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Increased secretion of adrenal progesterone explains the lack of response of oviductal embryo transport to a short intravenous infusion of estradiol in the rat

Un aumento en la secreción adrenal de progesterona explica la falta de aceleración del transporte de embriones después de una infusión corta de estradiol

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Administration of estradiol (E_2) as a single subcutaneous injection, but not as a short intravenous infusion (< 150 min), accelerates oviductal embryo transport in pregnant rats although the first mode determines lower E_2 circulating levels. Since progesterone (P) can antagonize the effect of E_2 on embryo transport we examined the circulating P levels under these two modes of E_2 administration.

Rats were treated on day 1 of pregnancy with 5 μg E_2 given s.c. or i.v. (10 min infusion). Other groups were either hypophysectomized (HPX), adrenalectomized (ADX) or ovariectomized (OVX) prior to E_2 treatment to prevent P rise, or were treated with E_2 plus RU486 to block the action of P. Some groups were autopsied at short intervals following treatment to measure P levels and others 24 h later to assess the effect of treatments on embryo transport.

P was increased several fold by i.v. infusions of E_2 or vehicle alone in intact and OVX rats but not in HPX or ADX rats, whereas s.c. administration of E_2 did not change P levels unless it was given concomitantly with i.v. infusion of vehicle. The short i.v. infusion of E_2 accelerated embryo transport in HPX, ADX, or RU486 treated rats but not in intact rats. The s.c. injection of E_2 accelerated embryo transport even when it was accompanied by an i.v. infusion of vehicle.

The data does not exclude the participation of glucocorticoids in the above phenomena but agrees with the view that it is the transient increase in adrenal P secretion which blunts the oviductal response to a brief pulse of E_2.

A single subcutaneous (s.c.) injection of 17-B-estradiol (E_2) given to rats on day 1 of pregnancy abbreviates the oviductal transit of embryos from 94 h to less than 24 h (Ortiz *et al.*, 1979). The same dose of E_2 given as an intravenous (i.v) infusion can be fully effective or totally ineffective in accelerating embryo transport, depending on the time taken for total delivery. For example, a short i.v. E_2 infusion (10-50 min) caused a high amplitude plasmatic pulse of E_2, which was ineffective in eliciting the response; however, the same total dose (5 μg) administered as a prolonged i.v infusion (200-300 min) or as a single s.c. injection, produced a lower but sustained rise of circulating E_2 followed by accelerated embryo transport (Forcelledo *et al.*, 1986). The E_2 tissue content, attained by either mode of administration, showed that differences in oviductal E_2 availability did not explain the occurrence of response with one mode and the lack of response with the other.

Since progesterone (P) can antagonize the effect of E_2 on egg transport (Fuentealba *et al.*, 1988) it was considered that changes in P production caused by either treatment could explain the different response of the oviduct.

The aims of this work were: 1) to determine whether or not an increased

secretion of P is involved in the blunted response of embryo transport to a brief estrogenic pulse and 2) to identify the source of P increase.

MATERIAL AND METHODS

Animals

Female Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 220-250 g were used. They were kept under controlled temperature (21-24°C) and with lights on from 07.00-21.00 h. Vaginal smears were taken daily and proestrous females were caged overnight with fertile males. Mating was verified the following morning by observation of spermatozoa in the vaginal smear. This day was designated as day 1 of pregnancy (P1).

Experimental Designs

Effect of two rates of delivery of E_2 on embryo transport

Eight rats were anesthetized with ether on P1 and were implanted with a polyethylene cannula (Intramedic, id 0.001 inch, od 0.024 inch) in the jugular vein, through which they were infused for 10 min with 5 μg 17-B-estradiol (E_2) (Sigma Chemical Co., St. Louis, M0) dissolved in 0.5 ml of aqueous vehicle (ethanol-glycerol-saline in the proportion 20:30:50). These animals also received 0.5 ml vehicle s.c. Another group of 8 rats was injected s.c. with 5 μg E_2 and received aqueous vehicle i.v. for 10 min. Eight control rats were infused i.v. and injected s.c. with vehicle. These animals were killed 23 h later to assess the effects of treatment on embryo transport.

