

LETTER TO THE EDITOR

EFFECT OF MET-ENKEPHALIN ON BLOOD GLUCOSE LEVEL

Sir,

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Evidence of endogenous opiate like compounds on the brain was proved by Hughes, in breaking Paper about 20 years ago (1, 2). Physiological Function's of these compounds have been described in diverse areas such as nociception, change in behaviour, appetite, psychiatric stress and shock (3). Plasma metenkephalin levels were significantly high in type 1 diabetes (15) and the presence of opioid peptides within pancreatic islets suggests that these peptides play a role in pancreatic endocrine secretion influencing glucose metabolism (16).

Stress can be induced in experimental animals in various forms e.g. forced immobilization stress, starvation, exposure to cold or heat. The various physiological changes seen in response to stress are due to increased hypothalamo pituitary action, activation of pituitary adrenal system and secretion of various hormones e.g. catecholamines, T.S.H., enkephalins and endorphins (6). During Mental Arithmetic Test (MAT) exercise stress, increase in met-enkephalin secretion occurred along with hemodynamic and various endocrine secretions noradrenalin, endothelin I etc. Individual difference in haemodynamic and endocrine responses to MAT may depend on a different activation of the endogenous opioid system (13).

The present study was done with an aim to study the effect of Met-enkephalin on

blood glucose level. Till date a few work has been done on the role of Met-enkephalin on blood glucose and its relation to liver and muscle glycogen content. The present study discussed the possible mechanism involved in hyperglycaemic effect of Met-enkephalin.

The present study was conducted on 108 albino rats of Charles Foster strain, each weighing between 150-250 gms. Animals were obtained from Central Animal facility of J.N. Medical College Animal House. The rats were acclimatized to the environment for atleast a week prior to experimentation. The animals were allowed to free access to tap water and food pellets (soaked Bengal gram) and they were maintained on a 12 hr light/dark cycle at $22 \pm 1^\circ\text{C}$ in separate iron cages. The animals were divided into three groups. Each group of animals contained 36 rats. Each group were further subdivided into six subgroups. Each subgroups had six rats ($6 \times 6 = 36 \times 3 = 108$). All drugs were given by intraperitoneal route and doses calculated per 100 gm body weight per 0.1 ml physiological saline. Group I (control, saline) received 0.1 ml physiological saline. Group II received i.p. injection of 20 μg of Met-enkephalin (Sigma Chemicals, U.S.A.) Group III received i.p. Naloxone (Sigma Chemicals, U.S.A.) 20 μg followed by Met-enkephalin dose as mentioned above.

In the first subgroup of 6 rats, the blood sample was collected for blood glucose

estimation at 10 min of drug administration, the animals were than sacrificed and tissue samples were collected for glycogen estimation. Similarly in the subground 2, 3, 4, 5 & 6, blood and tissue samples were collected at 20, 30, 60, 90 and 120 minutes respectively. Blood glucose estimation was done by orthotoludine Method by Dubowski (4). Glycogen estimation was done by Pfiliger's Method cited by Good et al (5). Results were evaluated by Students's 't' test (unpaired).

Tissue glycogen was determined by some modification of the original method of Pfiliger. Excised tissue was placed first in strong KOH solution and heated until it is disintegrated and dissolved. Then glycogen was precipitated from the solution by the addition of alcohol, centrifuged off and hydrolysed to glucose with fuming sulphuric acid. The glucose formed from the glycogen is estimated at OD 470 nm using Spectrophotometer.

Interpretational injection of Met-enkephalin caused hyperglycaemia ($P < 0.001$). Which persisted upto 90 minutes (10 min; $P < 0.05$; 120 min: $P < 0.01$). Naloxone blocked Met-enkephalin effect significantly ($P > 0.1$; Naloxone vs Saline) (Table I).

Liver glycogen contents were decreased in intraperitoneal injection of Met-enkephalin treated group. These changes were significant at 10 min ($P < 0.05$), 90 min ($P < 0.05$) and 120 min ($P < 0.05$) (Table II).

This showed the periodic decrease in liver glycogen contents. Naloxone blocked the effect in all but 20 min naloxone effect were significant ($P < 0.001$). On muscle glycogen Met-enkephalin effect were significant at 60 min ($P < 0.001$) and 90 min ($P > 0.05$). At the other time intervals effects were not significant ($P > 0.1$). Naloxone blocked Met-enkephalin effect on muscle glycogen were significant at 60 and 90 min ($P < 0.001$).

TABLE I : Blood glucose level mg%.

