Effects of stress upon plasma estradiol and progesterone levels and the rate of oviducal embryo transport in the rat

HUGO CARDENAS *

Laboratorio de Endocrinología, Facultad de Ciencias Biológicas,
Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

The possibility that changes in sex steroid levels associated with stress could alter the rate of oviducal embryo transport was investigated in the rat. To this end, the effect of cold-swimming and cold-restraint upon estradiol (E_2) and progesterone (P) serum levels and embryo transport were assessed. Swimming in water at 16° C for 10 min two or four times between 16:00 and 22:00 h on day 3 of pregnancy caused a modest acceleration of embryo transport that was not associated with decreased fertility. Restraint at 10° C for 2 h between 13:00 and 15:00 h on the first 4 days of pregnancy did not affect embryo transport. Both stimuli increased corticosterone serum levels. Cold-swimming produced a severe hypothermia as compared to cold-restraint and increased serum E_2, decreasing significantly the ratio P/E_2. Cold-restraint increased the P/E_2 ratio. When rats swam in cold water for 10 min twice and were rewarmed by immersion in water at 38° C during 20 min, embryo transport was accelerated despite that no changes occurred in the blood levels of sex steroids. It is concluded that oviducal embryo transport is minimally affected by stress in the rat and that the effect of acute immersion may be independent of alterations in circulating sex steroid levels.

INTRODUCTION

Transport of fertilized ova through the oviduct follows a complex pattern whose purpose is not well understood. The time of transfer of ova to the uterus needs to be coordinated with embryonic and uterine changes, so that the embryos reach a receptive environment for implantation. Fertilized eggs arriving too early or too late to the uterus will not implant (Dickmann and Noyes, 1960; Noyes and Dickmann, 1960). It has been demonstrated that these processes are regulated in the rat by the blood levels of estradiol and progesterone. Treatment with estradiol accelerates ovum transport and causes premature entry of embryos in the uterus (Ortiz *et al.*, 1979). Interference with the production of endogenous estradiol has the opposite effect (Forcelledo and Croxatto, 1988). The accelerating effect of exogenous estradiol in the cycling rat is counteracted by the concomitant administration of progesterone (Fuentealba *et al.*, 1987), and blockade of progesterone receptors with RU486 accelerates ovum transport in cycling and pregnant rats (Fuentealba *et al.*, 1987). So, the rate of passage of ova through the oviduct appears determined by the interplay between the accelerating and delaying influences of estradiol and progesterone, respectively. Therefore, it can be expected that physiological conditions implying different progesterone/estradiol ratios in serum should exhibit different rates of ovum transport; this is the case for the rat during pregnancy, pseudopregnancy and the estrous cycle (Forcelledo *et al.*, 1981). Stressful stimuli may also induce changes in the blood levels of sex steroids (Nequin *et al.*, 1975; Boehm et al., 1982; Bruce *et al.*, 1984). Stressful stimuli are likely to alter the rate of ovum transport in the rat through changes in the circulating levels of progesterone and estradiol. In this paper we examined this possibility studying the effects of cold-swimming and cold-restraint upon blood levels of progesterone and estradiol and upon the rate of embryo transport in the rat.

* Present address: Departament of Physiology and Biophysics, University of Illinois at Urbana-Champaign, 524 Burrill Hall, 407 So. Goodwin Ave., Urbana, Illinois 61801, USA. FAX: (1-217) 333-1133.

Biol Res 25: 15-20 (1992)

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing 220 g were kept in rooms with 14 h light and temperature around 25° C. On proestrus they were caged with males of proven fertility and mating was verified by observation of spermatozoa in the vaginal smear on the following morning (day 1 of pregnancy). Control animals were handled back and forth between the animal house and the laboratory, contemporaneously with experimental goups subjected to the stressful stimuli. Different groups of rats were used to assess the effects of stress on embryo transport, rectal temperature and blood hormonal levels.

