# Modulation of Post-Prandial Insulin Release by Ingested Opiate-Like Substances in Dogs

V. Schusdziarra, A. Holland, R. Schick, A. de la Fuente, M. Klier, V. Maier, V. Brantl[1] and E. F. Pfeiffer

Department of Internal Medicine I, University of Ulm, and [1]Department of Neuropharmacology,
Max Planck Institute of Psychiatry, Munich, FRG

**Summary.** We have previously demonstrated that opiate-like substances in food protein (exorphins), contained in the peptic digest of gluten, stimulate insulin and glucagon release in dogs and that this effect is inhibited by the opiate antagonist naloxone. The present study was designed to evaluate the possible rôle of ingested opiate-like substances in the modulation of post-prandial insulin release. Similarly, the addition of synthetic $\beta$-casomorphins, which are the opioid-active material of bovine casein peptone, elicit a stimulation of post-prandial insulin release during a liver extract-sucrose test meal. The addition of met-enkephalin to a liver extract-sucrose test meal also augmented the post-prandial insulin response. Both stimulatory effects were reversed by oral naloxone, as was the post-prandial increase of insulin following ingestion of bovine casein peptone (casopeptone). The post-prandial insulin response to digested and undigested liver extract was not affected by naloxone, suggesting that the foregoing effects are likely to be specific to opiate-like materials contained in foodstuff (exorphins). In view of previous findings, the present data are compatible with a role of opiate-like substances contained in ingested nutrients in the regulation of post-prandial insulin secretion.

**Key words:** $\beta$-Casomorphin, enkephalin, naloxone, insulin release, exorphins, opiates, dog.

Opiate-like substances such as $\beta$-endorphin, and enkephalin, have been shown to be present in the gastrointestinal tract and pancreas [1–6], and have widespread potent effects on gastric, intestinal and pancreatic exo- and endocrine functions when administered intravenously [7–18]. With the discovery of substances with opiate-like activity in foodstuff (exorphins), such as wheat gluten, bovine and human milk and bovine casein peptone [19–22], it is important to establish whether oral ingestion of such compounds might regulate post-prandial gastrointestinal and pancreatic endocrine functions. Recently we have shown that intragastric instillation of exorphins contained in the peptic digest of gluten stimulate insulin and glucagon release in dogs [23], and the demonstration of naloxone-induced inhibition of these effects indicates that opiate-receptors are activated in the gastrointestinal tract and/or pancreas. The present study was designed to extend the examination of post-prandial insulin release in response to test meals that contain opiate-like substances. To demonstrate the involvement of opiate receptors, naloxone, a specific opiate receptor antagonist [24], was added to the test meals.

## Materials and Methods

### Animals

The studies were performed in a total number of 12 conscious normal dogs (body weight 25–35 kg). After an overnight fast the dogs received an intragastric test meal according to the following protocols. All experiments were carried out in randomized order and each dog served as its own control: (1) In eight dogs, 25 g of bovine casein peptone (casopeptone, C/TLG 16300, Serva Feinbiochemica, Heidelberg, FRG), together with saline (5 ml, 0.15 mol/l) or naloxone (8 mg, Endo Laboratories, Garden City, USA) were given orally. This test meal was chosen because it has been shown previously to contain approximately 25 mg of opioid-active $\beta$-casomorphins [20, 21]. (2) In a second group of eight dogs, a mixture of synthetic $\beta$-casomorphins (3 mg $\beta$-casomorphin-7, 3 mg $\beta$-casomorphin-5, 4 mg $\beta$-casomorphin-4 and 4 mg $\beta$-casomorphin-4-amide) (Peninsula, La Jolla, USA and Bachem, Bubendorf, Switzerland) was added to a test meal consisting of 50 g undigested liver extract (Reheis Chemicals, Chicago) and 25 g sucrose. For comparison the same meal contained either saline or the mixture of casomorphins + 10 mg naloxone. (3) In eight dogs, met-enkephalin (10 mg) (Serva, Heidelberg, FRG) was added to a test meal consisting of 50 g undigested liver extract and 50 g sucrose. The same meal was also given containing either saline or 10 mg met-enkephalin + 10 mg naloxone. (4) The foregoing and previously reported effects may be due to the fact that the peptic digest of protein meals contains more amino acids and/or oligopeptides than the undigested form, which could increase post-prandial insulin release per se.
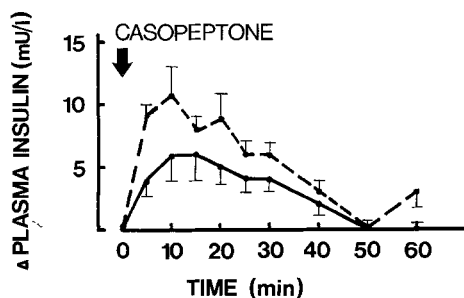
Fig. 1. Effect of the intragastric administration of naloxone (8 mg) (●———●) or saline (●---●) together with the casopeptone test meal (25 g in 300 ml water) on the increase in post-prandial peripheral vein plasma insulin levels (± SEM) above the mean of the three baseline values in eight conscious dogs

