Aust. J. Biol. Sci., 1982, **35**, 63–72

Lectins, Calcium Ionophore A23187 and Peanut Oil as Deciduogenic Agents in the Uterus of Pseudopregnant Mice: Effects of Tranylcypromine, Indomethacin, Iproniazid and Propanolol

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Abstract

Intraluminal injections of lectins, including concanavalin A (Con A), wheatgerm lectin, and soybean lectin, Con A–Sepharose 4B beads, calcium ionophore A23187 or peanut oil into the left uterine horns of mice on day 4 of pseudopregnancy induced the formation of deciduomata and significantly increased the weight and alkaline phosphatase activity of uterine tissue on day 7 of pseudopregnancy. In contrast, injections of these materials into the uterine horns of non-pseudopregnant mice that had not been previously mated failed to induce similar responses.

Tranylcypromine blocked the decidual cell reaction artificially induced by lectins, calcium ionophore A23187 and peanut oil in pseudopregnant mice. However, uterine responses observed after individual and concurrent injections with indomethacin, iproniazid, propanolol or progesterone indicated that this deciduoma-blocking effect may not be solely related to the ability of tranylcypromine to inhibit prostacyclin biosynthesis but may also involve catecholamines and luteolytic prostaglandins which interfere with decidualization on day 4 and day 6 of pseudopregnancy, respectively. A role for prostaglandins and uterine β-adrenergic receptors, however, was implicated in the induction of decidualization because both indomethacin and propanolol blocked the response to peanut oil.

The results suggested that the embryonic signal responsible for the induction of the decidual cell reaction in mice may involve surface interactions between the embryo and uterine luminal epithelium resulting in a stimulation of the uterus via glycoprotein receptors. A role for calcium was implicated in this phenomenon.

Introduction

The uterine decidual cell reaction in rodents, involving processes of hyperplasia, hypertrophy and differentiation of endometrial stromal cells, is induced during early pregnancy by a stimulus supplied by the blastocyst and is an essential reaction if implantation is to occur (Finn and Porter 1975). Similar transformations resulting in the formation of a deciduoma can also be induced in an appropriately sensitized uterus by various artificial stimuli (DeFeo 1967; Finn and Porter 1975).

Because the embryo assumes an intimate relationship with the luminal epithelium in the pre-implantation stages of development, it is widely accepted that the epithelial cells first respond to the blastocyst signal and then, in turn, stimulate the transformation and proliferation of the underlying stromal cells (Lejeune *et al.* 1981). Jonsson *et al.* (1979) indicated that prostacyclin (PGI_2) may be a product of the uterine epithelial cells involved in this process. In addition, there is increasing evidence to suggest that the embryo–maternal relationship established during early pregnancy

involves interactions between glycoproteins at the level of the cell surface (Pietras and Szego 1979; Surani 1979; Fishel and Surani 1980; Wu 1980). Many important cellular responses are now recognized to be mediated through interactions involving cell surface glycoproteins (Rapin and Burger 1974; Warren *et al.* 1978) and it is possible that similar surface interactions between the embryo and uterine luminal epithelial cells may provide the initial stimulus for the stromal decidual cell reaction.

In the present study the ability of concanavalin A (Con A), wheatgerm lectin, soybean lectin, calcium ionophore A23187 and peanut oil to act as deciduogenic agents in the pseudopregnant mouse was investigated. It was reasoned that, because the lectins have specific affinities for various carbohydrate residues in glycoproteins (Goldstein and Hayes 1978) and may cause alterations in cellular metabolism on binding to membrane receptors (Czech and Lynn 1973), they may be able to mimic the embryonic stimulus for the decidual cell reaction if in fact interactions at the level of the cell surface are involved in this phenomenon. Calcium ionophore A23187 was included for study because it has been reported recently that lectins, and in particular Con A, influence cellular functions by promoting both the uptake of calcium (Larner *et al.* 1980) and the intracellular release of this cation (Mikkelsen and Schmidt-Ullrich 1980). The possibility that the effects of these agents are mediated by prostaglandins (Jonsson *et al.* 1979) was examined by treating some animals with tranylcypromine [an inhibitor of PGI_2 biosynthesis and of monoamine oxidase activity (Gryglewski *et al.* 1976)] and indomethacin (an inhibitor of prostaglandin biosynthesis). Other animals were treated with iproniazid (another monoamine oxidase inhibitor) and propanolol (a β-adrenergic blocking agent) to gauge the specificity of tranylcypromine effects.