Embryo transport

In order to assess changes in the mean rate of embryo transport, rats were killed at 17:00 h on day 4 of pregnancy or at 12:00 h on day 5 of pregnancy, after been exposed to cold-swimming or cold-restraint (see below) on the previous days. Oviducts were flushed with saline and the flushings were examined under low power magnification to count the embryos. Acceleration of ovum transport would be detected as a decrease in the number of embryos recovered from the oviducts on day 4 of pregnancy, whereas a delay would be detected as an increase in that number on day 5 of pregnancy, with respect to control animals.

Stressful stimuli

Rats were submitted to one of two stressors: cold-swimming or cold-restraint. A total of 46 rats were submitted to one out of three schedules of cold-swimming: one (18:00 h), two (18:00 and 20:00 h) or 4 (16:00, 18:00, 20:00 and 22:00 h) 10 min swimming sessions in tap water at 16° C on day 3 of pregnancy. Another group of rats was subjected to 2 swimming sessions as above, but each followed by immersion (without swimming) in tap water at 38° C, for 20 min, procedure that normalized rectal temperature. Additional 22 rats were restrained in small boxes made of a wooden frame and wire sticks that can be adjusted to avoid displacement of the animals. From 13:00 to 15:00 h on days 1 to 4 of pregnancy they were maintained in the restraint boxes inside the cold room at 10° C.

Collection of blood

In order to measure corticosterone in serum, some rats were killed by decapitation and their blood was collected one hour after the first or the fourth swimming session began, or after one hour of cold-restraint. To assess the influence of swimming on serum progesterone and estradiol levels, trunk blood was collected from rats decapitated at different times after the initiation of the first or the second swimming session. To assess the effect of cold-restraint on progesterone and estradiol levels, trunk blood was collected from rats killed at different times during or after cold-restraint on day 1 of pregnancy. Contemporaneously with the experimental rats, blood was also collected from control animals. Serum was stored frozen until the assays.

Hormone assays

Corticosterone was measured by HPLC as described by Hofreiter *et al.* (1982). Intra and interassay coefficients of variation were 8% and 9% respectively; sensitivity was about 10 ng. Progesterone was measured according to the methods and reagents provided by the WHO Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology (Sufi *et al.*, 1985). Intra and interessay coefficients of variation were 6% and 17% respectively. Sensitivity was 6 pg, the volume of extraction was 0.1 ml and the recovery was 73%. Estradiol was also measured with the methods and with the reagents provided by the WHO Programme except that estradiol-(2, 4, 6, 7-^{3}H(N)) (NEN Research Products, Boston, USA) was used instead of the radioactive estradiol provided by the WHO Programme. Sensitivity was 2 pg, volume of extraction was 0.4 ml and recovery was 92%. Since estradiol and progesterone effects on ovum transport depend on the relative amount of each, the changes of both steroids were also expressed as the molar ratio progesterone/estradiol.

Embryo implantation

Rats subjected to 4 swimming sessions on the evening of day 3 of pregnancy were killed on day 15 of pregnancy to assess the number of implanted embryos.

Measurement of rectal temperature

Rectal temperature was measured in experimental rats at various times during and after the aplication of the stressful stimuli and in control animals, through a rectal thermoprobe connected to a telethermometer.

Statistical analysis

Data were analyzed using non-parametric statistics because their distribution was not always normal. Analysis of variance was applied to the ranks of the data, according to the recommendations of Conover and Iman (1981). Significance level was set at $p < 0.05$.

RESULTS

Effects of stressful stimuli upon rectal temperature

Each one of four successive swimming sessions applied every two hours on the evening of day 3 of pregnancy was followed by an acute and pronounced hypothermia (Fig. 1). Body temperature did not return to normal values until several hours later. However, immersion in water at 38° C during 20 min immediately after swimming normalized rectal temperature (data not shown). Cold-restrained rats developed mild hypothermia, which disappeared within one hour (Fig. 2).