P levels in serum following two rates of E_2 delivery

Fifteen animals in the morning on P1 were infused i.v. with 5 μg E_2 for 10 min and other 15 rats were injected s.c. with 5 μg E_2. Other 18 rats were injected s.c. with 5 μg E_2 and infused i.v. with vehicle. Lots of 5-6 rats from each group were killed by decapitation at 10, 30 and 60 min after treatment. Controls (three lots of 6 rats each) were decapitated at the same intervals after the administration of vehicle by both routes. Trunk blood was collected without heparin and serum was frozen at −20°C until P assay.

Effect of P blockage with RU486 on embryo transport in rats treated with E_2

Rats on P1, treated i.v. or s.c. with E_2 as described above, and grouped in lots of 5-6 animals received, in addition, RU486 (Roussel Uclaf, Paris) or vehicle. Two mg RU486 dissolved in 0.1 ml propylenglycol was administered s.c. 2 h before and after E_2 treatment. Five control rats were injected s.c. and i.v. with the corresponding vehicle and treated with RU 486 as above. All rats were killed 24 h later to assess the effect of treatment on embryo transport.

Effect of hypophysectomy on P serum levels and embryo transport in rats treated with E_2

Hypophysectomy (HPX) or sham hypophysectomy (SHPX) were performed in the morning of P1 through the parapharingeal approach, under ether anesthesia. Two hours after surgery these animals received E_2 i.v. infusion, E_2 s.c. injection and the vehicles i.v. or s.c. in the appropriate combinations. Lots of 5-6 rats were killed by decapitation either before or 10 or 60 min after the onset of treatment to collect trunk blood without heparin. Serum was frozen at − 20°C until P assay. Other groups of HPX and SHPX rats treated in the same way were killed 24 h later to assess embryo transport. The completeness of HPX was checked in all animals by visual inspection under magnification.

Glandular source of P

The source of increased plasmatic levels of P was studied by ablating the two principal P secretory glands. Ovariectomy (OVX) or adrenalectomy (ADX) and their respective sham operations (SOVX and SADX) were performed in the morning of P1 under ether anesthesia. Groups of 5-6 animals were treated with i.v. estradiol infusion, E_2 s.c. administration and/or the corresponding vehicle 3 h after surgery.

Lots of 5-6 rats were killed by decapitation before, 10 or 60 min after the onset treatment to collect trunk blood without heparin. Serum was frozen at −20° until P assay. Other groups of 5-9 SHADX or ADX rats treated in the same way were killed 24 h later to assess embryo transport.

Techniques

Embryo transport

Oviducts removed 22-24 h after treatment were flushed with saline and the flushings were examined under low power magnification to count the ova. A reduced number of oviductal embryos as compared with control rats, was considered evidence of accelerated transport.

Progesterone RIA

Serum progesterone was measured using a monoclonal antibody raised in mouse against progesterone-3-carboxymethyloxime conjugated with BSA. 20 μl of serum were extracted with 1.5 ml of ether after adding a tracer amount of tritiated progeste-

rone to correct for procedural losses. Average recovery was between 80% and 95%. The antibody was used at a final dilution of 1:210000 and the minimim detectable dose was 3 pg/tube. Coefficients of variation intra-assay were 5.2% and 12.8% for the low and high pool respectively. This antiserum cross-reacted less than 0.02% with testosterone, 1,6% with 17-α-hydroxyprogesterone and 0.04% with 20-α-dihydroprogesterone at 50% binding. Reagents and the antibody were provided by the WHO Programme for the Provision of Matched Reagents for the Radioimmunoassay of Hormones in Reproductive Physiology.

Statistics

Differences in the mean recovery of oviductal embryos were analyzed by the Mann Whitney test. Temporal variations in P levels after E_2 administration were analyzed using the Student's t-test.

RESULTS

Ovum transport after s.c. injection and i.v. infusion of E_2

Five μg E_2 were given as a single s.c. injection or as a 10 min i.v. infusion. The mean number of embryos recovered from the oviducts on the next day is shown in Fig. 1. The number of embryos in rats treated with E_2 was statistically lower than in the control group, only when the hormone was given s.c. This confirms that the two rates of delivery of E_2 have a different capacity to elicit this biological response.

