normal subjects receiving naloxone. These results seem to suggest that sensitivity to endogenous opiates may play some part in non-insulin-dependent diabetes.

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Endogenous substances with opioid activity (endorphins) are putative neurotransmitters in the central nervous system.[1] Recent evidence indicates the presence of endogenous opiates in neural and epithelial sites of gastrointestinal tissues.[2] In human endocrine pancreas, immunoreactive beta-endorphin has been found by radioimmunoassay and gel chromatography,[3] which suggests that opiate compounds may play some role in modulating pancreatic hormone release. A number of studies suggest a relationship between opiates and carbohydrate metabolism. The hyperglycemic effect of morphine has been a well recognized phenomenon throughout the century[4] and recently, beta-endorphin has been shown to increase insulin and glucagon levels in humans.[5] Moreover, sensitivity to enkephalins is thought to play some role in the pathogenesis of non-insulin-dependent diabetes (NIDD), since chlorpropramide alcohol flushing (CPAF), which occurs in some subjects with NIDD, can be blocked by naloxone, a specific opiate receptor blocker.[6]

The present study was undertaken to determine whether endogenous opiates could be implicated in the impaired insulin secretion to intravenous glucose in human diabetes. Evidence has been presented that in NIDD endogenous substances are present which impair insulin secretion.[7,8]

SUBJECTS AND METHODS

Twenty-two patients (14 females and 8 males) with NIDD and 10 normal subjects were studied. The diabetics ranged from 39 to 64 yr of age and at the time of the study were consuming weight-maintaining diets with at least 250 g of carbohydrate/day. They were taking no drugs. Sixteen subjects were within 15% of their ideal body weight (Metropolitan Life Insurance Tables, 1959) and six were overweight. The normal subjects were inpatients convalescing from minor disease (age range 30–45 yr) who were nonobese and without a personal or family history of diabetes. All subjects gave informed consent before voluntary participation in the study.

All studies were performed in the morning, in the postabsorptive state, after a 12–14-h fast. Intravenous lines were inserted in both antecubital veins, for blood sampling and the infusion of the various test agents. Patency was preserved by a slow saline drip. After the subjects had been resting for 30 min, four basal samples were obtained at 10-min intervals before glucose stimulation (only the mean of these four points is shown in the figures). Glucose stimulation was provided by injecting intravenously two pulses of glucose (0.33 g/kg, in less than 1 min), 120 min apart. Naloxone (Narcan, Crinos, Italy) was given as in i.v. bolus of 0.4 mg followed by the infusion of either 0.4 mg (seven diabetics), 2 mg (seven diabetics), or 4 mg (eight diabetics) for the subsequent period of the study (90 min). Control studies with saline instead of naloxone were performed in seven di-

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abetics. Some diabetics were studied twice; the interval between the two tests in the same diabetic was at least 7 days. Normal subjects received the two glucose pulses before and during the infusion of the intermediate dose of naloxone (0.4 + 2 mg).

Basal insulin level was calculated as the mean of the four samples drawn before the first pulse. The acute insulin response (AIR) to glucose was calculated as the mean of 3, 5, 8, and 10 min postglucose injection values minus the insulin level immediately before the pulse. Second phase insulin secretion was calculated as the insulin area from 10 to 60 min after the pulse which was above the insulin level (mean of two points) immediately before the pulse. Glucose disappearance rates were calculated with the method of the least squares taking the natural logs of the glucose concentrations of 15, 30, 45, and 60 min. The methods for the determination of plasma glucose and immunoreactive insulin have been previously described.[9] All samples from one subject were measured in the same assay. Statistical analysis of the results was performed by the Student's *t*, paired *t* test, and Wilcoxon rank sum test. Results are presented as mean ± SE.

RESULTS

All diabetics had fasting plasma glucose levels above 130 mg/dl. After the first glucose pulse in the diabetics receiving the smaller dose of naloxone (Figure 1), there was a weak and poor acute insulin response (AIR = 1.7 ± 1 μU/ml). The infusion of naloxone failed to alter significantly this response (4 ± 2.5 μU/ml, P = NS). Second phase insulin secretion was 180 ± 37 μU/ml · min^{-1} after the first pulse and 205 ± 40 μU/ml · min^{-1} after the second (Table 1). There was a slight but not significant increase in the glucose disappearance rates after naloxone (Table 1). However, even if insulin responses to glucose were expressed as the insulinogenic index in order to exclude the influence of different glycemic stimuli, this ratio was similar after both the first and the second glucose pulses. In control studies (Figure 1), in which saline rather than naloxone was infused, the acute insulin response to both the first and the second glucose pulses was quite similar, nor was there a difference in second phase insulin secretion as well as in glucose disappearance rates (Table 1).