Effects of stressful stimuli upon corticosterone levels in serum

One hour after a 10 min swimming session, corticosterone serum levels increased from ($\bar{x}$ ± SD) 440 ± 100 ng/ml (n = 4) in control rats to 900 ± 240 ng/ml (n = 4) in experimental rats. Similarly, one hour after the last of four swimming sessions serum corticosterone had increases from 192 ± 119 ng/ml (n = 5) in control rats to 1197 ± 279 ng/ml (n = 5). One hour of cold-restraint applied from 13:00 on day 1 of pregnancy led also to a significant increase of corticosterone serum levels from 33 ± 6 ng/ml (n = 3) in control rats to 521 ± 69 ng/ml (n = 3) in experimental rats. Differences between the 3 control groups are ac-

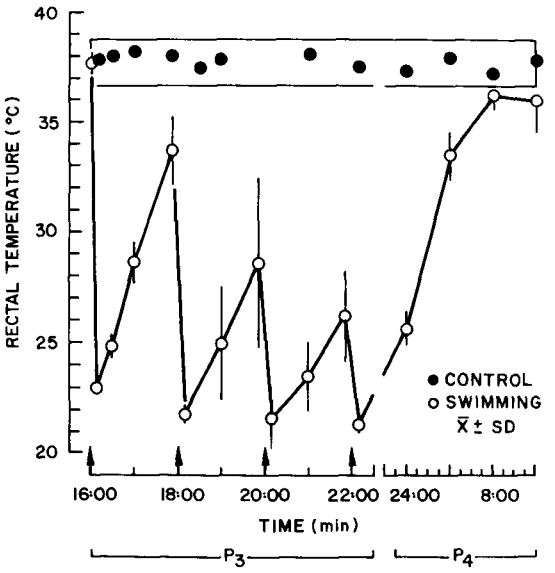

Figure 1: Effect of cold-swimming upon rectal temperature. Stressed rats (empty circles, n = 5) subjected to four successive swimming sessions in cold water (black arrows) on the evening of day 3 of pregnancy (P3). Rectangle, 95% normal distribution of rectal temperature of control rats (filled circles; grand mean ± SD = 37.8 ± 0.6° C, n = 32) maintained at ambient temperature (around 25° C).

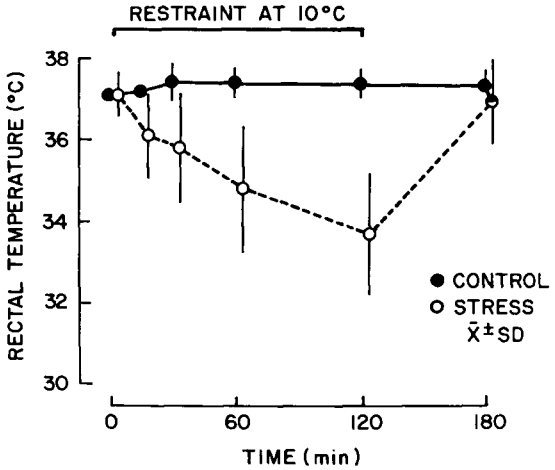

Figure 2: Effect of cold-restraint upon rectal temperature. Stressed rats (empty circles) subjected to cold-restraint from 13:00 to 15:00 h on day 1 of pregnancy. Controls (filled circles) maintained at ambient temperature (around 25° C).

Biol Res 25: 15-20 (1992)

counted by the circadian rhythm of corticosterone in the rat (for example see D'Agostino *et al.*, 1982).

Effects of stressful stimuli upon progesterone and estradiol levels in serum.

Progesterone levels decreased significantly 30 and 60 min but not 120 min after one swimming session (Fig. 3a). There was no difference between experimental and control rats 120 min after the stimulus (Fig. 3a). A second swimming session applied two hours later produced a similar decrease, but in this case the difference between experimental and control rats was also significant 15 min after the beginning of swimming (Fig. 3a). On the other hand, serum estradiol levels were higher in experimental than in control rats 15, 30 and 60 min after the first and 15 and 30 min after the second swimming session (Fig. 3b). The effect of swimming was a decrease in the molar ratio progesterone/estradiol, which was significant 15 and 30 min after the beginning of the first and 15, 30 and 60 min after the second swimming session (Fig. 3c) The area under the curve progesterone/estradiol *vs* time was 28% lower in experimental than in control rats (Fig. 3c). However, there were no differences in blood progesterone and estradiol levels between control and experimental rats one hour after one cold-swimming session when hypothermia was corrected by immersion in water at 38° C. Progesterone levels were ($\bar{x} \pm$ SD) 52 ± 23 ng/ml (n = 10) and 51 ± 15 ng/ml (n = 10) in control and experimental rats, respectively. Estradiol levels were 45 ± 13 pg/ml (n = 10) and 41 ± pg/ml (n = 10) in control and experimental rats, respectively.