Progesterone levels after estrogen treatment

Circulating P was measured at various times after i.v. or s.c. E_2 treatments or i.v. infusion of vehicle. The results are shown in Fig. 2. Following i.v. or s.c. delivery of 5 μg E_2 and/or vehicle, P levels increased four-fold at about 30 min and remained elevated at about 1 h. Serum P levels in rats receiving E_2 as s.c. injection only showed minor fluctuations that were not different from pretreatment values.

The results demonstrate that serum P increases following a brief i.v. infusion of E_2 or its vehicle.

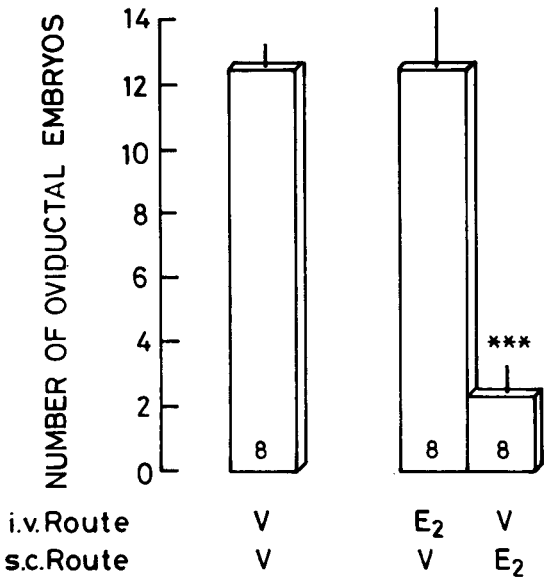

Fig. 1: Number of oviductal embryos on day 2 in rats given E_2 s.c. or i.v. on day one of pregnancy. 5 μg E_2 infused i.v. during 10 min did not accelerate transport. Bars represent the mean ± S.E.M. of eight rats.
***, $P < 0.001$ compared to vehicle treated controls.

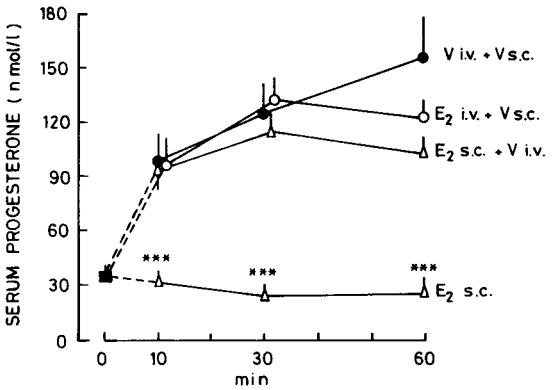

Fig. 2: Serum levels of progesterone after i.v. and/or s.c. administration of 5 μg estradiol 17B (E_2) and its vehicle (V) to rats on day one of pregnancy. In all groups treated with i.v. infusions there was a marked increase of progesterone not seen after s.c. E_2 administration alone. Each point represents the mean ± S.E.M. of 5-6 rats.
***, $P < 0.001$ compared to i.v. infusions at same time point.

Pituitary mediation of plasma P increase produced by i.v. infusion

Fig. 3 shows the number of embryos recovered from oviducts and the serum levels of P in rats hypophysectomized shortly before s.c. or i.v. vehicle or E_2 administrations.

