Binding of ^{125}I-HCG to rat ovary homogenates at various stages of development

Age	Number of animals	pg HCG bound/ 5 mg wet weight*		Number of binding sites**		K_D ($\times 10^{10}$)
		Total	Non-specific	moles/mg wet weight ($\times 10^{15}$)	moles/ovary ($\times 10^{15}$)	
Prenatal	40	40.8	35.4	0	0	–
Newborn	250	45.0	40.6	0	0	–
5-day-old	250	40.7	33.4	0	0	–
10-day-old	100	113.0	37.0	2.36	1.65	4.37
21-day-old	12	94.5	27.0	2.59	15.18	6.18
31-day-old	10	115.0	38.5	5.55	61.05	11.66
Spleen adult		41.3	36.5			

*Determined with 4 ng ^{125}I-HCG in the incubation assay. **Determined by Scatchard[22] analysis.

HCG was determined from samples containing a 1000fold excess of unlabelled hormone. The specific uptake of HCG was calculated from the difference between the radioactivity bound by samples incubated with ^{125}I-HCG alone and the radioactivity bound nonspecifically. Iodination of purified HCG (biological activity of 11,000 IU/mg) was performed according to the modified method C of Leidenberger and Reichert[15] with a time of exposure to chloramine-T of 20 sec. Specific radioactivity of the ^{125}I-HCG was 30–50 μCi/μg.

Results and discussion. As can be seen from the table, ovarian tissues of prenatal, newborn and 5-day-old rats do not specifically bind radioactively labelled HCG. Specific binding of HCG is first observed in ovaries of 10-day-old animals. These results are supported by the observation of Presl and Figarová[8] that accumulation of injected radioactively labelled HCG is only demonstrable in ovaries of rats older than 7–8 days. The number of binding sites, as well as the dissociation constants (K_D) of the receptor-hormone complex, are similar in 10–21-day-old rats. Twice the number of binding sites as in the 21-day-old rat were demonstrable in rats 31 days of age. The values for K_D and number of binding sites in the 31-day-old rat are largely consistent with those obtained by Lee and Ryan[14] in 35-day-old rats. It might be assumed that the lack of HCG binding in ovarian tissue of rats younger than 10 days is due to masking of the receptors by endogenous LH. This assumption can be abandoned, since specific HCG receptors are first demonstrable in ovarian tissue when the endogenous LH level has attained its maximum[3, 4, 16, 17].

These results strongly suggest that the HCG insensitivity of the early postnatal rat ovary is due to the lack of specific receptors. With the first appearance of the LH receptors in the ovary, the animals become sensitive to LH. Thus, in the female rat, in contrast to the male rat[18], LH receptor is not a constitutive protein. This difference between male and female is well understandable from a biological point of view. While steroidogenic activity of the testis is an absolute prerequisite for the development of the male phenotype, feminine differentiation occurs in the absence of gonadal steroids[19, 20]. Only during the postnatal development do ovarian steroids become necessary. From the observation that in 10-day-old female rats only the interstitial tissue of the ovary is steroidogenic[21], one might assume that interstitial cells are the first cells in the ovary to become endowed with LH receptors. The higher number of binding sites in the ovaries of 31-day-old rats compared with ovaries of 10-day-old rats might indicate that besides interstitial cells also granulosa and theca cells are equipped with LH receptors at this developmental time.

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The effect of epinephrine and the hyperglycemic factor of the scorpion's cephalothoracic ganglionic mass (CTGM) on the phosphorylase activity of hepatopancreas of the scorpion, Heterometrus fulvipes C. Koch

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Summary. Injection of epinephrine and CTGM extract showed different effects on hepatopancreatic phosphorylase activity and levels of total carbohydrate and glycogen in Heterometrus fulvipes. The former hyperglycemic principle involves phosphorolysis of glycogen whereas the latter does not.

The role of neuroendocrine system in the metabolite regulation has been explored in Arachnida only to a limited extent[2]. A hyperglycemic principle has been identified in the cephalothoracic ganglionic mass of the South Indian scorpion Heterometrus fulvipes[3, 4]. The vertebrate hormone, epinephrine also has been found to cause hyperglycemia in Heterometrus fulvipes.

