Embryo implantation in the rat uterus induced by epidermal growth factor

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The present study was undertaken to determine whether the mechanism of embryo transfer is a factor in the action of epidermal growth factor (EGF) in initiation of implantation. Unilateral intrauterine infusion of 3 μl buffered saline, or saline containing 1.5 μg EGF, plus i.v. injection of 100 μg EGF 2 h later resulted in implantation sites in all animals within 48 h. In several animals implantation was also initiated in the non-injected uterine horn. Administration of indomethacin 1 h before the intrauterine injection completely blocked the effect of EGF but not that of 25 ng oestradiol. The results confirm that EGF can replace oestrogen for initiation of implantation provided that the uterine trauma associated with embryo transfer, that is puncture, is provided. The mechanisms involved remain to be resolved.

Introduction

Oestrogen is considered essential for initiation of implantation in rodents, but the mechanisms involved remain unclear (Psychoyos, 1973; Finn, 1977; Weitlauf, 1988). Growth factors, especially epidermal growth factor (EGF) and the closely related transforming growth factor α (TGF-α), have recently received attention as possible mediators of oestrogenic action. In support of this contention, several studies have demonstrated increased abundance of EGF and EGF receptors in the uteri of mice and rats exposed to oestrogen (references by Adamson, 1990). Furthermore, exogenous EGF from a subcapsular kidney implant increased mitotic activity in uterine and vaginal epithelia of mice (Nelson et al., 1991); TGF-α has a similar action (Ignar-Trowbridge et al., 1992). Recent studies from the latter group (Nelson et al., 1992) indicated that EGF may function via the oestrogen receptor, because a potent anti-oestrogen prevented the effect of the growth factor on mitogenesis. Although EGF failed to initiate implantation in ovariectomized mice, it reversed the inhibitory effect of indomethacin in oestriol-treated animals (Paria et al., 1991). Exogenous EGF also failed to initiate implantation in delayed implanting ovariectomized or hypophysectomized rats, even if a subliminal i.v. dose of oestradiol was provided (Johnson and Chatterjee, 1993). In contrast, this study showed that EGF initiated implantation of embryos transferred into progesterone-primed hypophysectomized rats; greatest success was obtained when the embryos were obtained from a hypophysectomized donor that had been exposed to oestradiol. No explanation for the effectiveness of EGF for implantation when coupled to embryo transfer was offered other than to suggest the possible activation of the embryo in the transfer process. The present studies were undertaken to examine further the effects of EGF on initiation of implantation. The results indicate that the process of embryo transfer, i.e. puncture of the uterus, is an important factor for the success of implantation initiated by EGF.

Materials and Methods

Animals and treatments

Virgin female rats (200–250 g) of the Holtzman strain (Harlan Sprague Dawley Inc, Madison, WI) were housed in temperature (23 ± 1°C) and light (lights on 06:00–20:00 h daily) controlled conditions with free access to Purina laboratory chow and tap water. Protocols for all experiments were approved by the University of Kansas Medical Center Animal Care and Use Committee in accordance with guidelines set forth by the Public Health Service.

A pro-oestrous female rat was placed in a cage with two males of proven fertility of the same strain. The day on which a vaginal plug or spermatozoa was found in the vaginal lavage was considered day 1 of pregnancy. On day 3 of pregnancy, the animals were anaesthetized with diethyl ether and hypophysectomized using the parapharyngeal approach. After this operation, the animals were provided with a 5% solution of glucose for drinking and soft laboratory chow. In one group the ovaries and adrenals, but not the hypophysis, were removed on day 3 of pregnancy. A dorsolateral incision was used to exteriorize the ovaries, great care being taken to avoid damage to the oviducts. Adrenalectomized animals were given a 0.5% solution of NaCl for drinking. At the time of hypophysectomy or ovariectomy and daily thereafter each animal was injected (s.c.) with 2 mg progesterone dissolved in 0.1 ml of a mixture of benzyl benzoate and sesame seed oil (20:80 v/v). On day 9 of the delayed implanting pregnancy (4 days of delay) animals were anaesthetized with diethyl ether and one uterine horn was exteriorized through a dorsolateral incision. After careful examination for the absence of implantation sites, murine EGF or saline was injected into one uterine horn near the oviduct–uterine junction. Two hours later animals were injected (i.v.) with saline, EGF dissolved in saline, or oestradiol dissolved in saline. Forty-eight hours after the last treatment and 15 min before autopsy, the animals were injected i.v. with 0.5 ml of a 1% solution of Chicago Blue B in saline. Extravasation of the macromolecular dye owing to increased capillary permeability
indicated the site of embryo implantation (Psychoyos, 1961). Uteri were flushed with saline to determine the presence of unimplanted embryos. If no implantation sites or blastocysts were found, the animal was assumed not to be pregnant and was removed from the study.

**Chemicals**

Progesterone, oestradiol, Chicago Blue B, indomethacin, benzyl benzoate and sesame seed oil were purchased from Sigma Chemical Co. (St Louis, MO). Murine EGF (reagent grade; 99% pure) was purchased from Bioproducts for Science (Indianapolis, IN) and dissolved in sterile filtered phosphate-buffered saline (pH 7.4). Indomethacin was dissolved in benzyl benzoate and sesame seed oil (20:80 v:v).

**Results**

Intravenous injection of 25 ng oestradiol on day 9 of pregnancy 2 h after a unilateral intrauterine injection of saline resulted in implantation sites in both uterine horns in all animals (group 1) (Table 1). There was no difference in the number of sites per horn, i.e. the intrauterine saline did not reduce the number of sites. Control animals that were treated only with progesterone before receiving intrauterine and intravenous saline had no implantation sites (group 2).