Cold-restraint produced an increase in serum progesterone which was significant from 15 min up to 120 min of restraint. The major difference between control and experimental rats was observed after 60 min of restraint, when progesterone serum levels had increased from 19 ± 5 ng/ml in control to 78 ± 14 ng/ml in experimental rats (Fig. 4a). Serum estradiol was also higher in experimental than in control rats at 60 and 120 min of cold-restraint (Fig. 4b). The net effect of this stimulus was a rise in the serum levels of both steroids with a significant increase in

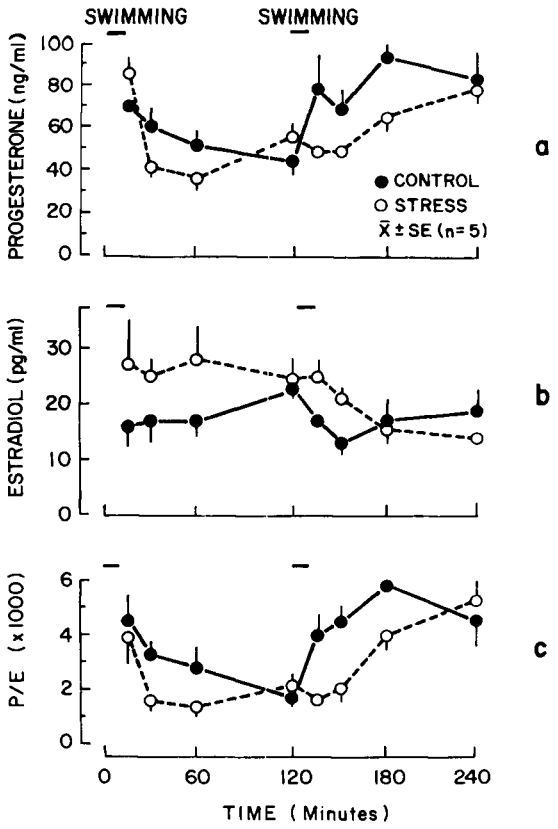

Figure 3: Effect of cold-swimming upon blood levels of progesterone and estradiol. Groups of stressed (empty circles) and control (filled circles) rats sacrificed at different times from beginning of first or second swimming session in cold water. Each swimming session significantly depressed both progesterone and molar ratio progesterone/estradiol, and increased estradiol in serum.

the molar ratio progesterone/estradiol, which was maximal at 30 min of restraint (Fig. 4c). The area under the curve progesterone/estradiol *vs* time was 68.5% higher in experimental than in control rats (Fig. 4c).

Effects of stressful stimuli upon the rate of embryo transport and implantation

Two or four swimming sessions in cold-water led to a similar slight but statistically significant decrease in the proportion of embryos recovered from the oviducts (Table I). One swimming sesion did not affect the rate of embryo transport. The decrease induced by two swimming sessions was not affected when rats were rewarmed by immersion in water at 38° C after each session (Table I). In addition, four swimming sessions did not modify

TABLE I

Effect of swimming in cold water upon oviducal embryo recovery

Condition	Oviducal embryos Mean ± SD	Number of rats
Control	11.5 ± 2.9	57
1 session	11.6 ± 3.7	17
2 sessions	8.7 ± 4.2 *	15
4 sessions	9.4 ± 4.4 *	14
Control	13.3 ± 1.7	10
2 sessions + rewarming	7.0 ± 1.3 *	9

Stressed rats subjected to 1, 2 or 4 swimming sessions on evening of day 3 of pregnancy. Another group of rats subjected to 2 swimming sessions but rewarmed by immersion in water at 38° C during 20 min after each session. Autopsy performed on following day at 17:00 h.
* Significantly lower than controls.