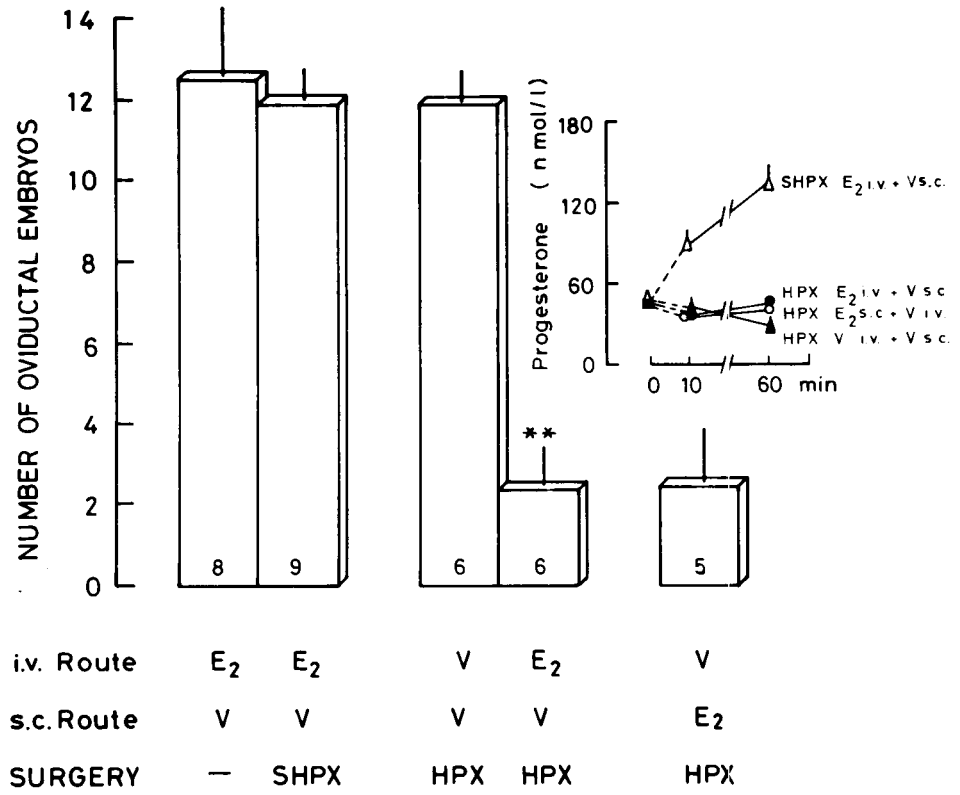

Fig. 3: Number of oviductal embryos and serum progesterone levels (inset) in rats submitted to hypophysectomy (HPX), sham hypophysectomy (SHPX), and treatment with estradiol 17B (E_2) and its vehicle (V) by i.v. and s.c. routes. Surgery and other treatments were done on day one of pregnancy. The number of oviductal embryos was assessed 24 h later. Number of animals indicated at the foot of the bars. Progesterone was measured in other groups of 5-6 rats each at the indicated times relative to the onset of E_2 treatment. Point and bar indicate mean ± S.E.M. Embryo recovery was decreased in HPX rats following i.v. E_2 administration. The increase of progesterone levels observed after i.v. infusions was suppressed by HPX.
**, $P < 0.02$ compared to SHPX plus i.v. E_2.

The number of oviductal embryos was the same in intact, SHPX and HPX plus vehicle administration. However, after HPX plus i.v. E_2 infusion the number of oviductal embryos was significantly decreased and was nearly identical to that seen after s.c. E_2. HPX suppresed the increase of P levels observed after i.v. infusion of E_2 or vehicle. These data show that the increase in circulating P caused by i.v. infusion requires the pituitary gland and suggest that its suppresion allows the oviduct to respond to a brief increase in circulatin E_2.

Effect of RU486, on the oviductal response to i.v. or s.c. E_2 administration

In order to confirm that the failure of a brief E_2 pulse to accelerate transport was caused by increased levels of P, the progesterone receptor blocking agent RU486 was used. Results are shown in Fig. 4. The number of oviductal embryos recovered in control rats was not affected by treatment with RU 486 (compare with Fig. 1). Egg recovery in rats given i.v. E_2 and injected with RU486, was significantly lower than in rats given i.v. E_2 without RU486. Egg recovery in rats given s.c. E_2, with or without RU486 was significantly lower than in the controls. These data demonstrate that the increased levels of P counteracted the effect of a brief estrogen pulse on embryo transport.