Materials and Methods

Materials

Con A, wheatgerm lectin, soybean lectin, Sepharose 4B beads, and Con A–Sepharose 4B beads were obtained from Pharmacia South Seas Pty. Ltd., North Ryde, N.S.W. Other materials were obtained from the following sources: calcium ionophore A23187 and progesterone from Calbiochem-Behring Australia Pty Ltd, Carlingford, N.S.W.; iproniazid phosphate, indomethacin and tranylcypromine from Sigma Chemical Co., St. Louis, Mo., U.S.A.; propanolol hydrochloride (Inderal) from Imperial Chemical Industries Ltd., Macclesfield, Cheshire, U.K.; dimethyl sulfoxide (DMSO) from BDH Chemicals Ltd, Poole, U.K.; pentobarbitone sodium (Nembutal) from Abbott Laboratories Pty Ltd, Sydney, N.S.W.

Animals

Female Quackenbush strain mice, aged 8–12 weeks, were used in all experiments and were housed as previously described (Murdoch *et al.* 1978). Pseudopregnancy was brought about by pairing the females with vasectomized males. The females were examined for copulation plugs each morning and the day of finding a plug was designated as day 1 or the first day of pseudopregnancy.

Induction of Deciduomata

The lectins and Sepharose beads described above were dissolved or suspended in 0·9% (w/v) NaCl and their ability to induce uterine deciduomata was examined by injecting either 30 μl of lectin solution (containing 25 or 125 μg of lectin) or 3 μl of Sepharose beads (containing 12 beads) at the uterotubal junction into the left uterine horns of mice between 1500 and 1600 h on day 4 of pseudopregnancy, after anaesthesia with Nembutal. The Sepharose beads were injected using the embryo-transfer technique described by Mullen and Carter (1973). The ovaries of all day 4 pseudopregnant mice were examined under a dissecting microscope before the intrauterine injections

were administered, and animals were used in experiments only when distinct corpora lutea were apparent.

Calcium ionophore A23187 was dissolved in DMSO and subsequently diluted in 0·9% (w/v) NaCl to permit 0·001, 0·01, and 0·1 μmol of ionophore in 30 μl of solution to be injected into uterine horns as described above. Solutions of 0·9% (w/v) NaCl either containing or not containing DMSO in amounts (0·0011, 0·011, and 0·11 nmol in 30 μl of solution) comparable to those of the ionophore solutions were similarly injected into the uterine horns of pseudopregnant mice to allow for a more confident assessment of ionophore and lectin effects.

Peanut oil is a potent stimulus of the decidual cell reaction in pseudopregnant mice (Finn and Hinchliffe 1964; Murdoch *et al.* 1978) and, because its use in this respect is so widely documented, it was used in the present study to standardize and gauge the effectiveness of other treatments. The oil was administered as described above using 30-μl volumes in each uterine horn. The right uterine horn in all animals received no treatment and acted as a control in the experiments.

Experiments with pseudopregnant mice were also repeated using animals that had not been previously mated. These animals, designated as non-pseudopregnant, were selected without regard for oestrous cycle activity and were used to determine the effects of the various treatments on uteri that were not suitably hormonally conditioned for a decidual response (see Finn and Porter 1975).

Administration of Tranylcypromine, Indomethacin, Iproniazid, Propanolol, and Progesterone

Pseudopregnant mice receiving a peanut oil stimulus in the left uterine horn on day 4 were randomized into 17 groups of equal size for treatment with the above compounds as follows:

Group 1: The animals in this group received no further treatment and were used as controls.

Group 2: Tranylcypromine was dissolved in 0·9% (w/v) NaCl and administered intraperitoneally (20 mg per kilogram body weight) 0·5 h before the deciduogenic oil stimulus on day 4.

Group 3: Animals in this group received the same dose of tranylcypromine as those in group 2, but were injected at 1100 h on day 6 of pseudopregnancy.

Group 4: This group received two subcutaneous injections of indomethacin (150 μg) dissolved in peanut oil at 6 h and again at 0·25 h before the deciduogenic oil stimulus on day 4.