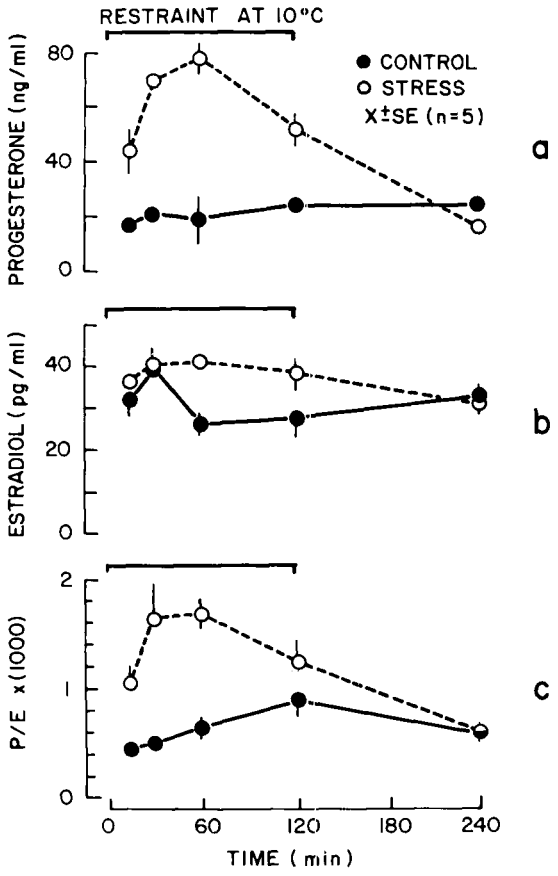

Figure 4: Effect of cold-restraint upon blood levels of progesterone and estradiol. Groups of stressed (empty circles) and control (filled circles) rats sacrificed at different times from beginning of cold-restraint. Progesterone, estradiol and molar ratio progesterone/estradiol were significantly increased in stressed animals.

the rate of implantation. On day 15 of pregnancy there were 12.4 ± 2.0 and 13.3 ± 1.4 implanted embryos in control and experimental rats, respectively.

Cold-restraint from 13:00 to 15:00 h applied on the first 4 days of pregnancy did not modify the rate of embryo transport (Table II).

TABLE II

Effect of cold-restraint upon oviducal embryo recovery

Day of Pregnancy	Condition	Oviducal embryos Mean ± SD	Number of rats
4	Control	11.6 ± 2.8	16
	Restraint	10.8 ± 3.6	17
5	Control	0.5 ± 0.8	8
	Restraint	0.3 ± 0.5	6

Stressed rats subjected to 2 h of cold-restraint from 13:00 to 15:00 h on first 4 days of pregnancy. Autopsies performed on days 4 or 5 of pregnancy. No significant differences between control and experimental rats.

DISCUSSION

Results presented in this paper show for the first time that stressful stimuli can elicit changes in circulating levels of sex steroids in the early pregnant rat, and that these changes, in contrast to what has been described previously for plasma corticosterone levels, are stimulus specific: cold-restraint produced an increase in the progesterone/estradiol ratio and cold-swiming led to a decrease in that ratio. The increases in blood progesterone and estradiol induced by cold-restraint could be of adrenal origin as demonstrated in other stressful conditions (Nequin *et al.*, 1975; Boehm *et al.*, 1982).

The results also show that different stressful stimuli may induce different effects upon oviducal embryo transport. The estrogenic predominance of cold-swimming was associated with accelerated transport and the progestational predominance of cold-restraint was associated with no changes in the rate of oviducal transport. These relationships are to be expected in view of the accelerating effect of exogenous estradiol (Ortiz *et al.*, 1979) and

the lack of effect of exogenous progesterone in the pregnant rat (Fuentealba *et al.*, 1987). However, when the severe hypothermia induced by two swimming sessions was reverted by immersion in water at 38° C the acceleration of ovum transport was maintained despite that estradiol and progesterone changes disappeared. So, acute cold *per se* induced a discrete acceleration of ovum transport independent of any change in circulating sex steroid levels. This suggests that the rich innervation of the oviduct (for example see Sjöberg, 1967; Papka *et al.*, 1985), or some other stress-associated hormonal change mediated the acceleration of oviducal embryo transport induced by swimming in cold water.