Endogenous source of P increase

Since the P increase appeared related to the anesthesia and surgical procedure utilized for i.v. infusion rather than E_2 administra-

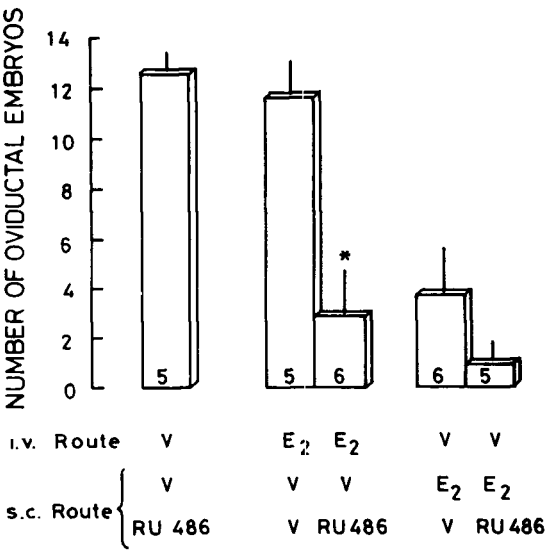

Fig. 4: Number of oviductal embryos on day 2 in rats treated with RU486, estradiol 17B (E_2) s.c. or i.v. or their combinations on day one of pregnancy. The recovery of embryos was significantly reduced following the administration of RU486 plus i.v. E_2. Bars represent the mean ± S.E.M. of the number of rats indicated at the foot.
*, $P < 0.05$ compared to i.v. E_2.

tion, an adrenal source of P was suspected. We measured P levels in rats ADX or OVX shortly before giving vehicle or E_2 as i.v. infusion or s.c. injection. The serum levels of P in ADX, OVX and sham operated controls and the number of embryos in ADX and SADX rats are shown in Fig. 5.

OVX or SOVX plus i.v. or s.c. E_2 administration were followed by an increase of P levels, albeit less pronounced than in intact rats receiving i.v. infusions. In contrast, ADX but not SADX prevented completely the P rise after E_2 or vehicle i.v. infusion. The i.v. E_2 infusion accelerated oviductal embryo transport in ADX but not in SADX rats.

These results indicate that the adrenals are the main source of increased P levels after the i.v. infusions and confirm that i.v. E_2 can accelerate oviductal embryo transport when P increase is prevented.

DISCUSSION

The present results show that the failure of a brief elevation of E_2 in serum that accompanies its i.v. infusion to accelerate oviductal embryo transport in rats is asociated with a concomitant increase in circulating P levels. This pituitary-mediated, increased secretion of P by the adrenals forms part of the stress response to the intravenous infusion procedure itself.

Surgical ablation of the pituitary or the adrenal prior to i.v. infusion prevented this P increase. These procedures or concomitant treatment with RU486 allowed the oviductal response to the brief elevation of E_2 in serum. Since stress is known to increase glucocorticoid secretion and RU486 is endowed also with antiglucocorticoid activity one cannot exclude a role of these steroids in the lack of accelerated embryo transport.

Based upon the response to a variety of E_2 oscillations in plasma we had previously suggested that the geometric profile of the oscillation was critical for determining the oviductal response (Forcelledo *et al.*, 1986). The present data do not support that interpretation. Instead, the critical factor appears to be the behaviour of P concomitant with the elevation of circulating E_2.

Elevated P serum levels were observed every time intact animals were subjected to i.v. infusion regardless of the E_2 content of the infused solution. This is true also when E_2 was given s.c. and vehicle was given i.v. to the same animals. Notwithstanding, this s.c. E_2 accelerated oviductal embryo transport in spite of increased levels of P. The reason why increased P levels counteract the effect of E_2 given by a short i.v. infusion but not by s.c. injection may be related to the time that circulating E_2 remains elevated. E_2 levels remain elevated twice as long after s.c. than i.v. administration (Forcelledo *et al.*, 1986) and increased P levels outlast the E_2 elevation seen after i.v. E_2 but not after s.c. E_2.

Intravenous E_2 accelerated embryo transport when P action was interfered by RU486. The possibility that this is due to increased secretion of E_2 is unlikely. Although, circulating E_2 was not measured in the present work, it was shown before that treatment with RU486 does not

