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SOME HORMONAL AND BIOCHEMICAL PARAMETERS IN THE SERA AND MAMMARY TISSUES OF MAMMOGENIC AND LACTOGENIC RATS AFTER TREATMENT WITH ALLOXAN AND / OR INSULIN

By

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SUMMARY

Seventy two gonadectomized male and female albino rats were used in the present study. They were divided into six groups, group A served as control, group B injected with Es+Prog. (mammogenesis), group C treated with Es+ Prog. +PRL (lactogenesis). The last three groups are lactogenic and treated with alloxan (D); insulin (E) and alloxan and insulin (F). Serum TSH, T_3, T_4, Ca, P, glucose, total protein and total lipids were estimated. Mammary tissues were isolated for determiation of Ca, P, total protein and total lipids.

Lactogenesis in the present study elevated release of thyroid hormones and the data proved the active role of insulin during this process. Induction of diabetes during lactogenesis increased TSH without any change in serum T3 and T4. Lactogenesis and insulin injection after alloxan increased serum and tissue total tipids and tissue total protein. Phosphorus measurement showed a significant increase after injection of insulin in lactogenic males and in lactogenic males and lactogenic females treated with alloxan and insulin. Calcium content of mammary tissues was increased in female lactogenic animals and lactogenic males and females treated with insulin. It is concluded from above data that diabetes in lactogenic animals have an adverse effect on hormonal and biochemical levels in the mammary tissues and sera and injection of insulin may change this effect.

INTRODUCTION

Morphological and functional differentiations of the mammary gland were shown to be complex processes which involve multiple hormonal interactions (Vonderhaar, 1984). prolactin (PRL), in concert with insulin and cortisol, has been shown to accelerate the production of milk constituents (Rillema, 1980). In addition, Rillema et al. (1985) reported that biosynthesis of milk casein and lipids started 6-10 hrs. after exposure of the mammary tissues to PRL.

Insulin was found to be an essential hormone for the induction of in vitro casein synthesis by mammary tissues of mice and rats as well as for induction of - lactalbumin activity (Topper et al., 1984). Prosser et al. (1987)added that insulin enhance the rate of carrier-mediated glucose transport by mammary epithelial cells. Accordingly, witharawal of insulin level of lactating rats resulted in immediate depression of lactational performance and decreased mammary synthesis of lactose, casein and lipids (Martin and Baldwin, 1971).

Several reports suggested that the breast playes an active role in the thyroid hormones dynamic of the lactating animals. Thyroid hormones as T_3 and T_4 (Oberkotter and Tenore, 1983) as well as TSH (Tenore et al., 1981) are secreted normally via the mammary gland. These hormones were found to affect mammary gland morphology (Vonderhaar and Greco, 1979), responsiveness to PRL (Bhattacharjee and Vonderhaar, 1979), milk production (Bhattacharjee and Vonderhaar, 1984) and secretion (Vonderhaar, 1977).

In the present investigation trials were made to answer the question of how could diabetes and treatment with insulin affect some metabolites and mammary tissue constituents of experimental mammogenic rats. Estimation of the metabolic hormones TSH, T_3 and T_4 were also encountered.

MATERIAL AND METHODS

Seventy two mature male and female albino rats were used for the present study. They were fed a diet composed of barley, bran, green fooder, vitamins and mineral mixture. Food and water were offered adlibitum. Rats were gonadectomized and after that left for 15 days then divided accoraing to sex into six equal groups and treated as follows:-

1- **Group A**: Rats were injected subcutaneously (s. c) daily with 0.1 ml olive oil for 10 days and starting from the eighth day, rats were injected with 0.5 ml saline daily for 2 days.

2- **Group B**: Rats were injected (s. c) with 0.1 ml olive oil containing 5 µg estradiol benzoate (Es.) (Folone, Misr Co.) and 1.0 mg progesterone (Prog.) (Lutone, Misr Co.) for five successive days then with 10 µg Es. Plus 0.5 mg prog. for five days, they were then injected with 0.5 ml saline for another five days (Mammogenic).

3- **Group C**: as the same regimen of group B in addition to five daily (s. c) doses of 50 µg PRL instead of saline (National Institute of Arthritis and Metabolic Disease, NIAMD, MSDA-B-1, AFP 5300). 24 hrs from last injection, the rats were injected with 0.5 ml saline (Lactogenic).