The rather discrete change in the rate of embryo transport induced by the deep hypothermia associated with cold-swimming which did not affect the implantation rate, and the lack of effect of repetitive exposures to cold-restraint, demostrate the efficacy of the regulation of the oviducal ovum transport in the rat, which ensures that fertilized ova reach the uterus at the appropriate time in order to accomplish successful implantation. In this regard the rat appears to be different to the mouse in which it has been reported that repetitive restraint applied on early pregnancy induces a decrease in progesterone plasma levels associated to luteolysis, acceleration of ovum transport and decrease in the implantation rate (Wiebold *et al.*, 1986).

ACKNOWLEDGMENT

This work was part of a Ph. D. Thesis approved by the Pontificia Universidad Católica de Chile. The financial support of the Rockefeller Foundation and the P. Universidad Católica de Chile is gratefully acknowledged.

REFERENCES

BOEHM N, PLAS-ROSER S, ROOS M, ARON C (1982) How different procedures of blood removal affect blood progesterone concentration in the cyclic female rat. *J Steroid Biochem 16:* 339-342

BRUCE N W, WILLCOX D L, MEYER G T, WADDEL B J (1984) Effects of handling, anesthesia, ovariectomy and adrenalectomy on serial measurements of serum progesterone in 16-day pregnant rats. *J Endocrinol 100:* 189-193

CONOVER W J, IMAN R L (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *Am Stat 35:* 124-129

D'AGOSTINO J B, VAETH G F, HENNING S J (1982) Diurnal rhythm of total and free concentrations of serum corticosterone in the rat. *Acta Endocrinol 100:* 85-90

DICKMAN Z, NOYES R W (1960) The fate of ova transferred into the uterus of the rat. *J Reprod Fert 1:* 197-212

FORCELLEDO M L, CROXATTO H B (1988) Effects of 4-hydroxyandrostenedione and exogenous testosterone on blood concentrations of estradiol and oviductal embryo transport in the rat. *J Endocrinol 118:* 93-100

FORCELLEDO M L, VERA R, CROXATTO H B (1981) Ovum transport in pregnant, pseudopregnant, and cyclic rats and its relationship to estradiol and progesterone blood levels. *Biol Reprod 24:* 760-765

FUENTEALBA B, NIETO M, CROXATO H B (1987) Ovum transport in pregnant rats is little affected by RU486 and exogenous progesterone as compared to cyclic rats. *Biol Reprod 37:* 768-774

HOFREITER B T, ALLEN J P, MIZERA A C, POWERS C D, MASI A M (1982) High-performance liquid chromatography and radioimmunoassay of rat serum corticosterone. *Steroids 39:* 547-555

NEQUIN L G, ALVAREZ J A, CAMPBELL C S (1975) Alterations in steroid and gonadotropin release resulting from surgical stress during the morning of proestrus in 5-day cyclic rats. *Endocrinology 97:* 718-724

NOYES R W, DICKMAN Z (1960) Relationship of ovular age to endometrial development. *J Reprod Fert 1:* 186-196

ORTIZ M-E, VILLALON M, CROXATTO, H B (1979) Ovum transport and fertility following postovulatory treatment with estradiol in rats. *Biol Reprod 21:* 1163-1167

PAPKA R E, COTTON J P, TRAURIG H H (1985) Comparative distribution of neuropeptide tyrosine-, vasoactive intestinal polypeptide-, substance P-immunoreactive, acetylcholinesterase-positive and noradrenergic nerves in the reproductive tract of the female rat. *Cell Tissue Res* 242: 475-490

SJÖBERG N-O (1967) The adrenergic transmitter of the female reproductive tract: distribution and functional changes. *Acta Physiol Scand, suppl 305:* 1-32

SUFI S B, DONALDSON A, JEFFCOATE S L (1985) Method Manual, W.H.O. Special Programme of Research, Development and Research Training in Human Reproduction. 9th ed

WIEBOLD J L, STANFIELD P H, BECKER W C, HILLERS J K (1986) The effect of restraint stress in early pregnancy in mice. *J Reprod Fert 78:* 185-192