Effect of oestradiol delivered from a perioviducal device on ovum transport in mice

J. Zenteno, C. Silva, H. Cárdenas and H. B. Croxatto

Laboratorio de Endocrinología, Facultad de Ciencias Biológicas, Universidad Católica de Chile, Santiago, Chile

Summary. Silastic devices impregnated with oestradiol and blank devices were placed around both oviducts and around skeletal muscle bundles in the forelegs to attain local and systematic delivery, respectively. Another group of mice received an oestradiol-impregnated device around one oviduct and a blank device in the contralateral oviduct. Implantation of blank devices around the oviducts and in the forelegs did not alter ovum transport. Devices impregnated with oestradiol placed around both oviducts produced a dose-dependent delay of ovum transport, which was more pronounced than the effect of devices located in the forelegs. Oviducts receiving an oestradiol-loaded device had a larger retention of ova than did the contralateral oviducts receiving a blank device. These results demonstrate a direct action of oestradiol upon the oviduct to delay ovum transport in the mouse.

Keywords: oviduct; egg transport; oestradiol; mouse

Introduction

Previous work showed that the action of oestradiol on ovum transport in the mouse is of unusual complexity. If the hormone is administered in low doses during the 1st day of pregnancy it delays the passage of embryos to the uterine horn, but if high doses are administered the opposite effect is observed. Moreover, low doses of oestrogen administered during the 2nd day of pregnancy cause accelerated transport of the majority of the embryos followed by oviducal retention of the remaining eggs (for review see Moscoso *et al.*, 1984).

Oestrogens affect several functions acting on a variety of target organs. Among them they induce release of oxytocin and its associated neurophysins (Amico *et al.*, 1981) and exert positive as well as negative feedback on the secretion of gonadotrophins (Daughaday, 1985), all of which could indirectly affect the oviducal transport function.

The present investigation attempts to ascertain whether oestradiol acts directly or indirectly upon the oviduct to delay the transport of embryos. For this purpose, a silicone rubber device was designed to allow the local delivery to the oviduct of a very small dose of oestradiol which is not effective when administered systemically. It was reasoned that if the local application succeeded in altering ovum transport under such conditions, it would provide experimental evidence that exogenous oestradiol delays ovum transport in the mouse by a direct action on the oviduct.

Materials and Methods

Preparation of the perioviducal devices. Silastic tubing (Dow Corning Corporation, Midland, Michigan, USA; 1.47 mm i.d.; 1.95 mm o.d.) was cut into pieces of 2–2.5 mm length. Half of the pieces were soaked in ethanol and the rest in solutions of oestradiol in ethanol (4 pieces/ml solution). After 3 h the pieces were removed to allow evaporation of the ethanol. Once dried, each piece was covered on the outer surface with a Teflon® tape (Thomas Catalog No.

Fig. 1. Illustration of the perioviducal device. (a) The final steps in preparation of the device: under a dissecting microscope each piece of Silastic tubing (A) is covered with a thin Teflon material (B) to reduce diffusion of oestradiol through the external surface. The two materials are sealed with Silastic medical adhesive. A longitudinal cut turns the tubing into a C-shaped device (C). (b) The mouse genital tract with a perioviducal device in position.

9088-T50) by means of silicone adhesive (Dow Corning). Finally, a longitudinal cut was made for opening and placing the device around the oviduct or in a distant location (Fig. 1).

Implantation of the devices. Female mice of the Swiss Rockefeller stock, 3–5 months old, on the first day of pregnancy (the day a vaginal plug was found) were utilized. The devices were implanted around the oviducts and around a bundle of skeletal muscle in the forelegs (systemic location) while the animals were anaesthetized with ether.

Experimental design. Two experimental series were carried out. In Exp. 1, mice were randomly separated into 5 groups: (1) intact controls which were not operated; (2) treatment with perioviducal and systemic devices without oestradiol; (3) treatment with systemic devices with oestradiol and perioviducal devices without steroid; (4) treatment with perioviducal devices with steroid and systemic devices with no steroid; and (5) treatment with systemic and perioviducal devices with oestradiol. For this experiment the oestradiol-loaded devices had been incubated in an oestradiol solution containing 1 µg/ml.

In Exp. 2, only perioviducal devices were implanted. Each animal was provided with a device containing oestradiol on the left side, and a device free of hormone on the right side. For this experiment the oestradiol-loaded devices had been incubated in solutions containing 0.5 or 2.0 µg oestradiol/ml.

