Effect of an enkephalin-analogue (FK 33-824) on glucose tolerance in man

X. Jeanrenaud, E. Maeder, E. Del Pozo and J. P. Felber

Division of Endocrinology and Clinical Biochemistry,
Department of Medicine, C. H. U. V. 1011 Lausanne, Switzerland and
Experimental Therapeutics Department1, Sandoz Ltd., Basel, Switzerland

Abstract. The purpose of the present work was to study the effect of a methionine-enkephalin analogue (FK 33-824) on glucose tolerance in man. Groups of 5 to 8 normal subjects were given a 0.5 mg im injection of the drug or placebo just before a 100 g oral glucose load or a 0.5 g/kg iv glucose load. In the enkephalin analogue treated subjects, diminished insulin response to glucose was observed following the oral glucose load, with insulin values significantly lower than in the controls from time 10 to 90 min, but no corresponding change in the glucose curve. This effect was not observed when glucose was given iv in another group of 5 subjects in whom the significant blunting of the insulin response was accompanied by a significant decrease in glucose tolerance.

These observations demonstrate that in man, enkephalin produces a decrease in insulin secretion in response to both oral and iv glucose loads. The absence of any marked impairment in glucose tolerance in the oral test in spite of the decreased insulin response suggests that enkephalin might have an additional effect in delaying glucose absorption.

On the basis of their recent studies (Pyke & Leslie 1978; Leslie & Pyke 1978; Leslie et al. 1979) suggested that a genetically determined hypersensitivity to enkephalin might play a role in the genesis of some cases of non-insulin-dependent diabetics. Previous studies have already demonstrated the hyperglycaemic effects of opiates, by action on the central nervous system (Feldberg & Shaligram 1972; Feldberg & Gupta 1974). Ipp et al. (1978), working on isolated dog pancreas and Pierluissi et al. (1981) on rat islets of Langerhans showed that endorphin and enkephalin possess stimulating action on insulin secretion. Kanter et al. (1980) on the contrary, showed an inhibitory action of methionine- and leucine-enkephalin on the release of insulin and glucagon by cultured pancreatic islets. Green et al. (1980), using isolated islets of Langerhans of rats showed an inhibition of insulin secretion at a high enkephalin dose, but a stimulation at a low dose.

These different observations might be explained knowing that the different opioid peptides have different receptors (Lord et al. 1977). The effects of β-endorphin and morphine, µ-receptors agonists, have been shown to be stimulatory (Ipp et al. 1978; Kanter et al. 1980) while those of enkephalins, possibly δ-receptor agonists, were shown to be inhibitory (Kanter et al. 1980; Green et al. 1980). Moreover, differences in concentrations of the peptides, as shown by Green et al. (1980) might also explain these differences.

Studies in the human did not show similar effects. Stubbs et al. (1978) and del Pozo et al. (1980) found no changes in fasting glycaemia or insulinaemia after an injection of the FK 33-824 enkephalin analogue. Morley et al. (1980) showed no effect of naloxone on insulinaemia or glucagonaemia in the course of an iv glucose tolerance test, with the only exception of a slight increase of basal
insulinaemia and of the glycaemia peak, without any alteration of the rate of glucose disappearance.

The present work was undertaken to verify a possible role of enkephalin, not only on basal glycaemia and insulinaemia, but mainly on glucose and insulin response to oral and iv glucose loads in man.

Materials and Methods

Subjects
The study was carried out on 3 groups of normal male subjects.

Group A consisted of 8 subjects, mean age 25 ± 1 years and mean body weight 94 ± 4% of their ideal body weight (IBW). Group B was composed of 8 subjects, mean age 25 ± 1 years, and mean body weight 92 ± 3% of their IBW. Group C consisted of 5 subjects, mean age 24 ± 1 years and mean body weight 94 ± 2% of their IBW.

The ideal body weight was calculated on the base of the Metropolitan Life Insurance Tables 1959.

The protocol was submitted to and accepted by the human investigation committee of the Department of Medicine of the University of Lausanne, Switzerland. All subjects gave their informed consent prior to the study.