The present communication examines the effect of epinephrine and scorpion's CTGM extract on the levels of total carbohydrates, glycogen and phosphorylase activity

Effect of injection of epinephrine or CTGM extract on the levels of total carbohydrate, glycogen, active (a) and total (ab) phosphorylase activities in the hepatopancreas of the scorpion H. fulvipes

Treatment	Total carbohydrate	Glycogen	Phosphorylases a	ab	a · 100/ab
1 Control	6.95 ± 1.12 (10)	3.31 ± 0.88 (10)	8.0 ± 1.25 (10)	28.2 ± 3.26 (10)	28.37
2 Epinephrine	5.85 ± 1.21 (10)	2.82 ± 0.80 (10)	22.2 ± 3.22 (10)	46.2 ± 6.82 (10)	48.06
3 CTGM	9.69 ± 1.29 (10)	4.34 ± 0.76 (10)	8.5 ± 0.95 (10)	29.2 ± 4.28 (10)	29.11

Comparison of means (Student's t-test) and changes in percent

	p	Percent	p	Percent	p	Percent	p	Percent
1–2	<0.05	− 15.8	NS	− 14.8	<0.001	+177.5	<0.001	+63.8
1–3	<0.001	+39.4	<0.02	+31.1	NS	+ 6.3	NS	+ 3.5
2–3	<0.001	+65.6	<0.001	+53.9	<0.001	− 61.7	<0.001	− 36.8

Values for total carbohydrate and glycogen: mg/g dry weight; for phosphorylase: μmole P_i/mg protein h. Number of determinations in brackets. NS, not significant.

of the hepatopancreas of the scorpion H. fulvipes. Collection, maintenance and details of preparation of experimental animals have been described earlier[4]. Cephalothoracic ganglionic mass extracts in aqueous medium were injected into a batch of animals at the rate of one CTGM-equivalent (in 0.25 ml) per animal. For a second batch, epinephrine as adrenalin chloride (Parke-Davis, India), 1:1000, in saline solution was injected in 50 μg-dose per animal. Controls received 0.25 ml of distilled water or saline. In the hepatopancreas of control, epinephrine injected and CTGM-extract injected scorpions, levels of total carbohydrates and glycogen were estimated by the anthrone method[5], and the phosphorylase activity was estimated by the method of Cori et al.[6], and liberated inorganic phosphate was estimated according to Fiske and Subba Rau[7]. Protein content was estimated with Folin-Phenol reagent.[8].

The data and statistical treatment[9] (table) reveals a fundamental difference between the mode of action of the vertebrate hormone and the scorpion's own hyperglycemic principle on the hepatopancreatic carbohydrate, glycogen and phosphorylase activity. Epinephrine leads to a significant ($p < 0.05$) decrease (–15.8%) of the total carbohydrate level of hepatopancreas, whereas CTGM extract injection results in a significant ($p < 0.001$) increase (+ 39.4%). The difference (+ 65.6%) between the total carbohydrate content of epinephrine- and CTGM extract-injected hepatopancreas also is significant ($p < 0.001$). Glycogen content shows a non-significant decrease on epinephrine treatment and CTGM extract leads to a significant ($p < 0.001$) elevation (+ 31.1%). The difference between the 2 treatments (+ 53.9%) is significant ($p < 0.001$), with regard to glycogen content also, as is the case with total carbohydrates. A 'prima facie' difference appears to exist in the modus operandi of hepatopancreatic carbohydrate metabolization between the 2 hormonal principles under consideration. This is further illustrated by the different influences exerted by these principles on the hepatopancreatic phosphorylase activity. Epinephrine elevates the levels of both active (a) and total (ab) phosphorylase levels significantly. Also this hormone brings about an alteration in the ratio of active to total phosphorylase. This ratio is elevated by 69.4% over control. But CTGM extract has no appreciable action on this enzyme system. Yet both these principles lead to development of significant levels of hyperglycemia[4].

The principle of CTGM-extract is not only different from epinephrine, as has been demonstrated here, but also from the hyperglycemic principle of the insect corpora cardiaca extract[10–13] which seems to influence the glycogenolytic pathway for the augmentation of haemolymph carbohydrate level through stimulation of fat body phosphorylase system. It is of interest to note that the millipede Spirostreptus asthenes possesses in its brain a hyperglycemic principle the action of which appears to be essentially similar to the CTGM-principle of the scorpion in its mode of action on the fat body carbohydrate levels and phosphorylase system[14].

In view of its significant accumulation under the influence of CTGM principle, the carbohydrate content of hepatopancreas of the scorpion may not be implicated as the important causal source for the hyperglycemia resulting under the same aegis. Under brain-extract injection in the millipede, Spirostreptus asthenes[14], and under CTGM-extract injection in the scorpion, H. fulvipes[4], decreases in total lipid content of fat body and hepatopancreas were 49.4% and 19.2% respectively.

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