Group 5: The same dose of indomethacin as in group 4 was administered to animals in this group, again in two injections, at 1100 and 1600 h on day 6 of pseudopregnancy.

Group 6: Iproniazid was dissolved in 0·9% (w/v) NaCl and administered subcutaneously (150 mg per kilogram body weight) 1 h before the deciduogenic oil stimulus on day 4.

Group 7: Animals in this group received the same dose of iproniazid as those in group 6, but were injected at 1100 h on day 6 of pseudopregnancy.

Group 8: Propanolol (3 mg per kilogram body weight) was administered intraperitoneally to animals in this group directly from sealed, sterile vials prepared by the manufacturer. Injections were given 0·5 h before the deciduogenic oil stimulus on day 4 of pseudopregnancy.

Group 9: This group was injected with propanolol at 1100 h on day 6 of pseudopregnancy using the same dose as in group 8.

Group 10: Animals in this group received a single subcutaneous injection of progesterone (500 μg) in peanut oil 1 h before the deciduogenic oil stimulus on day 4.

Group 11: The same dose of progesterone as in group 10 was administered to animals in this group, at 1100 h on day 6 of pseudopregnancy.

Group 12: This group received injections of indomethacin 6 and 0·25 h and tranylcypromine 0·5 h before the deciduogenic oil stimulus on day 4 of pseudopregnancy as described above.

Group 13: Animals in this group received injections of indomethacin at 1100 and 1600 h, and tranylcypromine at 1400 h on day 6 of pseudopregnancy as described above.

Group 14: Tranylcypromine and propanolol were administered to animals in this group 0·5 h before the deciduogenic oil stimulus on day 4 as described above.

Group 15: Animals in this group received injections of tranylcypromine and propanolol at 1100 h on day 6 of pseudopregnancy as described above.

Group 16: This group received injections of progesterone 1 h and tranylcypromine 0·5 h before the deciduogenic oil stimulus on day 4, as described above.

Group 17: Tranylcypromine and progesterone were administered to animals in this group at 1100 h on day 6 of pseudopregnancy as described above.

Procedures at Autopsy

Mice were killed by cervical dislocation at 1000 h on day 7 of pseudopregnancy or 65–66 h after receiving their appropriate deciduogenic stimulus. Previous studies (Murdoch *et al.* 1978) showed that the morphological features of decidualized uterine horns could be easily distinguished from non-decidualized horns at this stage and were accompanied by significant increases in uterine weight and maximal alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) activities. Thus, at autopsy in the present study the right (untreated) and left (treated) uterine horns were excised, dissected free of all connective and fatty tissues, and examined for evidence of decidualization by checking their morphological appearance, weight and alkaline phosphatase activity.

Alkaline phosphatase activities were assayed in individual uterine horn homogenates as described previously (Murdoch *et al.* 1978). Unless stated otherwise, units of enzyme activity are defined as nanomoles of substrate hydrolysed per minute at 37°C. The protein concentration of samples was determined by the method of Lowry *et al.* (1951) using standards of bovine serum albumin.

Replication of Experiments and Statistical Methods

All experiments were conducted using eight mice in each treatment group. When necessary, the significance of results was assessed by analysis of variance after converting the primary data to logarithms. In most cases, however, comparisons between treatments were made by using Student's *t*-test. All values in the tables are means ± standard error of the mean of unconverted data.

Results

Deciduogenic Effects of Various Materials

The results of experiments designed to assess the deciduoma-inducing ability of 0·9% (w/v) NaCl, Con A, wheatgerm lectin, soybean lectin, Con A–Sepharose beads, Sepharose beads, calcium ionophore A23187, DMSO and peanut oil following intraluminal injection into the left uterine horns of day 4 pseudopregnant mice are shown in Table 1.

The uterine horns of pseudopregnant mice receiving injections of either lectin solutions, Con A–Sepharose beads, calcium ionophore A23187 or peanut oil on day 4 were distinctly decidualized on day 7 of pseudopregnancy as judged by their morphological appearance and increased weight and alkaline phosphatase activity. In contrast, the morphological features, weight and alkaline phosphatase activity of uterine horns of animals receiving injections of 0·9% (w/v) NaCl, Con A-free Sepharose beads or low doses of DMSO remained essentially unchanged on day 7 of pseudopregnancy and did not display any evidence of deciduoma formation. The right, untreated uterine horns in all pseudopregnant animals also remained unchanged between days 4 and 7 of pseudopregnancy, at least in terms of the above criteria selected as indices of the decidual reaction.