4- **Group D**: The same regimen of group C was used in addition to one single (s. c) dose of 21 mg / 100 gm body weight alloxan monohydrate (Sigma Co) dissolved in saline (Schneider and Schedl, 1972).

5- **Group E**: Rats were treated as group C then were injected (s. c) with insulin (Nilab Retard NPH) at a does of 10 iu dissolved in 0.1 ml saline daily for each rat for five successive days instead of saline (Hough et al., 1982).

6- **Group F**: Rats were treated as group D, 48 hrs after alloxan injection, they were injected with insulin typically as in group E.

After 24 hrs from the last injection, blood samples were collected from the inner canthus of the eye. The animals were then decapitated, the mammary glands were separated and collected in sterile vials. Separated serum samples and mammary tissues were stored at - 20°C till use.

Total proteins and lipids were measured in mammary tissues using Soxhlet extraction and Kjeldal method respectively (A. O. A. C., 1975). Serum glucose was determined accordig to the method of Carawy (1976). Total protein in sera was estimated by the method of Doumas et al.

Table (1): Serum TSH, T3 and T4 levels of male and female rats after insulin and / or alloxan treatment of lactogenic and mammogenic animals.

Groups	TSH (ng/ml)		T_3 (ng/ml)		T_4 (µg %)	
	♂	♀	♂	♀	♂	♀
Control (A)	0.73 ±0.15	0.91 ±0.18	2.59 ±0.65	3.11 ±0.72	4.21 ±1.03	2.59 ±0.63
Es+Prog. (B)	0.64 ±0.10	0.76 ±0.21	0.14 ±1.05	3.64 ±0.94	5.11 ±1.13	4.93 ±1.29
Es+Prog.+PEL(C)	0.99 ±0.27	0.96 ±0.18	4.92[c] ±0.54	4.63 ±1.08	5.63 ±1.20	5.68[c] ±1.13
Es+Prog. +PRL + Alloxan (D)	2.31[b] ±0.51	2.65[a] ±0.30	2.64 ±0.74	3.74 ±0.66	2.50 ±0.66	2.34 ±0.66
Es+Prog. +PRL+ Insulin (E)	0.89 ±0.16	1.10 ±0.26	3.26 ±0.96	5.99[a] ±0.43	5.68 ±1.26	3.41[c] ±0.94
Es+Prog. +PRL+ Alloxan + Insulin (F)	0.74 ±0.22	0.64 ±0.05	6.22[c] ±1.27	6.56[c] ±1.12	4.19 ±1.01	6.84[c] ±1.43

Mean ± Standard error
a: Significantly different from control value at P<0.001
b: Significantly different from control value at P<0.01
c: Significantly different from control value at P<0.05

Table (2): Serum analysis of male and female rats after insulin and / or alloxan treatment of lactogenic and mammogenic animals

Groups	Glucose mg%		Total protein gm%		Total lipids gm%		Ca mg %		p mg %	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Control (A)	112.3 ±8.2	117.4 ±12.6	7.43 ±1.48	7.84 ±1.10	1.31 ±0.21	1.22 ±0.29	11.32 ±2.71	11.62 ±1.33	5.12 ±0.64	6.27 ±1.13
Es+Prog. (B)	123.6 ±7.1	151.5 ±14.9	6.94 ±2.16	8.31 ±0.96	1.45 ±0.41	1.55 ±0.16	10.11 ±1.99	9.18 ±1.64	6.23 ±1.53	5.11 ±0.90
Es+Prog.+PRL(C)	109.1 ±6.3	119.6 ±6.13	8.34 ±1.10	7.33 ±1.40	2.21[c] ±0.30	2.53[c] ±0.25	8.55 ±2.08	9.22 ±2.13	5.14 ±0.85	5.36 ±0.84
Es+Prog. +PRL + Alloxan (D)	236.4[a] ±11.9	248.9[a] ±13.4	6.19 ±1.16	6.98 ±1.06	3.45[a] ±0.42	2.15 ±0.25	9.53 ±2.14	7.32[c] ±1.12	5.50 ±0.74	6.20 ±1.02
Es+Prog. +PRL+ Insulin (E)	108.5 ±6.4	115.8 ±4.4	6.54 ±0.11	7.33 ±1.1	2.11 ±0.63	1.56 ±0.08	10.22 ±2.66	10.51 ±2.27	6.44 ±1.34	8.51[c] ±0.74
Es+Prog. +PRL+ Alloxan + Insulin (F)	142.5 ±15.3	118.3 ±5.9	6.93 ±2.06	7.43 ±0.84	2.51 ±0.28	2.27 ±0.80	9.17 ±1.23	8.93 ±2.04	7.50 ±0.55	5.83 ±1.20