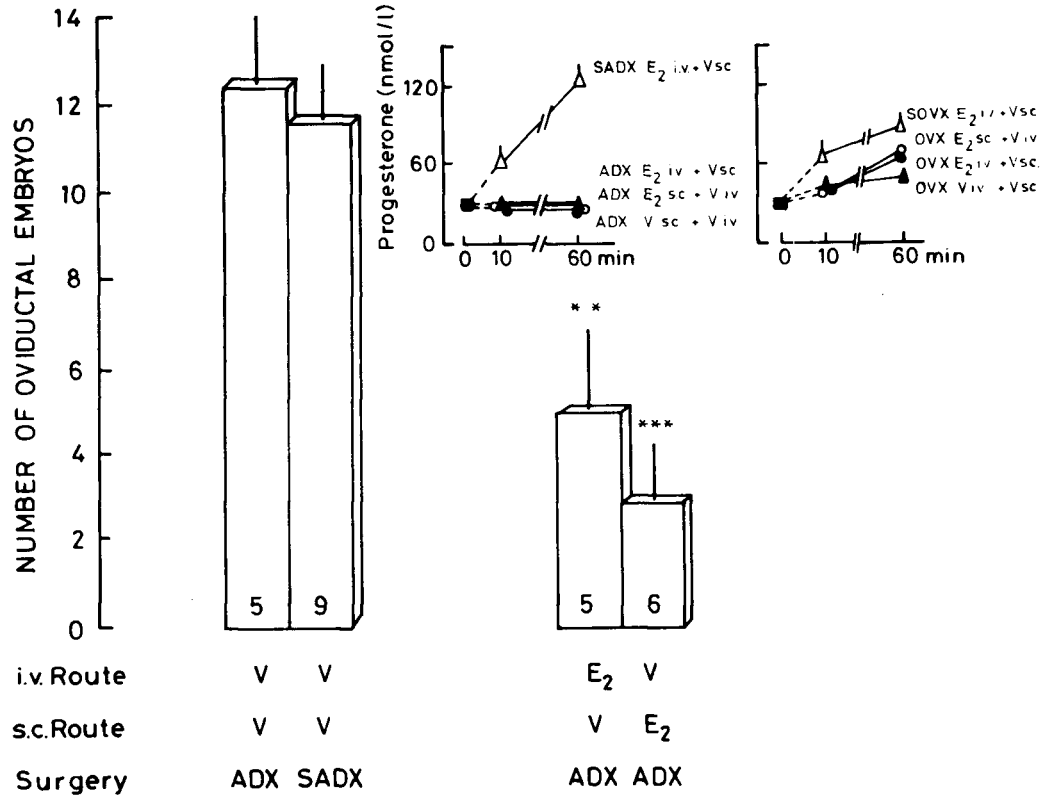

Fig. 5: Number of oviductal embryos and serum progesterone levels (inset) in rats submitted to ovariectomy (OVX), adrenalectomy (ADX) or sham operations (SOVX, SADX), and treatments with estradiol 17B (E_2) and its vehicle (V) by s.c. or i.v. routes. Surgery and treatment were done in the morning of day 1 of pregnancy and embryo transport was assessed in ADX and SADX 24 h later. Number of rats indicated at the foot of the bars. Progesterone was measured in other groups of 5-6 rats each at the indicated times relative to the onset of E_2 treatment. Point and bars indicate mean ± S.E.M. The recovery of embryos was decreased in ADX rats following s.c. or i.v. E_2 administration. The increase of progesterone levels observed after i.v. infusions (E_2 or vehicle) was totally suppressed only by ADX.
**, $p < 0.02$ and ***, $P < 0.005$ compared to vehicle infused operated animals.

elevate plasma E_2 levels during the first 3 days of pregnancy in rats (Fuentealba *et al.*, 1987).

The amplitude and duration of the P increase following i.v. infusion under ether anesthesia in pregnant rats, appears very similar to the P increase observed in pregnant rats under other stressful stimuli such as cold-restraint or swimming in cold water (Cárdenas and Croxatto, 1988). Although catecholamines, ACTH or cortisol were not measured in this work, we assume that P increase was due to stress rather than to E_2 positive feedback upon the pituitary.

In summary, the failure to accelerate ovum transport of a brief but high estrogen pulse in serum associated with E_2 i.v. infusion, can be accounted for by a pituitary-mediated enhancement of adrenal steroid secretion. When this was abolished by HPX, ADX or progesterone and glucocorticoid receptors were blocked by RU486, the oviductal response was restored. Since P is known to antagonize the effect of E_2 on oviductal embryo transport in the rat (Fuentealba *et al.*, 1988) increased P secretion is sufficient to account for these results.

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