Therefore, another test meal with presently unknown content of opiate-like material – bovine liver extract – was employed in the present study. Twenty-five g undigested liver extract (eight dogs) or 25 g digested liver extract (six dogs) were administered intragastrically with oral naloxone or saline, respectively. The peptic digest of liver extract was prepared as described by Zioudrou et al. [19] and after the 2-h incubation period the reaction was stopped by neutralization and subsequent lyophilization.

All test meals were dissolved in 300 ml water just before the instillation via a gastric tube. Blood samples were drawn from a crural vein before and at 5 min intervals for 30 min and at 10 min intervals until 60 min after the instillation of the test meal, and blood was collected into chilled tubes containing 500 KIU Trasylol and 6 mg EDTA. All samples were centrifuged at 2000 rev/min at 4 °C for 20 min and the separated plasma was frozen until the time of assay. Plasma insulin levels were determined by radioimmunoassay as described previously [25] 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

### Response to Casopeptone

Plasma insulin levels rose in response to casopeptone and saline from a mean baseline of 5 ± 0.5 to 16 ± 4 mU/l at 10 min. The addition of naloxone to the casopeptone meal elicited a maximal increase by only 6 mU/l from similar baseline levels (Fig. 1). The incremental insulin level during the first 20 min was 23 ± 3 mU/l after casopeptone and naloxone, significantly below the level of 49 ± 3 mU/l in response to saline and casopeptone ($p < 0.01$). Plasma glucose levels did not rise during the casopeptone meal and were not affected by oral naloxone.

### Effect of Oral β-Casomorphins Upon the Insulin Response to a Liver Extract-Sucrose Test Meal

In the saline control animals plasma insulin levels rose from a mean baseline of 10 ± 2 to a maximum of 37 ± 6 mU/l at 15 min, returning thereafter to baseline levels
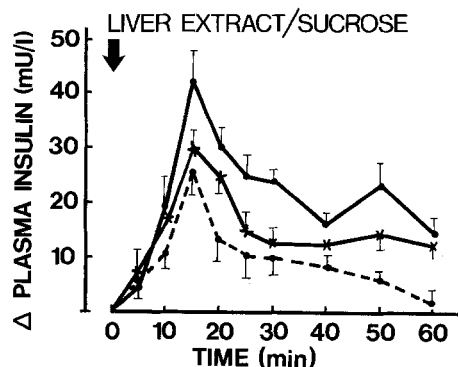


Fig. 2. Effect of the intragastric administration of a mixture of synthetic β-casomorphins (●———●), β-casomorphins + naloxone (10 mg) (x———x), or saline (●---●) together with a liver extract-sucrose test meal (50 g liver extract and 25 g sucrose in 300 ml water) on the increase in post-prandial, peripheral vein plasma insulin levels (± SEM) above the mean of the three baseline values in eight conscious dogs



Fig. 3. Effect of the intragastric administration of met-enkephalin (10 mg) (●———●), met-enkephalin (10 mg) + naloxone (10 mg) (x———x), or saline (●---●) together with a liver extract-sucrose test meal (50 g liver extract and 50 g sucrose in 300 ml water) on the increase in post-prandial peripheral vein plasma insulin levels (± SEM) above the mean of the three baseline values in eight conscious dogs
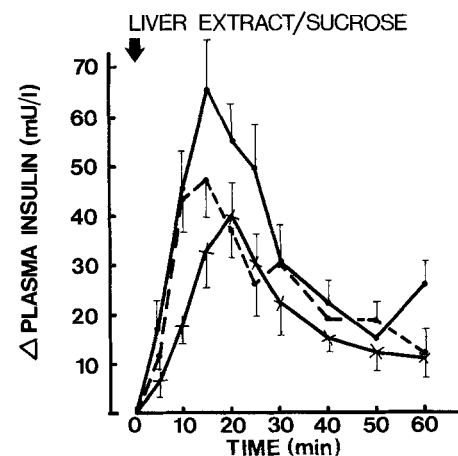
(Fig. 2). The addition of β-casomorphins elicited a stimulation of insulin release from a mean baseline of 8 ± 1 to 50 ± 4 mU/l; insulin levels remained above those of the controls for the entire experimental period of 60 min. The incremental insulin level during the 60 min was 222 ± 40 mU/l, significantly above the 104 ± 28 mU/l of the controls ($p < 0.025$). The addition of naloxone to the test meal attenuated this stimulatory effect of β-casomorphins (159 ± 25 mU/l; $p < 0.05$ and not significantly greater compared with the controls). The rise of peripheral vein plasma glucose levels by 0.5 mmol/l in the controls was not altered by the addition of β-casomorphins.

