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امكانية تداخل المواد البروتينية الرابطة على ديناميكية المنشطات المنسلية خلال دورة الشبق في الفئران

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أجريت الدراسة الحالية لتوضيح الدور الذي تلعبه المواد البروتينية الرابطة على ديناميكية المنشطات المنسلية خلال دورة الشبق في الفئران •

وأوضحت النتائج أن فترة نصف الحياه لكل من هرموني مصل الفرس الحامل والمشيمة الآدمي في الفئران في مرحلة الشبق كانت أقل منها في مرحلة الهدوء الجنسي وأدى حقن كل من جزيئي الالبيومين والجلوبيولين المستخلصة من الفئران في مرحلة الهدوء الجنسي الى تطويل فترة نصف الحياه لكل من هرموني مصل الفرس الحامل والمشيمة الآدمي عند حقنه للفئران في مرحلة دورة الشبق وخلال فترة الهدوء الجنسي كانت فترة نصف الحياه لهرمون المشيمة الآدمي أطول من التي عولجت بالالبيومين والجلوبيولين في الفئران في مرحلة الشبق•

وكانت فترة نصف الحياه لهرمون مصل الفرس الحامل في الفئران في مرحلة الهدوء الجنسي المعالجة بجزئ الجلوبيولين المستخلص من فئران في مرحلة الشبق شبيهه بمثيلتها في الفئران في مرحلة الشبق بالاضافة الى أن حقن جزئ الالبولين المستخلص من الفئران في مرحلة الشبق الى فئران في مرحلة الهدوء الجنسي أدى الى نقص فترة نصف الحياه لهرمون مصل الفرس الحامل على عكس حالة حقن الجلوبيولين •

ولذلك نستطيع أن نستخلص أن المواد البروتينية الرابطة ممكن أن تلعب دورا في ديناميكية المنشطات المنسلية وبالذات هرمون مصل الفرس الحامل خلال دورة الشبق •

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A POSSIBLE INVOLVEMENT OF COMPETITIVE PROTEIN BINDING SUBSTANCES IN THE DYNAMICS OF GONADOTROPHINS DURING THE ESTROUS CYCLE IN RATS

(With 4 Tables)

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SUMMARY

The present study was undertaken to clarify the role played by protein binding substances in the dynamics of gonadotrophins during the estrous cycle in rats. The results indicated that the half life ($t_{1/2}$) of both PMSG and hcG estrous-rats were shorter than in diestrus ones. Injection of both albumin and globulin fractions obtained from diestrus rats into estrous ones prolonged the $t_{1/2}$ of both PMSG and hcG. During diestrous the half-life of hcG was proplonged in rats treated with albumin or globulin obtained from estrous-ones.

The half-life of PMSG in diestrous-rats treated with globulin-fraction obtained from estrous-ones was similar to its $t_{1/2}$ in estrous-rats. In addition albumin fraction obtained from estrous-rats when administered into diestrous-ones led to a decrease in $t_{1/2}$ of PMSG but not as in case of globulin.

Thus we can conclude that protein binding substances may play an important role in the dynamics of gonadotrophins especially PMSG during estrous phase.

INTRODUCTION

Several mechanisms have been accepted as regulators of oestrus cycle in rats. The Ovarian functions are mainly under the control of pituitary gonadotrophins (SCHALLEY, et al. 1973). Generally, the net control of these gonadotrophins are the hypothalamus-pituitary ovaries - axis (SOLIMAN, et al. 1980). In these regards, non steroidal factors called inhibins, produced by ovarian follicles (HENDERSON and FRANCHIMONT, 1981). These factors selectively suppress plasma FSH and to some extent LH (FINDLAY, et al. 1985). Inhibins may act directly on the pituitary (GREEF, et al. 1983) or indirectly through its action on the hypothalamus (LUMPKIN, et al. 1981).