The infusion of the 2-mg dose of naloxone in seven diabetics (Figure 2) caused a partial restoration of the acute insulin response to glucose (AIR = 4.3 ± 1.5 μU/ml after the first pulse vs. 12.5 ± 3.5 μU/ml after the second glucose pulse, P < 0.05) and a slight but significant augmentation of the second phase insulin secretion (Table 1). Glucose disappearance rates were also significantly increased after naloxone (kG: first pulse = 0.48 ± 0.05; second pulse = 0.95 ± 0.1 %/min, P < 0.05).

The infusion of the highest dose of naloxone (4 mg) in seven diabetics (Figure 3) produced a fourfold augmentation of the acute insulin response to glucose (AIR = 3 ± 1 μU/ml after the first pulse vs. 13.5 ± 3.5 μU/ml, P < 0.01). In addition, second phase insulin secretion was augmented (first pulse = 220 ± 40 μU/ml · min^{-1}; second pulse = 432 ± 65 μU/ml · min^{-1}, P < 0.01). Moreover, there was a significant increase in the mean glucose disappearance rates (kG: first pulse 0.55 ± 0.05; second pulse = 1 ± 0.1 %/min, P < 0.01, Table 1.)

Those diabetics who were studied on two occasions pre-

FIGURE 1. Comparison of insulin responses to glucose pulses given before and during an infusion of naloxone (0.4 mg) or saline in non-insulin-dependent diabetics (N = 7).

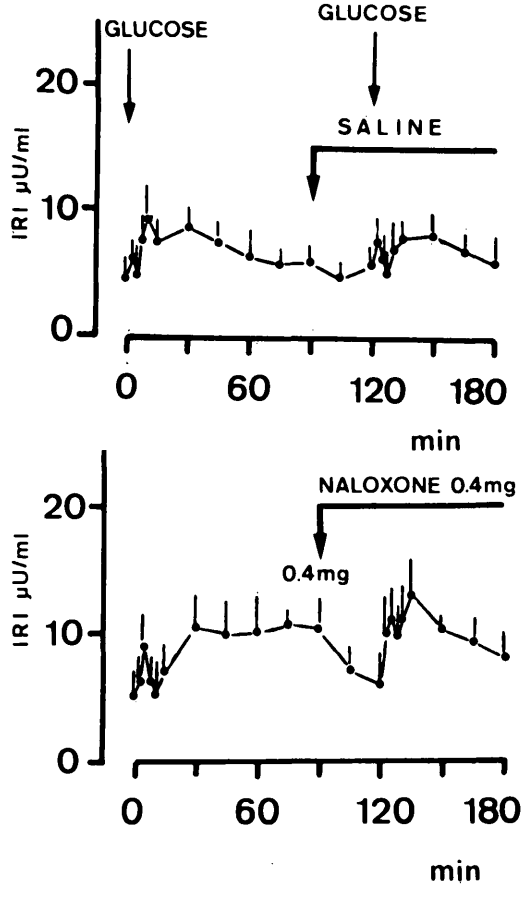


FIGURE 2. Comparison of insulin responses to glucose pulses given before and during an infusion of naloxone (2 mg) in non-insulin-dependent diabetics (N = 7).