Drug/Time	10	20	30	60	90	120
Group I (Saline)	65.0 ± 3.74	61.16 ± 5.6	55.8 ± 6.43	56.66 ± 7.0	61.66 ± 6.15	62.0 ± 6.03
Group II (Met-Enk.)	96.0 ± 2.38*	89.5 ± 6.92	96.5 ± 2.81*	86.16 ± 3.31	86.5 ± 6.83	68.0 ± 3.89
Group III (Naloxone + Met-Enk.)	57.6 ± 5.7	63.0 ± 4.69	65.2 ± 2.77	54.6 ± 4.27	61.2 ± 1.3	58.5 ± 5.00

TABLE II : Liver glycogen content mg/gm tissue.

Drug/Time	10	20	30	60	90	120
Group I (Saline)	4.56 ± 0.4	3.36 ± 0.38	4.76 ± 0.89	4.23 ± 0.34	3.85 ± 0.4	3.92 ± 0.18
Group II (Met-Enk.)	3.76 ± 0.15	2.39 ± 0.5	4.31 ± 0.46	3.93 ± 0.16	2.46 ± 0.26*	3.05 ± 0.27*
Group III (Naloxone + Met-Enk.)	4.42 ± 0.5	5.24 ± 0.46*	5.09 ± 0.25	3.6 ± 0.83	3.93 ± 0.39	4.5 ± 0.3

TABLE III : Muscle glycogen content mg/gm tissue.

Drug/Time	10	20	30	60	90	120
Group I (Saline)	2.77 ± 0.13	2.47 ± 0.04	2.14 ± 0.12	4.40 ± 0.1	2.01 ± 0.1	1.95 ± 0.12
Group II (Met-Enk.)	1.70 ± 1.01	1.62 ± 1.8	2.24 ± 0.04	1.14 ± 0.16*	1.26 ± 0.30*	2.29 ± 1.39
Group III (Naloxone + Met-Enk.)	2.58 ± 0.33	2.52 ± 0.08	2.24 ± 0.24	2.28 ± 0.1*	3.09 ± 0.1*	3.43 ± 0.2

The result showed Met-enkephalin raised blood glucose level and caused decreased in level of muscle and liver glycogen contents.

Medicinal value of natural opioids like morphine, synthetic opioid compound like Pentazocin, endogenous opioid peptides like Met-enkephalin and opioid antagonist compounds like Naloxone are metamorphosing towards the end of 20th Century. In 21st Century, it is bound to find more newer compounds of natural endogenous opioids peptide origin. We selected the Met-enkephalin in our study, which is pentapeptide, isolated and identified in the brain in 1975 by Hughes and Hughes and Tereneus Walhstrom in 1977 (1, 2). Pituitary gland secrete many peptides and compute drastic modulation of blood glucose level. This was enlightened by Luna et al (14) that endocrine changes were noted in cerebrospinal fluid and peripheral provided the basis of our present study.

In present model, intraperitoneal injection of Met-enkephalin in 0.1 ml physiological saline (6), raised blood glucose level. Naloxone caused significant reversal of the effect of Met-enkephalin. Morphine and many opioid compounds has hyperglycaemic effect as reported earlier (7, 8) agreed with our findings.

We found in newer compound that Met-enkephalin has potent and similar hyperglycaemic effect in rats. This simple study, correlate the simultaneous changes taken place at liver and muscle glycogen content, which show marked decrease in liver and muscle glycogen content at 10, 60, 90 minutes after Met-enkephalin i.p. injection (20 µg/100 gm of body weight).

Opioids regulate glucagon and insulin secretion by intraislet regulation of somatostatin (9), via opioid receptors in islets (10). Morphine and betaendorphin both inhibit somatostatin secretion in islets (9). Met-enkephalin caused hyperglycaemia in present study may be due to inhibitory effect on somatostatin and modulate alpha and beta cells in islets of langerhans in pancreas.

Alternatively Met-enkephalin effect may be mediated through the activation of β-adrenergic system (11), hyperglycaemic effect was reported by Matsumara et al (8). Decrease in glycogen content may be due to (i) direct effect of glucagon on hepatic glucose output via opioid receptors not linked to CAMP (11); (ii) via glycogenolysis and gluconeogenesis (12) via CAMP mediated activity via adenylate cyclase (12).

Hyperglycaemic effect and effect on liver and muscle glycogen content may be due to

central sympathetic discharge (7). This explained the possible mechanism involved in hyperglycaemic effect of Met-enkephalin in the present study. This finding gave us tremendous importance in understanding

many clinical related disorders due to stress like diabetes, hypertension, atherosclerosis and lipid glucose abnormalities, those are the prime cause leading to system failure.

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