A new model of gonadotrophins control is the protein binding substances that discussed previously in rats by SOLIMAN, et al. (1983); in buffaloes and cows by EL-GHANDOUR (1981,

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1985) and in sheep by ATEIA (1985). These binding substances are localized mainly in the albumin fraction of serum proteins (EL-GHANDOUR, 1981, and ATEIA, 1985). In addition, the concentration of the protein binding substances differs according to the phase of the oestrus cycle. FSH binding substances were absent during proestrus and start to appear during estrus till they reached their peak levels during diestrus. LH binding substances were completely absent during estrus and start to appear at metestrus till they reached peak levels during diestrus.

The rate of disappearance of gonadotrophins from the circulation is an important parameter in studying the dynamics of hormones. This rate is most frequently expressed in terms of the half-life ($t_{1/2}$) (PARLOW, 1968).

The present investigation aimed to clarify the capacity and role played by serum proteins namely albumin and globulin fractions obtained during estrus and diestrus phases on the dynamics of exogenous gonadotrophins. Therefore binding substances obtained from animals in diestrus were injected into rats in estrus and the reverse.

MATERIALS and METHODS

The present investigation was done on 112 mature female rats of an average weight 150± 20 g., vaginal smears were obtained and examined daily. Rats which showed consecutive 4 or 5 days oestrus cycle for at least 3 weeks were selected. The animals were allocated into 14 group, 8 rats each. The first two groups were divided according to the phase of the oestrus cycle namely follicular phase represented in estrus and luteal phase represented in diestrus. Blood samples were obtained from these animals after decapitation under mild anaesthesia. Sera from rats of each group were pooled together and kept in a deep freeze at - 20°C. Albumin and globulin fractions of rats sera were obtained using the alcohol precipitation method of ONCLEY, et al. (1947). Just before use, phosphate buffer (pH: 7.2) was added to the former precipitate to bring it back to the original volume of serum. The other twelve groups were divided and treated as follows:

1 - Group A of 8 rats in estrus phase was injected intravenously (1/v) with 100 i.u PMSG (Gestyl of Organon Co., Holland) contained in 0.5 ml phosphate buffer.

2 - Group B of 8 rats in estrus phase was injected 1/v with 100 i.u hCG (Pregnyl of Organon Co., Holland) contained in 0.5 ml phosphate buffer.

3 - Group C of 8 rats in diestrus phase was injected with PMSG as in group A.

4 - Group D of 8 rats in diestrus phase was injected with hCG as in group B.

The four montioned groups were considered as control groups.

5 - Group E of 8 rats in estrus phase was injected intraperitoneally (1/p) with 1.0 ml of albumin fraction of diestrus rats sera.

6 - Group F of 8 rats in estrus phase was injected 1/p with 1.0 ml of globulin fraction of diestrus rats sera.

7 - Group G of 8 rats in diestrus phase was injected 1/p with 1.0 ml albumin fraction of estrus rats sera.

8 - Group H of 8 rats in diestrus phase was injected with the globulin fraction of estrus rats sers.

After 3 hours from the last injection, each rat was treated with 100 i.u of PMSG 1/v. Each rat in groups I,J,K and L was treated with 100 i.u. of hCG 1/v.

Individual blood samples were collected from the inner canthus of the eyes using heparinized cappillary tubes at intervals of 5, 20, 40 and 60 min after injection of PMSG or hCG., sera were obtained and kept on a deep freeze. Serum PMSG and hCG were assayed using the immunoassay method adopted by ALLEN (1969) and SCHURRS (1969).

The biological half-life of hormones was determined by obtaining clearance constant per minute (K_t). The standard error of mean was calculated according to (DIXON and MASSAY, 1957). "t" test was performed to evaluate the difference between groups.

RESULTS

The results obtained were summarized from tables (1) to (4). It is clear from tables (1 & 2) that maximal concentrations of PMSG and hCG in the sera of female rats in all groups were obtained after 5 minutes that followed by a gradual decrease according to the type of treatment and the phase of estrous cycle.

