genesis of schizophrenia [10, 11], it may just be worth while turning our attention to the gut flora in this context.

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Changes induced by DMet^2-pro^5-enkephalinamide in plasma insulin response to glucose in the rat

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From reports of several laboratories, it seems reasonable to assume that opioid peptides, endogenous ligands for opiate receptors, may operate as well as in pain modulation, in regulating extrapyramidal, limbic system and neuroendocrine functions (for a review see [1]).

Previous work from this laboratory [2, 3] has shown that leu-enkephalin stimulates a release of growth hormone as well as of prolactin from pituitary, and this agrees with results obtained with Met-enkephalin by Rivier *et al.* [4].

We now report an extrapituitary hormonal effect of an opioid. D-Met^2-pro^5-enkephalinamide, an analogue of met-enkephalin with long lasting analgesic activity [5], causes changes in insulin levels in plasma of glucose-loaded rats.

Male Sprague–Dawley rats weighing 220 ± 20 g were used, and housed under standard conditions (room temperature 20°, relative humidity 60 per cent). Studies were begun between 8 and 9 a.m. following an overnight fast. Administration of the drug and withdrawal of blood were accomplished in unanesthetized and unrestrained rats. This was made possible by using animals with permanent indwelling jugular cannula, according to the method of Cocchi *et al.* [6]. A group of ten rats (controls) received intravenously 1 ml/kg of a 33 per cent glucose solution in saline. Another group of ten animals received intravenously, immediately after an identical load of glucose, 0.4 mg/kg of D-Met^2-pro^5-enkephalinamide (Met-Enk-NH_2) dissolved in saline.

Blood samples were taken from each group of animals at various time-intervals, for the determination of plasma insulin by radioimmunoassay, according to the method of Keane *et al.* [7]. Rat insulin was used as a standard (Novo Industri, DK). From the same blood samples, glucose concentration was also determined, according to the method of Werner *et al.* [8].

The results reported in Table 1 indicate that a rise occurs, as usual, in plasma insulin levels of glucose-loaded rats, with a peak effect under our experimental conditions, at the 3rd min.

Table 1. Plasma insulin levels (IRI) of glucose loaded rats following the administration of a single dose of D-met^2-pro^5-enkephalinamide (Met-Enk-NH_2, 0.4 mg/kg/i.v.)

Time of observation (min)	IRI (μUnits per ml)	
	Glucose load (controls)	Glucose load + Met-Enk-NH_2
0	28.2 ± 1.7	26.4 ± 2.3
3	127.5 ± 8.3	76.3 ± 3.7*
8	52.3 ± 2.4	71.4 ± 2.9*
15	34.9 ± 1.9	29.8 ± 1.4
30	21.2 ± 1.1	26.3 ± 1.8
60	27.4 ± 1.7	32.8 ± 1.5

* $P < 0.01$ compared with control values at the same time-intervals. The values are the means of 10 animals ± S.E.M.

Table 2. Glycemic values of glucose loaded rats treated or not with D-met^2-pro^5-enkephalinamide (Met-Enk-NH_2, 0.4 mg/kg/i.v.)

Time of observation (min)	Glycemia (mg%)	
	Glucose load (controls)	Glucose + Met-Enk-NH_2
0	96.5 ± 5.2	88.3 ± 3.4
3	247.4 ± 12.6	322.9 ± 11.7*
8	143.2 ± 10.9	197.8 ± 9.5*
15	91.5 ± 5.3	164.4 ± 8.7*
30	102.7 ± 4.8	116.8 ± 5.9
60	108.3 ± 4.5	91.3 ± 4.1

* $P < 0.05$ compared with control values at the same time-intervals. The values are the means of 10 animals ± S.E.M.

Met-Enk-NH_2 administration results in a significant ($P < 0.01$) reduction of plasma insulin response compared with 3 min values of controls. In samples taken 8 min after intravenous glucose injection, the mean values of plasma insulin are significantly higher for opioid-treated rats than for controls. In the following time-intervals of observation, the values are substantially coincident for both groups of animals. Within a 30 min interval of observation, blood glucose concentration is higher in Met-Enk-NH_2 treated rats (Table 2), thus suggesting that this opioid duplicates the hyperglycemic effect reported for morphine [9]. Whether changes in plasma insulin levels by Met-Enk-NH_2 are due to direct action on pancreatic insulin secretion or whether they result from a modifying action on homeostatic feedback mechanisms, cannot be decided on the basis of our present findings. As far as glycemia is concerned, the increase in blood glucose levels is not always paralleled by an increase of insulin levels of corresponding intensity in the two groups of rats: at 3rd min of observation, when glucose concentration reaches its maximum, insulin values are higher in control animals than in those treated with Met-Enk-NH_2. It is also possible that changes in plasma insulin are mediated by endogenous monoamines, such as serotonin. On one hand, the inhibiting effect of serotonin in plasma insulin response to a glucose load has already been shown [10]; on the other hand, some evidence [11] suggests a serotonin involvement in another hormonal response of Met-Enk-NH_2, that is the release of prolactin from pituitary.

Moreover, results obtained at brain level by Algeri *et al.* [12] show that a similar opioid peptide, D-Ala^2-Met-enkephalin causes an increase of serotonin turnover in the rat. The higher insulin levels in opioid-treated rats compared with those of controls at the 8 min of observation could also be the result of a rebound phenomenon following the removal of the opioid due to its shortlived action. In any case, the results obtained seem to be promising for investigations on extracerebral effects of opioids.

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