Rapid Publication

Evidence for an Effect of Exorphins on Plasma Insulin and Glucagon Levels in Dogs

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SUMMARY

Recently, peptides with opioid-like activity have been demonstrated in peptic digests of dietary protein. The present study was designed to determine the effect of digested and undigested gluten on postprandial insulin and glucagon levels in conscious dogs. The intragastric instillation of digested gluten (25 g) elicited a more rapid and a significantly greater rise in postprandial peripheral vein insulin and glucagon levels compared with the effect of 25 g undigested gluten. The incremental insulin level was 104 ± 20 μU/ml after digested gluten and only 58 ± 7 μU/ml ($P < 0.01$) after undigested gluten; the respective values for glucagon are 426 ± 25 pg/ml versus 302 ± 20 pg/ml ($P < 0.01$). The intragastric administration of naloxone (4 mg), a specific opiate receptor antagonist, reduced the insulin response and augmented the glucagon response to the digested gluten test meal, whereas the response of both hormones to the undigested gluten meal was not affected by naloxone. Intravenously infused naloxone during the digested gluten meal did not influence insulin or glucagon levels. The present data suggest that in dogs the peptic digest of gluten contains an opioid-like material that stimulates postprandial insulin and glucagon release. DIABETES *30*: 362–364, April 1981.

Previous studies have demonstrated the presence of opiate-like activity in peptic digests of dietary proteins such as β-casein and wheat gluten.[1–3] These compounds have been called exorphins in analogy to the endogenously derived opiate-like materials. In view of the recent demonstration of the biologic activity of β-endorphin, enkephalin, and morphine upon pancreatic endocrine function,[4–6] the present study was designed to determine whether the ingestion of a peptic digest of gluten would be followed by an endocrine response that could be due to opiate-like materials.

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MATERIALS AND METHODS

The studies were performed in 8 conscious normal dogs (body wt 26–35 kg). After an overnight fast, each dog received an intragastric test meal of 25 g gluten or 25 g digested gluten together with the specific opiate receptor antagonist naloxone (4 mg)[7,8] (Narcanti, Winthrop), or saline, respectively. In another series of experiments the same dogs received naloxone intravenously (4 mg/h) during the intragastric instillation of digested gluten. All experiments were carried out in randomized order and each dog served as its own control.

The peptic digest of gluten was prepared as described by Zioudrou et al.,[1] and after the 2-h incubation period the reaction was stopped by neutralization and subsequent lyophilization. Twenty-five grams of gluten or digested gluten, respectively, was dissolved in 300 ml water just before instillation via a gastric tube. Frequent blood samples were drawn before and after the instillation of the test meal, and blood was collected into chilled tubes containing 500 KIU Trasylol and 1.2 mg EDTA. All samples were centrifuged at 2000 rpm at 4°C for 20 min and the separated plasma was frozen until the time of assay.

Plasma insulin[9,10] and glucagon[11] levels were determined as previously described and glucose was measured by the glucose-oxidase method using the Technicon autoanalyzer.

For statistical comparisons, Student's *t* test for paired data was employed and P values of 0.05 or less were considered significant. Incremental data were calculated as the sum of the values at each time point above the mean of the three baseline samples.

RESULTS

Effect of gluten or digested gluten on postprandial plasma insulin and glucagon levels. The intragastric instillation of 25 g gluten elicited an increase of plasma insulin levels from a mean baseline of 12 ± 3 μU/ml by 13 μU/ml to a maximum of 25 ± 4 μU/ml at 30 min (Figure 1).