The results obtained in table (3 & 4) showed that the half lives of PMSG and hCG in intact estrous rats were 13.37 and 16.05 min, respectively while their half lives in diestrous were 33.78 and 21.05 min, respectively.

The $t_{1/2}$ values of hCG were delayed in estrous and diestrous phases after injection of inverse fractions from both albumin and globulin. On the other hand albumin and globulin fractions from diestrous injected to estrous phase resulted in delayed $t_{1/2}$ of PMSG while this fraction obtained from estrous to diestrous phase resulted in retaining or shortage of $t_{1/2}$ as compared to $t_{1/2}$ of estrous phase.

DISCUSSION

The present study clarifies the role played by serum proteins in regulating estrous cycle in rats. It is clear that the $t_{1/2}$ of both PMSG and hCG in estrous rats were shorter than in diestrus ones, especially PMSG as reported previously by AZOUZ (1979) for hCG and ZAKI (1984) for PMSG. The shorter $t_{1/2}$ of PMSG in estrus may be due to the rapid uptake of the hormone by granulosa cells (NIMROD, et al. 1976 and SALOMON, et al. 1977) and hcG by theca and granulosa cells as reported by ZELEZNIK, et al. (1974) and RICHARDS (1980).

Injection of both albumin and globulin fractions obtained from diestrous rats in to estrous ones prolonged the $t_{1/2}$ of both PMSG and hcG. This result may be due to the high levels of protein binding substances in the albumin fraction of diestrous rats as reported by (SOLIMAN, et al. 1983 and TAHA, 1985), while the delayed $t_{1/2}$ resulted from administration of globulin fraction may be due to the presence of antireceptor substances which may prevent the uptake of the hormones by target organ as previously reported by REICHERT, et al. (1981) and JAMES, et al. (1982).

During diestrus, the half-life of hcG was prolonged in rats treated with albumin or globulin from estrous rats. This may be due to loss in LH-receptors in the target organ (newly formed corpus luteum) (HAOUR and SAXENA, 1974) or Saturation of the receptors by endogenous LH hormone as reported by RAO and RICHARDS (1976) and LEE and TAKAHASHI (1977).

Concerning PMSG, the present data showed that administration of the protein fractions into rats alterd the $t_{1/2}$ of hormone. This change was retain to $t_{1/2}$ of estrus by injecting globulin or decrease than diestrus one by injecting albumin fraction.

Lastly, we can conclude that protein binding substances may play an important role in the dynamics of gonadotrophins especially during estrous phase.

Table (1)

Dynamics of exogenous PMSG in the circulation of estrus and diestrus rats pretreated with albumin or globulin fraction of the other phase

Treatment	Phase	Minutes after administration of PMSG			
		5	20	40	60
Saline + PMSG	Estrus	4.69±0.50	2.77±0.40^{a}	1.20±0.50^{b}	0.30 ±0.20^{c}
Albumin of diestrus + PMSG	Estrus	3.07±0.90	2.17±0.32	1.54±0.31	0.59 ±0.12^{a}
Globulin of diestrus + PMSG	Estrus	4.51±0.50	2.31±0.60^{a}	1.80±0.4^{c}	0.59 ±0.19^{c}
Saline + PMSG	Diestrus	2.17±0.30	0.92±0.02^{c}	0.72±0.06^{c}	0.47 ±0.11^{c}
Albumin of estrus + PMSG	Diestrus	5.40±0.45	4.70±0.50	1.12±0.40^{c}	0.004±0.00^{c}
Globulin of estrus + PMSG	Diestrus	5.05±0.50	2.17±0.01^{c}	0.49±0.19^{c}	0.27 ±0.01^{c}

Table (2)

Dyamics of exogenous hCG in the circulation of estrus and diestrus rats pretreated with albumin or globulin fraction of the other phase

