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Neuropeptide FMRFamide induces hypoglycemia in rats

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Neuropeptide FMRFamide on intracerebroventricular administration reduced significantly ($P < 0.001$) the blood glucose levels in normal and diabetic rats. FMRFamide also increased the serum insulin levels in both the category of rats, thus eliciting hypoglycemic effect. Intracerebroventricular administration of FMRFamide antiserum enhanced significantly ($P < 0.01$) the blood glucose levels in normal rats, suggesting the involvement of neuropeptide FMRFamide in the regulation of blood glucose levels.

FMRFamide (Phe–Met–Arg–Phe–NH_2) was first isolated from the ganglia of clam *Macrocalista nimbosa*[1]. Subsequent findings have revealed the existence of FMRFamide in mammalian central nervous system (CNS) and gastrointestinal tract (GIT)[2]. The neuropeptide has been shown to be involved in a variety of physiological processes in mammals, including cardiovascular action[3], inhibition of morphine[4] and defeat-induced analgesia[5], selective amnesia[6], excessive grooming on intrathecal administration[7], inhibition of morphine- and amphetamine-stimulated locomotor activity[8] and stimulation of gastric acid secretion on intracerebroventricular (ICV) administration[9].

Relatively large quantity of immunoreactive FMRFamide material has been detected in the exocrine pancreas of the rats[2]. With the existence of FMRFamide in the CNS and pancreas, it was proposed to investigate the role of FMRFamide in the regulation of blood glucose levels.

Male albino rats (Haffkine strain, 180–200 g) maintained under 12 h light/dark cycle and 25 ± 2°C were selected for the study. Food (gold mohur, Bombay) and water was *ad libitum* up to the time of experiment. The following drugs were used: alloxan monohydrate (Loba Chemie, Australia), pentobarbitone sodium (Loba Chemie, Australia), FMRFamide antiserum (anti-FMRFamide, Incstar Inc., USA). FMRFamide was obtained from Prof. M. J. Greenberg, C. V. Whitney Laboratory, St. Augustine, Florida, USA. All the drugs were dissolved in 0.9% NaCl (saline).

In the first set of experiments, rats were fasted overnight and blood glucose levels were determined 15, 30 and 60 min after the administration of FMRFamide (5–10 µg, ICV). For ICV administration, rats were anaesthetized using pentobarbitone sodium (45 mg/kg, intraperitoneally) and polyethylene cannulae (outer diameter 0.75 mm, inner diameter 0.30 mm) were implanted stereotaxically[10]. The cannulae were fixed to the skull with dental cement. The coordinates for ICV administration were: 0.8 mm posterior from bregma, 1.8 mm lateral from midline and 3.3 mm ventral to the surface of skull. Animals were housed individually after the surgery and a recovery period of seven days was allowed before the start of experiments.

Blood glucose levels were measured with reflective photocell glucometer (Reflolux-II, Boehringer Mannheim, GmbH, Germany) using glucose strips. Blood samples were collected by milking of tail and were placed on the glucose strip for 60 s. The strip was then washed with distilled water, blotted and placed in the reflector photocell of the glucometer for direct reading of the blood glucose in mg/dl. The entire procedure took about 2 min for a single estimation.

Further, serum insulin levels were estimated 15 min after the injection of FMRFamide (5–10 µg, ICV). Radioimmunoassay kit (RIAK 1) developed by the Bhabha Atomic Research Centre (BARC), Bombay, was employed for the estimation[11] of serum insulin levels. In another set of experiments, diabetes was induced in rats by injecting alloxan monohydrate[12] (180 mg/kg) subcutaneously. After about two weeks, when the blood

Table 1. Effect of ICV FMRFamide on blood glucose levels in normal and diabetic rats

Group	No. of rats	Blood glucose level (mg/dl ± SE) 15 min	30 min	60 min
Normal				
Saline (10 µg, ICV)	6	66.33 ± 5.7	65.16 ± 4.7	67.52 ± 3.2
FMRFamide (ICV)				
5 µg	5	55.17 ± 3.0[a]	59.00 ± 1.4[c]	64.00 ± 1.8[c]
10 µg	5	46.20 ± 1.6[b]	51.80 ± 2.2[b]	65.60 ± 2.2[c]
Diabetic				
Saline (10 µg ICV)	6	246.6 ± 2.3	243.00 ± 3.0	248.00 ± 2.0
FMRFamide (ICV)				
5 µg	6	222.16 ± 5.4[b]	227.80 ± 3.8[b]	237.00 ± 2.3[c]
10 µg	6	212.50 ± 3.4[b]	221.50 ± 4.0[b]	240.00 ± 2.0[c]

[a] $P < 0.01$, [b] $P < 0.001$ and [c] not significant compared to saline-treated animals as controls (Student's *t*-test).

glucose levels of animals stabilized in the range 200–250 mg/dl, FMRFamide (5–10 µg, ICV) was injected and the blood glucose levels were determined at intervals of 15, 30 and 60 min. Similarly, serum insulin levels in diabetic rats were estimated[11] following FMRFamide administration.

To investigate the role of brain FMRFamide in the regulation of blood glucose, anti-FMRFamide (1 µl, ICV) was chronically administered for five days and the blood glucose level determined in normal as well as diabetic rats.