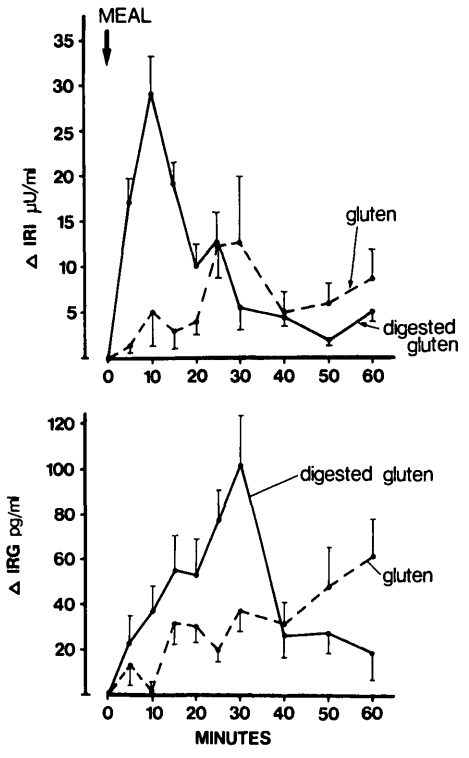


FIGURE 1. Effect of the intragastric instillation of gluten (25 g) or digested gluten (25 g) dissolved in 300 ml water on the increase in postprandial peripheral vein plasma insulin (IRI) and glucagon (IRG) levels above the mean of the three baseline values in 8 conscious dogs (mean ± SEM).

In response to digested gluten, insulin levels rose sharply by 30 μU/ml within 10 min from a mean baseline of 11 ± 3 μU/ml. The incremental insulin level during digested gluten was 104 ± 20 μU/ml, significantly above the value of 58 ± 7 μU/ml in response to the undigested gluten test meal ($P < 0.01$).

In response to gluten, plasma glucagon levels rose from a mean baseline of 86 ± 10 pg/ml by 65 pg/ml within the 60-min experimental period, whereas after digested gluten plasma glucagon levels increased by 100 pg/ml within 30 min from a mean baseline of 70 ± 7 pg/ml (Figure 1). The incremental plasma glucagon level was 302 ± 20 pg/ml in response to gluten and 426 ± 25 pg/ml in response to digested gluten ($P < 0.01$).

Effect of naloxone on postprandial plasma insulin and glucagon levels in response to gluten or digested gluten. The intragastric administration of naloxone to the digested gluten test meal reduced the postprandial insulin response substantially. The incremental insulin level was only 42 ± 7 μU/ml, significantly below the 104 ± 20 μU/ml of the saline controls ($P < 0.01$). Plasma glucagon levels rose more rapidly when naloxone was added to the digested gluten meal and remained elevated by 80–100 pg/ml above baseline for the entire experimental period (Figure 2). The incremental glucagon level with naloxone was 766 ± 25 pg/ml, significantly above the 426 ± 25 pg/ml of the saline controls ($P < 0.005$).

In response to the gluten meal, the intragastric addition of naloxone did not change postprandial insulin and glucagon levels (Figure 3). The incremental insulin level with naloxone was 42 ± 7 μU/ml versus 58 ± 7 μU/ml in the saline controls. The respective values for glucagon were 350 ± 16 pg/ml versus 302 ± 12 pg/ml ($P > 0.05$).

FIGURE 2. Effect of the intragastric administration of naloxone (4 mg) or saline together with the digested gluten test meal (25 g in 300 ml water) on the increase in postprandial peripheral vein plasma insulin (IRI) and glucagon (IRG) levels above the mean of the three baseline values in 8 conscious dogs (mean ± SEM).

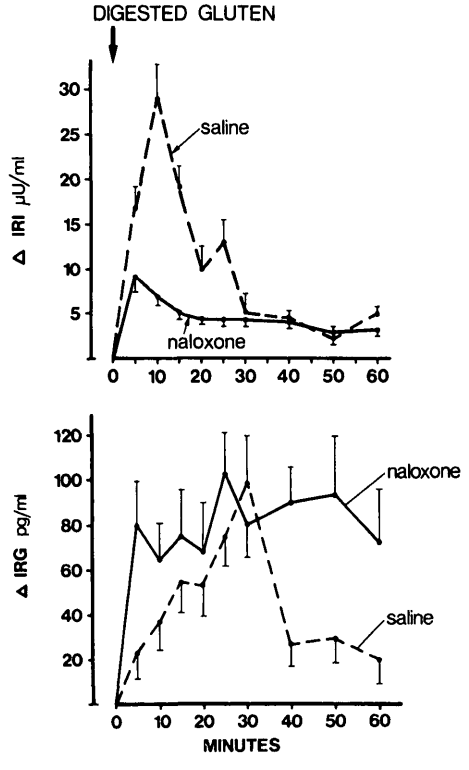
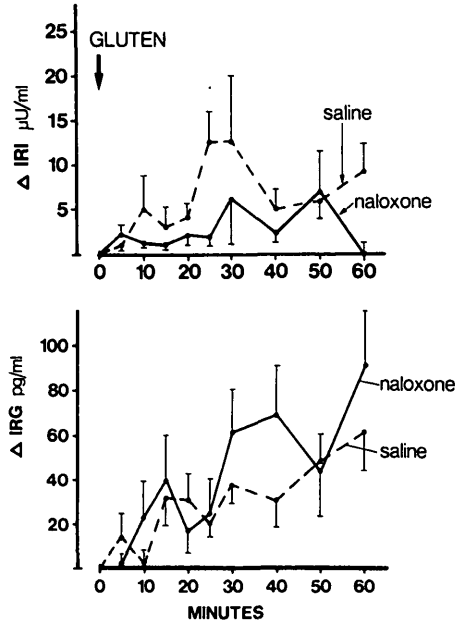