Treatment	Phase	Minutes after administration of hCG			
		5	20	40	60
Saline + hCG	Estrus	10.74±1.60	6.25±0.80^{a}	3.05±0.60^{c}	0.74±0.13^{c}
Albumin of diestrus + hCG	Estrus	10.94±1.02	10.31±0.40	9.49±0.60	7.22±0.70^{b}
Globulin of diestrus + hCG	Estrus	11.40±0.80	10.50±0.50	9.48±0.00	8.20±1.50
Saline + hCG	Diestrus	11.54±3.01	8.49±2.10	5.79±1.30	1.22±0.60^{b}
Albumin of estrus + hCG	Diestrus	12.11±0.40	9.49±0.00^{c}	8.22±1.5^{a}	6.06±0.50^{c}
Globulin of estrus + hCG	Diestrus	12.11±0.60	10.17±0.70	8.70±0.80^{b}	5.70±0.80^{c}

Mean ± standard error

a : Means significantly different from the initial concentration (5 mim) at P/ 0.05

b : Means significantly different from the initial concentration (5 mim) at P/ 0.01

c : Means significantly different from the initial concentration (5 mim) at P/ 0.001

Table (3)
Biological half-life of exogenous PMSG in the circulation of estrus and diestrus rats pretreated with albumin or globulin fraction of the other phase

Treatment	Phase	Duration of clearance from injection	Clearance constent (k_t)	Biological half life ($t_{1/2}$) min.
Saline + PMSG	Estrus	5 - 20	0.0351	19.7
		20 - 40	0.0418	16.5
		40 - 60	0.0692	9.9
		Over all mean		13.37
Albumin of diestrus + PMSG	Estrus	5 - 20	0.0232	29.74
		20 - 40	0.0171	40.35
		40 - 60	0.0474	14.56
		Over all mean		28.22
Globulin of diestrus + PMSG	Estrus	5 - 20	0.0447	15.4
		20 - 40	0.0122	56.6
		40 - 60	0.0552	12.5
		Over all mean		28.17
Saline + PMSG	Diestrus	5 - 20	0.0571	12.09
		20 - 40	0.0122	56.36
		40 - 60	0.0209	32.89
		Over all mean		33.78
Albumin of estrus + PMSG	Diestrus	5 - 20	0.0092	75.0
		20 - 40	0.0716	9.6
		40 - 60	0.2814	3.4
		Over all mean		29.33
Globulin of estrus + PMSG	Diestrus	5 - 20	0.0562	12.3
		20 - 40	0.0743	9.3
		40 - 60	0.297	23.18
		Over all mean		14.93

Table (4)
Biological half-life of exogenous PMSG in the circulation of estrus and diestrus rats pretreated with albumin or globulin fraction of the other phase

Treatment	Phase	Duration of clearance from injection	Clearance constent (k_t)	Biological half life ($t_{1/2}$) min.
Saline + hCG	Estrus	5 - 20	0.0362	19.14
		20 - 40	0.0358	19.25
		40 - 60	0.0707	9.76
		Over all mean		16.05
Albumin of diestrus + hCG	Estrus	5 - 20	0.00394	174.7
		20 - 40	0.00414	166.7
		40 - 60	0.0033	50.5
		Over all mean		130.6
Globulin of diestrus	Estrus	5 - 20	0.0055	126.0
		20 - 40	0.00505	136.6
		40 - 60	0.00729	94.56
		Over all mean		119.05
Saline + hCG	Diestrus	5 - 20	0.0204	33.82
		20 - 40	0.0402	17.16
		40 - 60	0.0566	12.19
		Over all mean		21.05
Albumin of estrus + hCG	Diestrus	5 - 20	0.0116	59.5
		20 - 40	0.0078	88.6
		40 - 60	0.0211	32.7
		Over all mean		60.27
Globulin of estrus + hCG	Diestrus	5 - 20	0.0162	42.6
		20 - 40	0.0073	94.5
		40 - 60	0.152	45.4
		Over all mean		60.83

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