FMRFamide reduced (Table 1) the blood glucose level significantly in normal as well as diabetic rats, with peak effect 15 min after the administration. Blood glucose levels tended to recover to normal after 30 min of FMRFamide administration. The observation that FMRFamide exhibits significant hypoglycemic effect in normal as well as diabetic animals implicates its regulatory role in glucose homeostasis. Immunoneutralization of brain FMRFamide by anti-FMRFamide enhanced the normal blood glucose levels (Table 2), indicating the involvement of brain FMRFamide in the maintenance of blood glucose levels. This observation strengthens further the speculation that FMRFamide acts in CNS for the regulation of blood glucose levels.

Table 2. Influence of anti-FMRFamide (1 µl ICV for 5 days) on blood glucose levels

Treatment	No. of rats	Blood glucose level (mg/dl ± SE) Normal rats	Diabetic rats
Saline (10 µl, ICV)	6	66.20 ± 1.1	243.00 ± 1.4
Anti-FMRFamide	6	83.00 ± 1.6[a]	245.20 ± 2.44[b]

[a] $P < 0.001$, and [b] not significant compared to saline-treated animals (Student's *t*-test).

Table 3. Effect of ICV FMRFamide on serum insulin levels in normal and diabetic rats

Group	Serum insulin (µU/ml ± SE)
Normal	
Saline (10 µl, ICV)	77.33 ± 1.8
FMRFamide (ICV)	
5 µg	93.50 ± 3.5[a]
10 µg	103.33 ± 2.2[a]
Diabetic	
Saline (10 µl, ICV)	40.83 ± 2.3
FMRFamide (ICV)	
5 µg	53.33 ± 1.8[a]
10 µg	56.00 ± 2.4[a]

Number of rats used in all the cases was 6.
[a] $P < 0.001$ compared to saline-treated animals as controls (Student's *t*-test).

Further, FMRFamide increased the serum insulin levels significantly ($P < 0.001$) after 15 min of administration (Table 3). This increase in serum insulin levels was evident in normal as well as diabetic rats, suggesting a role of FMRFamide in insulin secretion by exocrine pancreas.

In conclusion, the present study demonstrates that central administration of FMRFamide elicits hypoglycemic effect through the modulation of insulin secretion. The study also exhibits the involvement of neuropeptide FMRFamide in glucoregulation.

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Figure 1. Porcelain white bodies on dorsolateral last segment of the infected grub (× 40).

A new Rickettsia-like organism associated with white grubs (*Coleoptera: Scarabaeidae*)

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A new Rickettsia-like organism (RLO) has been recorded from the white grub *Holotrichia consanguinea* Blanchard and *Adoretus* sp. The natural incidence of the RLO varies from 5.76 to 11.54% on *H. consanguinea* in different localities of Central Gujarat, India.

THE white grubs (Coleoptera: Scarabaeidae) are a major set of pests of several agricultural crops and are widely distributed in India, with different species being important in different agro-climatic zones of the country. Several microbial agents have been reported to attack white grubs[1–3] and are being tried for their exploitation to manage white grubs.

During a survey for pathogens of white grubs in Gujarat, in August 1989, we came across an unusual symptom in grubs of *Holotrichia consanguinea* Blanchard and *Adoretus* sp. collected from four villages of Kheda district in Central Gujarat. The grubs carrying the symptom exhibited reduced feeding and appeared sluggish. Healthy larvae of *H. consanguinea* pupate in about 3 months, but the infected larvae extended their larval period up to 4–6 months; and upon death, they left a dry carcass. The dorsal and lateral portion of the last segments showed typical porcelain-type bright-white crystalline bodies (Figure 1). Moreover, the natural incidence of milky disease caused by *B. popilliae* and green muscardine disease caused by *M. anisopliae* were also recorded from the same localities. The milky-diseased and RLO-infected grubs can be easily differentiated in advanced stage of infection. The milky diseased grubs turn chalky-white and are filled completely and uniformly with a milky fluid, whereas the RLO-infected grubs show porcelain-type bright-white crystalline bodies on the dorsal and lateral side of the last few segments which are unevenly distributed. The cuticle of milky-diseased grub on death is soft, and on puncturing it with a needle, translucent milky fluid oozes out, which shows the presence of footprint-shaped bacteria with distinct spore and parasporal body under a light microscope. *M. anisopliae*-infected grubs become hard and show typical dark-green muscardine growth on the carcass. The RLO-infected grubs appear shrunken, containing the least amount of fluid, and the cuticle becomes hard. On grinding, it releases RLOs, as discussed below.

The grubs were ground in a hand tissue grinder (Kontes-Duall) in distilled water and spun at 9000 rpm for 20 min. The pellets were resuspended in distilled water and specimen grids were prepared for examination under an electron microscope. The only microorganism found in this examination was a new Rickettsia-like organism. The different forms of RLO observed were: (a) small forms of RLO (electron-dense elliptical bodies or elementary bodies (EBs)) which measured 0.14–0.26 μm × 0.5–0.75 μm (Figure 2); (b) the fragments of pointed forms of RLO, which swell to become reticulate bodies (RBs) with diameters up to 0.75 μm and filled with developing EBs (Figure 3).