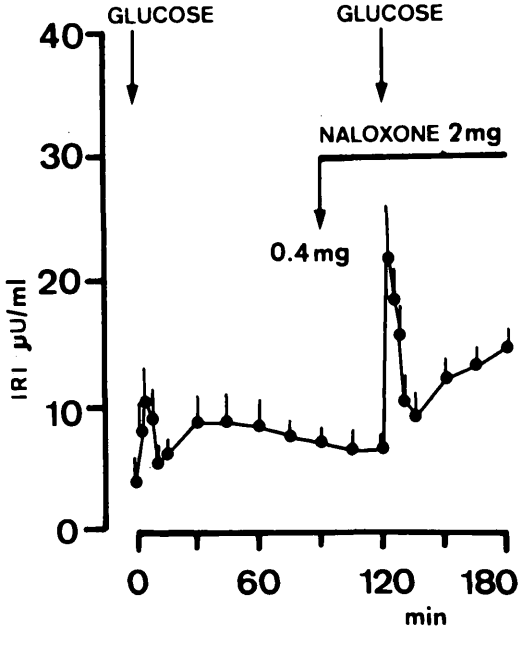


TABLE 1
Plasma glucose levels before (basal) and at various times during the experiments performed. Acute insulin responses (AIR), second phase insulin secretion (2nd), and glucose disappearance rates KG are compared

	Plasma glucose (mg/dl)					Pulse I			Pulse II		
	Basal	60 min	90 min	120 min	180 min	KG (%/min)	AIR (μU/ml)	2nd (μU/ml · min)	KG (%/min)	AIR (μU/ml)	2nd (μU/ml · min)
Nal. (0.4 mg) NIDD (N = 7)	175 ± 22	217 ± 24	198 ± 22	180 ± 24	225 ± 24	0.39 ± 0.07	1.7 ± 1	180 ± 37	0.54 ± 0.08	4.0 ± 2.5	205 ± 40
Nal. (2 mg) NIDD (N = 7)	185 ± 21	225 ± 22	204 ± 19	190 ± 19	200 ± 20	0.48 ± 0.05	4.3 ± 1.5	200 ± 34	0.95 ± 0.1*	12.5 ± 3.5*	280 ± 43*
Nal. (4 mg) NIDD (N = 8)	167 ± 18	205 ± 21	190 ± 20	179 ± 19	191 ± 18	0.55 ± 0.05	3.0 ± 1	220 ± 40	1.00 ± 0.1*	13.5 ± 3.5*	432 ± 65*
Saline NIDD (N = 7)	180 ± 24	222 ± 24	204 ± 20	175 ± 19	225 ± 20	0.45 ± 0.07	4.0 ± 1	171 ± 40	0.40 ± 0.06	2.0 ± 1.0	115 ± 30
Nal. (2 mg) Normals (N = 7)	85 ± 4	82 ± 5	78 ± 4	74 ± 4	70 ± 5	2.12 ± 0.17	42 ± 8		2.17 ± 0.19	44 ± 9	

* Indicates significant differences.

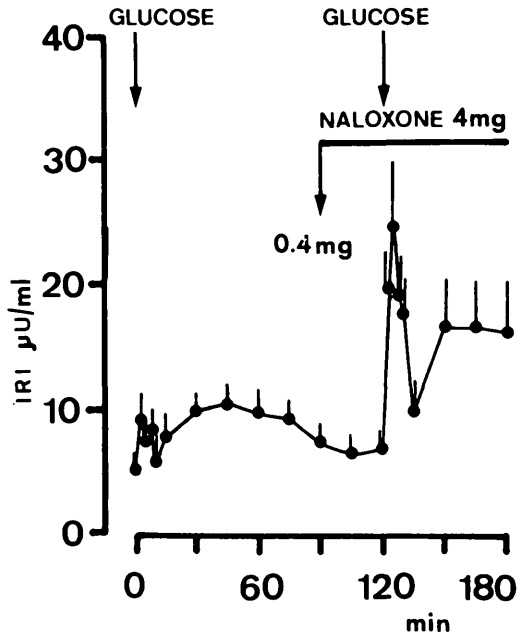
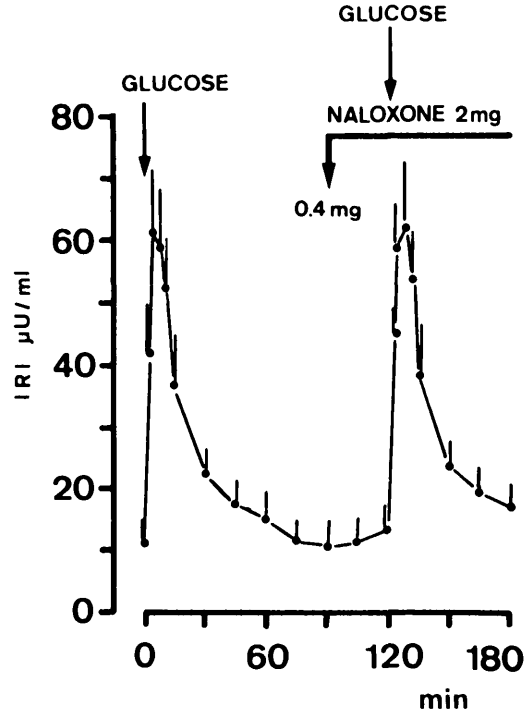


FIGURE 3. Comparison of insulin responses to glucose pulses given before and during an infusion of naloxone (4 mg) in non-insulin-dependent diabetics (N = 8).

sented similar insulin responses to the first glucose pulses during the two tests.