Mean ± Standard error
a: Significantly different from control value at P< 0.001
b: Significantly different from control value at P< 0.05

Table (3): Mammary gland analysis of male and female rats after insulin and / or alloxan treatment of lactogenic and mammogenic animals

Groups	Total lipids gm%		Total protein gm%		Ash %		Ca %		p %	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Control (A)	17.69 ±2.41	14.23 ±2.88	9.27 ±2.34	5.14 ±1.06	2.2 ±0.6	1.8 ±0.4	1.10 ±0.26	0.99 ±0.03	0.71 ±0.15	0.53 ±0.17
Es+Prog. (B)	18.36 ±3.18	15.32 ±3.95	8.15 ±1.93	7.22 ±1.85	3.1 ±0.8	2.3 ±0.4	1.41 ±0.42	0.84 ±0.25	0.83 ±0.09	0.62 ±0.11
Es+Prog.+PRL(C)	25.16[c] ±2.55	23.16 ±4.05	13.11 ±2.98	11.85b ±2.15	4.3 ±1.7	2.7 ±0.8	2.64[a] ±0.36	1.31 ±0.16	0.98 ±0.25	0.80 ±0.06
Es+Prog. +PRL + Alloxan (D)	19.15 ±4.60	17.83 ±4.46	7.99 ±1.82	8.93 ±2.07	2.5 ±0.5	2.4 ±0.3	1.32 ±0.35	1.46 ±0.29	0.74 ±0.27	0.83 ±0.13
Es+Prog. +PRL+ Insulin (E)	22.23 ±3.86	20.21 ±2.50	9.19 ±2.59	9.17 ±2.11	2.6 ±0.6	2.7 ±0.4	2.55[c] ±0.45	1.91[a] ±0.18	0.81 ±0.10	0.76 ±0.16
Es+Prog. +PRL+ Alloxan + Insulin (F)	28.17[c] ±3.22	27.58[c] ±4.18	11.91 ±2.58	12.11[c] ±2.46	3.1 ±0.6	3.3 ±1.0	1.98 ±0.50	1.63 ±0.35	1.13[c] ±0.8	0.93 ±0.24

Mean ± Standard error
a: Significantly different from control value at P< 0.001
b: Significantly different from control value at P< 0.01
c: Significantly different from control value at P< 0.05

(1981).

Total lipids of the sera were measured according to Girard et al. (1970). Calcium and phosphorus in the sera and ash of mammary tissues were measured using colorimetric methods of Chiamari and Herry (1958) and Gomorri (1942) respectively.(1981).

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Serum TSH, T_3 and T_4 were measured by enzyme immunoassay test kits (Gamma Trade) according to Chapra et al.1972).

RESULTS

The data obtained in table (1) showed that lactogenic state of female and male rats (group C) presented a signifincant increase is serum T_3 and T_4 respectively. Alloxan treatment of lactogenic animals (group D) increased serum TSH in both sexes while insulin treatment (group E) significiantly increased serum T_3 in male rats only. Insulin injection to alloxan-treated animals (group F) increased T_3 in both sexes and T_4 in male rats.

Table (2) showed the biochemical analysis of the sera measured. The illustrated results revealed that the highest glucose level measured was obtained in alloxan treated rats (group D). All treatments did not change the total protein level. Total lipids increased significantly in group C (lactogenic state) as well as in group D (lactogenic with alloxan) in females only. A significant decrease in serum Ca level was obtained after injection of alloxan in lactogenic rats (group D). Injection of insulin to lactogenic animals (group E) significantly increased serum phosphorus in male rats, while injection of insulin to alloxan treated animals (group F) significantly increased serum phosphorous levels in female rats.

Analysis of mammary tissues (table 3) showed a significant increase in total lipids in lactogenic female animals (group C) and lactogenic alloxan-treated animals besides insulin (group F) in both sexes. Total proteins significantly increased in males of lactogenic animals (group C) as well as in insulin and alloan-treated animals (group F). A significant increase in Ca of mammary tissue was obtained in lactogenic females (group C), and in both sexes of lactogenic animals treated with insulin (group E). Phosphorus level did not show any significant variation exceptin females of group (F) i. e. lactogenic females treated with alloxan and insulin. Statistical analysis of the obtained data was done using Students "t" test according to Snedecor and Cochran (1980).