Assessment of ovum transport. On the 4th day of pregnancy, the animals were killed by cervical dislocation. Animals in which the devices had been displaced were eliminated. Oviducts and uterine horns were removed and flushed separately with 0.2 and 0.5 ml saline (0.154 M-NaCl), respectively, to determine the number of ova.

Measurement of oestradiol contents and release from the devices. Unused devices and devices recovered from animals were processed simultaneously for measuring the oestradiol content by means of a specific radioimmunoassay (RIA). For this purpose, each device was extracted twice with 5 ml ethyl ether for 1 h. After evaporating the ether, the residue was resuspended in 0.5 ml saline phosphate buffer, and 0.2 ml of this solution was utilized for each RIA tube in duplicate. All the samples were processed in a single RIA run as previously described (Forcelledo *et al.*, 1981).

Statistical analysis. The analysis was done according to the recommendations of Conover (1980). Differences in the number of ova recovered from different groups of animals and their distribution in the genital tract were analysed by Kruskal–Wallis and χ^2 tests, respectively (Conover, 1980).

Results

The results of Exp. 1 are shown in Fig. 2. The mean number of embryos was 9.3 ± 2.8 ($\pm$ s.d., $n = 44$), without significant differences between groups. In all groups embryos were found in both segments of the genital tract but in different proportions. In the undisturbed control group and in

Fig. 2. Mean numbers ($\pm$ s.e.) of embryos recovered in Exp. 1 from oviducts and uteri on Day 4 of pregnancy in mice left undisturbed or fitted on Day 1 with oestradiol-free (C) or oestradiol-loaded (E₂) Silastic devices around the oviduct and systemically. N = number of animals.

Fig. 3. Mean number ($\pm$ s.e.) of embryos recovered in Exp. 2 from oviducts and uteri on Day 4 of pregnancy in mice fitted on Day 1 with an oestradiol-loaded (E₂) periovidual device on one side and an oestradiol-free (C) device on the opposite side. N = number of animals.

those groups with perioviducal devices free of oestradiol, most of the embryos were in the uterine horn while in the groups for which the perioviducal devices contained oestradiol most of the embryos were in the oviducts. The difference was statistically significant ($P < 0.001$).

The distribution of ova in the groups given oestradiol systemically did not differ from that observed in the corresponding group without oestradiol. Measurement of oestradiol content of used and unused devices gave 1750 pg (1335–2294, $n = 5$), 85 pg (46–158, $n = 5$) and 119 pg (66–213, $n = 3$) (geometric mean, 95% confidence interval) for unused, perioviducal and systemic devices respectively, and shows that the hormone was effectively delivered, regardless of the location in which they were implanted.

In Exp. 2, there were no differences in the total number of embryos recovered from the genital tract in the two groups of mice, the mean recovery was 9.3 ± 1.8 ($\pm$ s.d., $n = 17$). However, a statistically significant larger proportion of ova occurred in the oviducts treated with both doses of oestradiol than in the contralateral oviducts ($P < 0.001$, Fig. 3) but there were no differences between groups receiving devices with different loads of oestradiol.

Discussion

The results of this study provide convincing evidence that, at equal doses, oestradiol delivered to the serosal surface of the mouse oviduct is more effective in delaying oviducal transport of embryos than is oestradiol delivered at a distant site. This site-related difference can be best explained by admitting that oestradiol delays oviducal transport of embryos in the mouse by a direct action upon the oviduct; i.e. 1.7 ng oestradiol delivered from a distant site does not reach the oviduct in sufficient concentration to elicit the effect whereas the same amount delivered at the site of action attains above threshold concentration. When oestradiol-releasing devices were placed only on one side, a statistically significant retention of embryos was observed on that side in comparison with the opposite side fitted with an oestradiol-free perioviducal device, thus demonstrating a local action restricted to the treated side. This lends further support to the idea of a direct action on the oviduct over an indirect action mediated humorally through a distant or neighbouring organ.

The initial oestradiol content of the devices decreased one order of magnitude during use regardless of location. The different effects of the two locations are therefore unrelated to total release. A different rate of release at the two locations is unlikely since *in vivo* this is controlled largely by Silastic itself (reviewed in Tatum, 1970).

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