## Effect of Oral Met-Enkephalin upon the Insulin Response to a Liver Extract-Sucrose Test Meal

In response to the liver extract-sucrose test meal, plasma insulin rose from a mean baseline of 6 $\pm$ 1.5 to a maximum of 53 $\pm$ 8 mU/l at 15 min, returning thereafter to baseline levels (Fig. 3). The addition of 10 mg met-enkephalin elicited a rise from a baseline of 7 $\pm$ 1 to a maximum of 72 $\pm$ 12 mU/l at 15 min and remained above the levels of the saline-containing test meal for the first 30 min. The incremental insulin level during the first 30 min was 265 $\pm$ 40 mU/l, significantly greater than the 185 $\pm$ 25 mU/l in the controls ($p < 0.05$). The additional intragastric instillation of 10 mg naloxone reduced the stimulatory effect of met-enkephalin upon post-prandial insulin levels (170 $\pm$ 25 mU/l, $p < 0.02$).

Peripheral vein plasma glucose levels rose by 0.66 mmol/l above the mean baseline of 4.68 mmol/l and this response was not changed by the addition of met-enkephalin and naloxone.

## Response to Digested and Undigested Liver Extract

In response to digested liver extract insulin rose from a mean baseline of 5 $\pm$ 1 to 14 $\pm$ 4 mU/l at 15 min decreasing thereafter towards baseline levels. This response was not affected by oral naloxone. The incremental insulin level in response to digested liver extract and saline was 34 $\pm$ 3.5 mU/l – not significantly different from the 36 $\pm$ 4.7 mU/l after naloxone.

After the meal of undigested liver extract, insulin rose from the mean baseline of 9 $\pm$ 1.5 to 31 $\pm$ 6 mU/l at 20 min. The response was not significantly changed by naloxone. The incremental insulin level was 89 $\pm$ 6 mU/l in response to undigested liver extract and saline, and 83 $\pm$ 5 mU/l after the addition of naloxone.

In contrast to the greater insulin response to digested gluten compared with the undigested gluten meal [23], the incremental insulin level in response to undigested liver extract and saline (89 $\pm$ 6 mU/l) was significantly greater than that to digested liver extract and saline (34 $\pm$ 3.5 mU/l, $p < 0.005$), suggesting that peptic digestion per se does not raise post-prandial insulin secretion.

Peripheral vein plasma glucose levels did not rise in response to digested or undigested liver extract and were not affected by naloxone.

## Discussion

The present study demonstrates that test meals which contain opioid-active materials influence post-prandial insulin secretion in accordance with previously reported results [23]. Casopeptone, containing the opiate-like substances casomorphins [20, 21], stimulated insulin release and this effect was reduced by opiate receptor blockade. A similar effect can be obtained by the ad-

ministration of synthetic $\beta$-casomorphins together with a carbohydrate-protein test meal. Further support for a modulatory role of ingested opiate-like substances is derived from the experiments with the opiate agonist met-enkephalin, which stimulates insulin release specifically via opiate receptors. These and previously reported effects [23] are not the consequence of peptic digestion of any protein test meal, as shown by the results obtained with liver extract, and probably reflect more specific, opiate-receptor mediated effects, which are induced by certain test meals only.

The mechanism of action of exorphins on islet cell function is unknown. From the present data it cannot be decided whether exorphins affect opiate-receptors within the gastrointestinal tract, thereby influencing the hormonal and/or neural signal between gut and islets of Langerhans, or whether the effect is due to absorbed exorphins exerting their effect at the level of the B cell, as shown for two other opiates [7, 9]. The data are consistent with the hypothesis that not only the intravenous administration of opiates but also the oral ingestion of opiate-like substances contained in certain nutrients (exorphins) can influence the regulation of post-prandial insulin release. Whether these effects of exorphins are of any importance in the postulated role of opiates in nutrient intake [26, 27] remains to be established in further studies.

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Dr. V. Schusdziarra
Zentrum für Innere Medizin
University of Ulm
Steinhoevelstraße 9
D-7900 Ulm/Donau
FRG