FIGURE 3. Effect of the intragastric administration of naloxone (4 mg) or saline together with the undigested gluten test meal (25 g in 300 ml water) on the increase in postprandial peripheral vein plasma insulin (IRI) and glucagon (IRG) levels above the mean of the three baseline values in 8 conscious dogs (mean ± SEM).



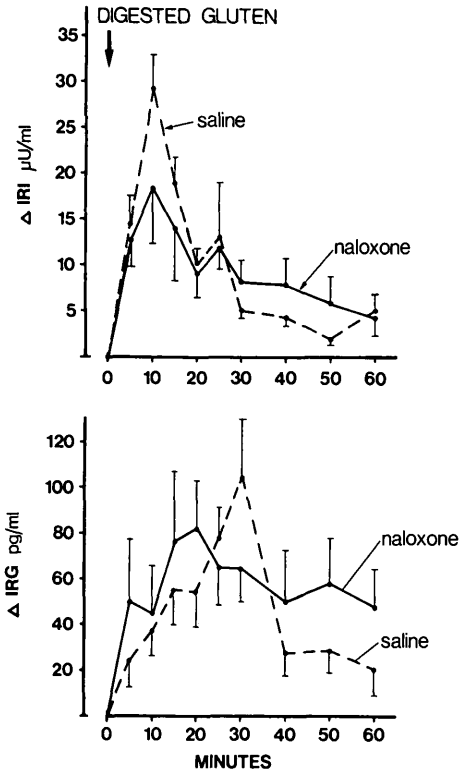


FIGURE 4. Effect of the intravenous infusion of naloxone (4 mg/h) during the intragastric instillation of digested gluten (25 g in 300 ml water) on the increase in postprandial peripheral vein plasma insulin (IRI) and glucagon (IRG) levels above the mean of the three baseline values in 8 conscious dogs (mean ± SEM).

To determine if the effects on insulin and glucagon levels observed during the meal of digested gluten could be influenced by naloxone when administered intravenously, naloxone was infused at a rate of 4 mg/h starting with the intragastric instillation of digested gluten. As shown in Figure 4, intravenously administered naloxone did not change the insulin and glucagon response significantly compared with the saline controls.

Peripheral vein plasma glucose levels did not change significantly from baseline levels in response to both test meals; this was not altered by the addition of naloxone.

DISCUSSION

The present study demonstrates that in dogs the intragastric instillation of digested gluten elicits a more rapid and greater rise of postprandial plasma insulin and glucagon levels, compared with the effect of undigested gluten. Recently, Zioudrou et al.[1] have demonstrated that the peptic digest of gluten contains peptides with opiate-like activity. Although it is conceivable that digested protein would give a greater response of pancreatic endocrine function because of more rapid absorption of oligopeptides and amino acids, the present data are compatible with an effect of exorphins because the addition of naloxone, a specific opiate-receptor antagonist, reduces postprandial insulin levels in response to digested gluten. In contrast, the rise in glucagon is more rapid and prolonged when naloxone is added to the digested gluten meal. This augmentation of the glucagon response does not necessarily disprove an opiate-specific effect of the digested gluten meal because it has to be considered that insulin, a potent inhibitor of glucagon release,[12] is reduced substantially—thereby possibly counterbalancing the opiate antagonistic effect of naloxone. In response to undigested gluten, naloxone did not alter either postprandial insulin or glucagon levels.

The intravenous infusion of naloxone did not change insulin or glucagon levels. Similar findings have recently been reported by Morley et al.[13] in response to various stimuli of pancreatic endocrine function.

ACKNOWLEDGMENTS

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