Intrauterine injection of 10 µl buffered saline containing 5 µg EGF resulted in initiation of implantation in three of six rats, but a total of only six sites was found (group 3). Although the material was infused slowly, there was a noticeable swelling of the uterus and in subsequent experiments the volume was reduced to 3 µl, which is still about three times the volume used for embryo transfer. Intravenous injection of 0.15 µg EGF in 3 µl did not initiate implantation in two animals, but one site was found in one of two animals infused with 3 µl containing 15 µg EGF (data not shown). These results suggested that intraluminal administration of EGF was not a promising protocol to follow. Subsequent experiments used i.v. administration of EGF in addition to intrauterine treatments. Two of nine rats had implantation sites when 1.5 µg of EGF was given by intrauterine injection and 25 µg was given by i.v. injection (group 4). Both animals had sites in the non-injected uterine horn. Doubling the i.v. dose of EGF (50 µg), but keeping the intrauterine dose the same, resulted in implantation sites in six of seven animals; sites were present in both horns in three of the animals (group 5). When 75 µg EGF was injected i.v., all five rats had sites in the injected horn and two animals had sites in both horns (group 6). Increasing the dose of EGF to 100 µg did not have a significant effect upon initiation of implantation (group 7); only one of the nine rats had one site in the non-injected horn.

The question arose as to the necessity of the intrauterine injection of EGF, that is, was manipulation and puncture of the uterus a sufficient adjuvant to EGF administered i.v.? This was determined by injecting buffered saline into one uterine horn 2 h before i.v. administration of EGF. Three of four animals given 75 µg EGF (group 8) had implantation sites in the injected horn and in one animal one site was found in the non-injected horn. With 100 µg EGF four of five animals had at least one implantation site in the injected horn (group 9). Simply puncturing the uterus with a needle (group 10) 2 h before injecting 100 µg of EGF produced sites in three of five animals and in two animals sites were bilateral. The specificity of uterine trauma was examined by use of a unilateral dorsolateral incision.
in the skin and peritoneum without touching the uterus (group 11). In these animals, EGF was without effect, although the uteri contained blastocysts.

Intrauterine injections could produce an inflammatory response and an increase in prostaglandin synthesis, we therefore attempted to block the latter with indomethacin. Indomethacin (1 mg in 0.1 ml oil) was injected (s.c.) 1 h before intrauterine injection of saline and 3 h before an i.v. injection of EGF or oestradiol. No implantation sites were found in the animals given EGF (group 12). In contrast, implantation sites were found in all five rats treated with oestradiol i.v. (group 13). In group 13 the injected horn had fewer implantation sites than did the contralateral horn but the reason for this is obscure.

The involvement of endogenous steroid in the response to EGF was tested using adrenalectomized plus ovariectomized rats. Three of four such animals given intrauterine and i.v. EGF had at least one site in each horn (group 14).

Discussion

The objective of the present study was to determine why i.v. administration of EGF was effective in initiating implantation of embryo transplants but not in situ delayed implanting embryos (Johnson and Chatterjee, 1993). The results of the study reported here strongly suggest that uterine trauma associated with embryo transfer is a significant adjunct for the action of EGF. When the growth factor was injected into the uterine lumen as well as given i.v., all 14 animals had implantation sites. However, 10 of the 14 animals had sites when the uterus was simply punctured or injected with saline before giving EGF. Furthermore, in about one-third of the animals (11 of 32) implantation sites were found in the non-traumatized as well as the traumatized horn. This finding suggests that the trauma to the uterus produced some factor that could be transmitted by the vascular system because the lumina of the uterine horns of the rat are not contiguous.

The nature of the augmenting factor(s) induced by uterine trauma is of considerable importance. Because of their association with trauma and inflammation and their known involvement with implantation (Weillau, 1992), prostaglandins may be involved. The finding that initiation of implantation induced by EGF and uterine trauma was inhibited by indomethacin is consistent with this contention. Several studies, reviewed by Glasgow et al. (1992) have emphasized the requirement of arachidonic acid metabolites for the mitogenic action of EGF. The importance of prostaglandin synthesis in response to EGF was also suggested by the studies of Paria et al. (1991). These authors used indomethacin to inhibit the initiation of implantation induced by oestriol in ovariectomized mice. This inhibition was overcome by the injection (s.c.) of EGF. Because EGF also increased prostaglandin synthesis by cultured mouse uterine tissue, these authors suggested that the role of the growth factor in implantation was induction of prostaglandin synthetase.

The results obtained in the study reported here with ovariectomized plus adrenalectomized animals given EGF indicate that the growth factor is not functioning via stimulation of endogenous steroid production. This model system is, however, flawed in that extensive trauma is induced by the surgical manipulations of the reproductive tract with the complications of adhesions. These problems are not present when hypophysectomized animals are used.

Whether expression of EGF is involved in normal implantation remains to be established. The present results confirm our previous findings (Johnson and Chatterjee, 1993) that EGF can initiate implantation without the need for exogenous oestrogen. Furthermore, the oestrogen receptor does not appear to be involved, because the effect of EGF is not prevented by prior exposure to a potent anti-oestrogen (ICI-182,780) (D. C. Johnson and S. Chatterjee, unpublished data). EGF may not be as efficient as oestrogen for implantation. The efficacy of the growth factor was quite variable and in only a few cases did the number of implantation sites equal the number found in oestrogen-treated animals. Unimplanted blastocysts could usually be flushed even from uteri that contained implantation sites. Whether the growth factor can replace other oestrogenic functions in the uterus during early pregnancy remains to be established.

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References