While the highest dose (0·11 nmol) of DMSO employed in the study only slightly but significantly ($P < 0·05$) increased the weight of treated uterine horns on day 7 of pseudopregnancy, alkaline phosphatase activity in these horns was nevertheless highly significantly enhanced ($P < 0·01$).

When the left uterine horns of non-pseudopregnant mice were injected with the various materials described above, no significant changes in weight or alkaline

phosphatase activity were detected and the morphological appearance remained unchanged indicating the absence of any decidual reaction (results not shown).

Effects of Tranylcypromine, Indomethacin, Iproniazid, Propanolol and Progesterone

Experiments described in this section were initiated to test the proposal of Jonsson *et al.* (1979) that PGI_2 may be an early mediator of the artificially induced decidual cell reaction. This was attempted by treating pseudopregnant mice with tranylcypromine as described in the Materials and Methods and using the same dose as employed by Jonsson *et al.* (1979).

Table 1. Changes in the weight and alkaline phosphatase activity of uterine horns of pseudopregnant mice after intraluminal injection of NaCl, lectins, Sepharose beads, ionophore A23187, DMSO and peanut oil

Values are the means ± standard errors for eight mice. All treatments were administered into the left uterine horn on day 4 of pseudopregnancy. The right uterine horn received no treatment. Autopsy was performed on day 7 of pseudopregnancy. *, ** Significantly different from the right uterine horn, $P < 0{\cdot}05$, $P < 0{\cdot}01$, respectively

Treatment	Wt of right horn (mg)	Wt of left horn (mg)	Alkaline phosphatase activity (units per mg protein) in: Right horn	Left horn
0·9% (w/v) NaCl (30 μl)	41 ± 3	42 ± 5	77 ± 4	81 ± 10
Con A (25 μg)	49 ± 5	121 ± 22**	98 + 12	529 ± 190**
Con A (125 μg)	58 ± 11	282 ± 73**	91 ± 21	862 ± 101**
Wheatgerm lectin (125 μg)	25 ± 3	187 ± 23**	58 ± 3	841 ± 83**
Soybean lectin (125 μg)	29 ± 3	131 ± 27**	76 ± 3	852 ± 91**
Con A–Sepharose beads (12)	31 ± 4	147 ± 24**	45 ± 7	528 ± 66**
Sepharose beads (12)	30 ± 4	32 ± 5	73 ± 9	68 ± 12
Ionophore A23187 (0·1 μmol)	39 ± 12	160 ± 6**	92 ± 24	798 ± 120**
Ionophore A23187 (0·01 μmol)	33 ± 5	225 ± 65**	103 ± 20	593 ± 100**
Ionophore A23187 (0·001 μmol)	30 ± 13	89 ± 18*	72 ± 9	462 ± 101**
DMSO (0·11 nmol)	29 ± 1	53 ± 9*	134 ± 13	647 ± 190**
DMSO (0·011 nmol)	30 ± 5	31 ± 4	93 ± 14	102 ± 21
DMSO (0·011 nmol)	27 ± 3	29 ± 2	91 ± 7	110 ± 15
Peanut oil (30 μl)	53 ± 16	392 ± 49**	98 ± 35	1078 ± 44**

Although the results of our preliminary experiments (not shown) clearly indicated that tranylcypromine was capable of blocking the deciduogenic effects of lectins, calcium ionophore A23187 and peanut oil in pseudopregnant mice, the extent to which this effect could be attributable solely to its inhibition of PGI_2 biosynthesis was questionable. Mice treated with the drug displayed symptoms attributable to the catecholamines that accumulated with the inhibition of monoamine oxidase and included tachycardia, piloerection, and increased aggression. The possibility that these catecholamines, and catecholamine-induced luteolytic prostaglandins (Ferreira *et al.* 1971), may be involved in the action of tranylcypromine was tested by treating mice with indomethacin, iproniazid, propanolol, progesterone, and combinations of these compounds with tranylcypromine on day 4 of pseudopregnancy near the time of giving a deciduogenic oil stimulus, and on day 6 when decidualization was already in progress.