DISCUSSION

Male and female rats behaved differently after gonadectomy and estrogen treatment (Ibrahim et al., 1986). According to this finding, both sexes were used for induction of mammogenesis and lactogenesis. These mammogenic and lactogenic rats were subjected for the study of the effect of diabetes and insulin treatment on some biochemical parameters and levels of TSH and thyroid hormones.

The data obtained from this investigation showed that after induced lactogenesis (group C), levels of serum T_3 of female and serum T_4 of male rats were significantly elevated as compared to control group. These results are consistent with those of Oberkotter and Tenore (1983) where these hormones are required for regulation of general metabolism, development, tissue differentiation and gene expression during lactional state. However, diabetic state of lactogenic animmals (group D) showed a significant increase in serum TSH without any change in serum T_3 and T_4. On the other hand, insulin treatment of diabetic lactogenic animals (group F) led to significant increase of serum T_3 (males and females) and serum T_4 (males) with normal level of TSH. These results proved the active role of insulin on the thyroid gland during lactogenesis. In addition, Tramontano et al. (1986) reported that insulin

greatly enhanced the mitogenic effect of TSH on thyroid follicle.

As regards total lipids, the significant increase detected in serum and mammary tissue during lactogenesis may be explained on the basis of the lipogenic action of prolactin (and consequently insulin). Heesom et al. (1992) reported also that lipogenesis was increased in isolated acini from lactating rat mammary glands by conversion of medium-chain fatty acids (the major precursor of which is blood glucose) into lipids. Moreover, Barber et al. (1992) showed that both of prolactin and growth hormones induce a wide variety of effects on mammary gland and adipose tissue metabolism, which would be expected to enhance milk synthesis and limit extramammary use of nutrients.

Insulin is considered as a key regulator of lipoprotein lipase activity in adipose tissue (Ong et al., 1988) which may interpret the lipogenic action of the injected insulin on mammary tissues of diabetic lactogenic animals.

The role of prolactin and insulin in protein synthesis in milk (Martin and Baldwin, 1971 and Topper et al., 1984) and in mammary glands (Beardsley et al., 1988) is proved by our finding of increased protein content of mammary tissues of lactogenic male rats as well as of diabetic group after insulin treatment. Moreover, Beardsley et al. (1988) revealed that diabetes severly depressed the activity of the enzymes of the salvage pathway of purine synthesis, but appeared to be without effect on the "de novo" pathway enzyme.

In reference to the non-significant variations in levels of serum total proteins obtained in this study which appeared to be independent of the mammary protein content. These data are consistent with the finding of Geursen and Grigor (1987) that ablumin being a major whey protein in the rat, it being synthesized at an extramammary site and transferred to the milk space by a paracellular mechanism from an extravascular mammary pool rather than directly from the serum.

As regards serum phosphorus, no significant changes were obtained as a result of the diabetic state of the lactogenic rats, a finding agrees with Mc-Nair et al. (1979) in human diabetic patients and with Ishida et al. (1985) in experimentally diabetic rats. However, insulin treatment increase in phosphorus either in serum of lactogenic male rats or in serum and mammary tissues of diabetic lactogenic female rats which may explained by the reduction of renal loss of phosphate (Schedl et al., 1978).

The hypocalcaemia obtained only in diabetic male rats, a result agrees with those of Ishida et al. (1985) and yacout et al. (1988). This result could be explained by the decrease in the duodenal calcium absorption, as a result of an insulin-dependent effect in the hydroxylation of 25-$(OH)_2$ vit-D in the kidney with the resultant decrease in the concentration of 1, 25 $(OH)_2$ vit-D leading to a detective synthesis of the carrier protein needed for calcium transport in the duodenal mucosa (Schneider and Schedl, 1972). An additional factor leading to hypocalcaemia is the increased urinary loss of calcium due to the osmotic diuresis resulting from glucosuria (Raskin et al., 1978), which is supported by the finding of Mc-Nair et al., (1979) that calcuria is directly proportional to glucosuria.

Calcium content of mammary tissues was significantly increased in lactogenic female rats and lactogenic animals injected with insulin. This finding is attributed to the enhancing effect of insulin on duodenal calcium absorption, by increasing the concentration of 1, 25 $(OH)_2$ vi-D, and the reduction of urinary calcium excretion (Schedl et al., 1978 and Raskin et al., 1978).

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