Methods

The enkephalin-analogue FK 33-824 (Sandoz D-Ala 2, MePhe 4 Met(0)5ol-enkephalin) was used throughout the study instead of native enkephalin, for its longer duration of action. Its biochemical and pharmacological characteristics have been reported previously (Roemer et al. 1977).

The subjects were given a weight-maintaining diet containing at least 250 g carbohydrates for 3 days before the study. The sequences of the test were randomised and the tests were carried out at least at 1 week interval. The tests were performed after an overnight fast. The subjects were placed in recumbent position. A short iv catheter (Venflon®) was introduced in an antecubital vein. Patency was maintained by means of a continuous iv infusion of saline.

After a 30 min rest, subjects of group A received an im injection of either 0.5 mg FK 33-824 in the thigh or of saline as placebo. Subjects of group B ingested 100 g glucose dissolved in 400 ml water flavoured with lemon juice which they drank within 5 min after the im injection of either 0.5 mg FK 33-824 or saline, given at time 0 (OGTT). Similarly, subjects of group C were given an iv glucose load (0.5 g/kg body weight) as a 3 min infusion after the same im injection of either FK 33-824 or saline at time 0 (IVTT).

Blood samples were taken at various times according to the different tests, for measurements of plasma glucose and immunoreactive insulin (IRI).

Analytic procedures

Plasma glucose was measured by the hexokinase method, plasma IRI according to the method described by Herbert et al. (1965).

All data are presented as mean ± SEM. The statistical comparisons were calculated by means of the paired t-test analysis. The area under the curves was calculated by triangulation. The mean glucose assimilation coefficient was calculated according to Lundbæk (1962) on the base of regression curves in the IVTT.

Side effects

All subjects experienced an unpleasant feeling of heaviness of the body, particularly in the legs and the thorax, within 30 s and 1 min after injection and lasting for 15 and 30 min with a maximum after 5 to 10 min. This was accompanied by an occasional feeling of heat in the face with conjunctival injection and rhinorrhea. No real facial flush was observed. Other side effects were borborygms, intestinal motility and variable degrees of somnolence within 1–2 min after injection, lasting for 15 to 30 min, with only one case of real sleep during 3 h.

Results

Group A: effects of FK 33-824 on basal plasma glucose and insulin levels (Fig. 1)

Plasma glucose and IRI levels did not show any significant modification during the 120 min following the injection of 0.5 mg FK 33-824 or placebo. A transient but not significant drop in plasma IRI was however observed at 15 min.

Group B: effects of FK 33-824 on plasma glucose, insulin and glucagon levels in response to a 100 g oral glucose load (Fig. 2)

Following the FK 33-824 injection the ascent of the glycaemia curve was significantly delayed during the first 15 min, in comparison to the control group (P < 0.025 at time 10 min, P < 0.01 at time 15 min). The glycaemic peak was almost identical in both cases at 30 min. The fall of the plasma glucose curve was slightly attenuated after FK 33-824 with levels significantly higher at time 180 min (P < 0.025).

A significant delay in the rise of plasma IRI was also observed after injection of FK 33-824 with values significantly lower than those of the control group from time 10 to time 90 min (P < 0.01 at time 10 min, P < 0.005 at time 90 min). The values were higher than those of the control at the end of the test at 180 min (P < 0.01). In the FK 33-824-
Mean plasma glucose (●, ○) and insulin (■, □) levels ± SEM, following a saline or a FK 33-824 injection at time 0 (†) in basal state, N = 8.

Mean plasma glucose (●, ○) and insulin (■, □) levels ± SEM, following a saline or a FK 33-824 injection at time 0 (†) during a 100 g oral glucose tolerance test. N = 8. (* P < 0.05; ** P < 0.025; *** P < 0.01; **** P < 0.005).
treated group, the surface area of the plasma IRI curve over baseline from time 0 to 120 min represented 55% of that of the control group (38 ± 4 vs 70 ± 9 µU IRI/ml/min, \( P < 0.005 \)).