The results presented in Table 2 show that individual injections of tranylcypromine, indomethacin, iproniazid and propanolol, and combined injections of tranylcypromine with either indomethacin or progesterone blocked the deciduogenic effects of a peanut oil stimulus in day 4 pseudopregnant mice. Tranylcypromine given together with propanolol, however, allowed the peanut oil to successfully stimulate a decidual response at this time. When the drugs were administered to

Table 2. Changes in weight and alkaline phosphatase activity of uterine horns of pseudopregnant mice after intraluminal injection of peanut oil and treatment with tranylcypromine, indomethacin, iproniazid, propanolol, and progesterone

Values are means ± standard errors for eight mice. Oil was administered into the left uterine horn on day 4 of pseudopregnancy. The right uterine horn received no treatment. Autopsies were performed on day 7 of pseudopregnancy after administering compounds on either day 4 or day 6 as indicated. a, Significantly different from the right uterine horns of animals in the same treatment group, $P < 0{\cdot}01$; b and c, significantly different from the left uterine horns of control animals, $P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively

Group	Treatment	Day of pseudo-pregnancy	Weight (mg) of: Right horn	Weight (mg) of: Left horn	Alkaline phosphatase activity (units per mg protein) in: Right horn	Alkaline phosphatase activity (units per mg protein) in: Left horn
1	Control	—	29 ± 3	279 ± 25[a]	132 ± 14	1080 ± 199[a]
2	Tranylcypromine	4	32 ± 4	41 ± 6[c]	91 ± 9	152 ± 19[c]
3	Tranylcypromine	6	26 ± 2	27 ± 7[c]	109 ± 15	183 ± 20[c]
4	Indomethacin	4	24 ± 2	22 ± 2[c]	84 ± 13	98 ± 19[c]
5	Indomethacin	6	26 ± 4	293 ± 33[a]	137 ± 19	1185 ± 201[a]
6	Iproniazid	4	24 ± 2	23 ± 3[c]	86 ± 11	102 ± 8[c]
7	Iproniazid	6	28 ± 2	356 ± 38[a]	126 ± 23	1151 ± 116[a]
8	Propanolol	4	28 ± 2	26 ± 2[c]	93 ± 11	99 ± 4[c]
9	Propanolol	6	32 ± 4	300 ± 29[a]	120 ± 22	999 ± 49[a]
10	Progesterone	4	30 ± 3	250 ± 41[a]	96 ± 17	987 ± 42[a]
11	Progesterone	6	30 ± 2	280 ± 37[a]	84 ± 5	560 ± 53[a,b]
12	Tranylcypromine + indomethacin	4	25 ± 1	29 ± 4[c]	93 ± 7	109 ± 10[c]
13	Tranylcypromine + indomethacin	6	33 ± 6	252 ± 13[a]	113 ± 23	1019 ± 96[a]
14	Tranylcypromine + propanolol	4	28 ± 3	274 ± 29[a]	106 ± 9	1029 ± 89[a]
15	Tranylcypromine + propanolol	6	22 ± 2	34 ± 3[c]	88 ± 5	102 ± 4[c]
16	Tranylcypromine + progesterone	4	21 ± 4	26 ± 2[c]	91 ± 7	110 ± 11[c]
17	Tranylcypromine + progesterone	6	22 ± 4	337 ± 10[a]	79 ± 17	801 ± 219[a]

mice on day 6 of pseudopregnancy, only tranylcypromine and tranylcypromine given together with propanolol caused a significant ($P < 0{\cdot}01$) regression of deciduomata. Furthermore, although the administration of progesterone did not block the decidual response to peanut oil, it did significantly ($P < 0{\cdot}05$) depress the alkaline phosphatase activity of stimulated uterine horns when administered on day 6.

The morphological appearance, weight and alkaline phosphatase activity of the right, unstimulated uterine horns were not significantly altered by any of the treatments.