All normal subjects had fasting plasma glucose levels below 100 mg/dl. After the first glucose pulse, there was an immediate insulin response to glucose; insulin returned to basal values by 90 min (Figure 4). Naloxone failed to alter significantly the acute insulin response to the second glucose pulse (Table 1). Similarly, there was no significant difference in glucose disappearance rates after both the first

FIGURE 4. Circulating insulin levels in responses to glucose pulses before and during an infusion of naloxone (2 mg) in normal humans (N = 7).



and the second glucose pulses. This pattern of response was reproduced in additional subjects receiving the 4 mg-dose of naloxone (data not shown). Naloxone was well tolerated by all subjects.

DISCUSSION

The results of these studies demonstrate that naloxone, a specific opiate receptor blocker, causes a partial restoration of the acute insulin response to glucose in NIDD and increases significantly glucose disappearance rates. These effects, however, were observed with the higher doses of naloxone (2 and 4 mg, respectively), no significant change in either acute insulin response, second phase insulin secretion and glucose tolerance being detected with the smaller dose (0.4 mg) of the drug. None of these effects of naloxone were observed in saline control studies, which suggests that the effects are presumably specific for the drug. In normal subjects, naloxone (2 mg) failed to alter significantly insulin responses and glucose tolerance.

Subjects with NIDD and fasting hyperglycemia lack acute insulin response to glucose, which is retained for a variety of other secretagogues, including glucagon[10] and amino acids.[11] This has led to the concept of the presence of endogenous substances that impair acute insulin response to glucose in this form of diabetes. Interestingly, both phentolamine, an alpha-adreneragic receptor blocker, and acetylsalicylic acid, an inhibitor of endogenous prostaglandin synthesis, cause a partial restoration of insulin responses to glucose in NIDD.[7,8] This suggests that an increased alpha-adrenergic drive and/or an increased synthesis of prostaglandins (E series) may be important in this respect. The demonstration that naloxone also causes a partial restoration of the acute insulin response in NIDD seems to suggest the inclusion of endorphins in the list of endogenous substances which impair insulin secretion in NIDD.

In the isolated and perfused dog pancreas, Ipp et al.[12] have shown that morphine increased insulin and glucagon secretion but inhibited somatostatin release. Subsequently, the same investigators noted that the hyperglycemic effect of morphine was augmented in alloxanized diabetic dogs as a consequence of opiate-induced glucagon secretion in the absence of accompanying insulin release.[13] Finally, beta-endorphin has been shown to increase insulin and glucagon concentrations in humans.[5] However, in goats, a potent analogue of methionine-enkephalin, as well as morphine, produces a decrease in plasma insulin levels,[14] and in man, the administration of a long-acting enkephalin (DAMME) induces a fall in plasma glucose levels without changing levels of insulin and glucagon.[6] In the isolated rat islets, enkephalins show a dose-dependent effect on insulin secretion, low concentrations (10^{-10}–10^{-8}) being stimulatory and high concentrations (10^{-5}) being inhibitory.[15] Indeed, Morley et al.[16] have reported that naloxone increased serum insulin but not glucagon levels, and did not change insulin response to glucose in normal subjects. In diabetics, naloxone infusion depresses beta-hydroxybutyrate and FFA levels by inhibiting lipolysis (increased insulin?).[17]

At present, a definite picture cannot be drawn as the physiologic, if any, role of endogenous opiates in the control of the endocrine pancreas. On the other hand, the observation that CPAF, which is an inherited dominant trait found in some NID diabetics, can be blocked by naloxone[6] and the results of our studies that naloxone can restore partially the insulin response to glucose in NIDD seems to suggest that sensitivity to endogenous opiates may play some part in this form of diabetes.

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