**Group C: effects of FK 33-824 on plasma glucose, insulin and glucagon levels in response to a 0.5 g/kg body weight iv glucose load (Fig. 3)**

The fall of plasma glucose levels from the peak at time 10 min was significantly attenuated from time 20 to 60 min after the FK 33-824 injection (\( P < 0.05 \) at time 20 min, \( P < 0.01 \) at time 60 min). The mean assimilation coefficient was 1.6 ± 0.3 with a variation coefficient of 0.99 significantly different compared to the control group with a mean assimilation coefficient of 2.9 ± 0.3 and a variation coefficient of 0.95 (\( P < 0.005 \)).

Plasma IRI levels showed a decreased response to the glucose load after FK 33-824, with IRI levels significantly lower than the control values from time 5 to time 30 min (\( P < 0.05 \) at time 5 min, \( P < 0.01 \) at time 30 min). The surface area of the plasma IRI curve over baseline, from time 0 to 40 min, was significantly different from that of the control group, with a value of 40.1 ± 7 vs 58 ± 11 µU IRI/ml/min (\( P < 0.025 \)).

**Discussion**

The present results demonstrate that insulin secretion in response to orally or iv administered glucose was diminished following an enkephalin injection, while basal insulinaemia remained unaffected. The inhibiting effect of the enkephalin-analogue FK 33-824 on the insulin release was perfectly demonstrated during the iv glucose tolerance test. In this test, the lowering of the insulin response was accompanied by a highly significant decrease in glucose tolerance. This may be explained by the fact that iv administered glucose is utilized mainly by peripheral tissue under insulin stimulation (De-
Fronzo et al. 1978). A decrease in glucose utilization in the periphery is therefore likely to be the consequence of the inhibitory effect of the enkephalin-analogue on insulin secretion.

The oral glucose tolerance test is more difficult to understand. The observation of a delay in the rise of the plasma glucose curve during the first 15 min after the FK 33-824 injection, with higher plasma glucose levels at the end of the test than in the control period, suggests at first an inhibitory effect of the enkephalin-analogue on the glucose absorption. Among all the factors able to modify glucose absorption, enkephalin, like morphine, is known to possess an inhibitory effect on gastric motility and emptying (Konturek et al. 1978a,b; Sullivan et al. 1981).

The diminished plasma insulin response to glucose observed from the 15th min after oral glucose administration under FK 33-8234 leads however to the following hypotheses. The first one would be that the delayed and prolonged insulin response would correspond to the delayed glucose absorption. The second one would be that, next to its inhibitory action on glucose absorption, the enkephalin-analogue is able also to modify the insulin response to the oral as to the iv administration of glucose. The delayed passage of glucose would prevent, at the beginning of the test, the higher glycaemic rise which would be expected from the lower insulin levels. The higher plasma glucose levels at the end of the test, rather than being representative of a delayed glucose absorption, could be a consequence of the decreased insulin secretion. The glucose tolerance curve observed during the oral test, after the FK 33-824 injection, might also reflect changes induced by the enkephalin analogue on the sensitivity of the tissues to the hormone, such as changes at the receptor or post-receptor levels, which would explain the small changes in glycaemia despite significant lowering of the insulin levels. This would imply that enkephalin, next to its effect on insulin secretion, could modulate the sensitivity to insulin in the splanchnic area. This would not be the case in peripheral tissues as seen from the iv glucose tolerance test.

The mechanism involved in the inhibition of insulin secretion by the enkephalin-analogue FK 33-824 remains unknown, although it confirms in man some observations reported in the animal (Kanter et al. 1980; Green et al. 1980). The presence of enkephalin has been reported in the pancreas and the gastro-intestinal track (Polak et al. 1977; Forssmann et al. 1977; Bruni et al. 1979). Functional interactions have also been demonstrated between the opioid peptides and neurointestinal factors (Konturek et al. 1978a,b, 1980) which could influence endocrine pancreatic secretion.

In conclusion, the present study shows that, in man, enkephalin produces a decrease in insulin secretion in response to glucose loads and suggests a possible role of the human endogenous opioid system in glucose tolerance. Moreover, the comparison between the oral and the iv results supports the hypothesis of an effect of enkephalin on glucose absorption.

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References


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