Discussion

The results of the present investigation clearly demonstrate that lectins and calcium ionophore A23187 act as deciduogenic agents in pseudopregnant mice and stimulate the formation of deciduomata containing significantly elevated levels of alkaline phosphatase activity. However, the ionophore solutions used in the present study contained DMSO which at the highest dose employed induced a decidual response by itself. This action of DMSO agrees with the findings of Humphrey and Martin (1968) and indicates that, in high doses, the effects of ionophore A23187 are confounded by the presence of DMSO. Ionophore solutions were effective, however, in inducing a decidual response when DMSO was present in amounts that were in themselves unable to stimulate deciduomata formation.

The interaction of lectins with the uterine epithelium to promote decidualization appears to involve surface glycoproteins of the epithelial cells rather than mechanisms requiring the penetration and uptake of lectin by the cells. This conclusion is supported by the results of experiments which showed that Sepharose beads containing covalently bound Con A effectively promoted the formation of deciduomata in pseudopregnant mice with high alkaline phosphatase activity. In addition, Enders and Schlafke (1974) found that Con A was capable of binding to surface glycoproteins of mouse uterine epithelial cells and showed that the binding properties of the lectin were abolished by α-methyl-D-mannoside. The present results are in conflict to some extent with those of Nilsson (1977). He found that Con A–Sepharose beads in the uterus of hormone-treated ovariectomized mice altered the ultrastructure of the uterine epithelium and elicited a local secretory activity, but did not cause any decidual reaction. The reason for this discrepancy between results is not clear, but differences in the number of beads injected or the general experimental conditions could be important factors. However, the failure of Con A-free Sepharose beads to cause a decidual reaction in pseudopregnant mice in the present study provides further evidence that it is not the physical presence of the blastocyst which stimulates the uterus under normal circumstances (McLaren 1968).

Although the results of the present investigation agree with the findings of Jonsson *et al.* (1979) that tranylcypromine is able to block the artificially induced decidual cell reaction in mice, it is questionable whether this affect is brought about by a singular action of the drug on PGI_2 biosynthesis. Thus, iproniazid, a monoamine oxidase inhibitor like tranylcypromine but without any influence on PGI_2 synthesis (Gryglewski *et al.* 1976), also blocked the decidual response when injected on day 4 of pseudopregnancy. These results indicate that catecholamines, which accumulate with the inhibition of monoamine oxidase, partly facilitate the adverse effects of tranylcypromine on the decidual response. While this claim is supported by our observation that propanolol neutralized the effects of tranylcypromine when administered on day 4, Jonsson *et al.* (1979) found that pargyline, another monoamine oxidase inhibitor, had no influence on the decidual cell reaction. The regression of deciduomata caused by tranylcypromine injected on day 6 of pseudopregnancy and the neutralization of this effect by indomethacin and progesterone indicate that prostaglandins capable of exerting luteolytic effects in mice (Horton and Poyser 1976; Lau *et al.* 1979) are also produced in response to the drug and cause progesterone levels to fall below those required for the maintenance of deciduomata. Hence, our

interpretation of the results in Table 2 is that tranylcypromine causes the production of both catecholamines and prostaglandins in pseudopregnant mice. The catecholamines interrupt the decidualization process on day 4 but not on day 6 of pseudopregnancy, while the prostaglandins are mainly disruptive on day 6. These results do not eliminate the possibility that PGI_2 is an early mediator of the decidual cell reaction in mice (Jonsson *et al.* 1979), but they do indicate that tranylcypromine is not a suitable drug for use in experimental work involving the study of physiological responses attributable to the specific inhibition of PGI_2 biosynthesis.

The inhibitory effects of indomethacin on the induction of deciduomata in day 4-pseudopregnant mice supports previous claims that prostaglandins may be involved in the early stages of the decidualization process (Jonsson *et al.* 1979; Kennedy 1977, 1979; Rankin *et al.* 1979). However, a similar requirement for prostaglandins on day 6 of pseudopregnancy, when decidualization is in progress, is not indicated by the present results since indomethacin by itself failed to exert any apparent influence when administered at this time. These results, and others obtained from the use of indomethacin, should perhaps be interpreted with caution since recent studies have indicated that this prostaglandin inhibitor acts to block the uptake of calcium ions by cells (Anderson *et al.* 1981). In addition, a role for factors capable of interacting with β-adrenergic receptors is indicated in the early induction stages of the decidual reaction since propanolol inhibited the response when administered on day 4 of pseudopregnancy. Again, these factors may not be required once decidualization is in progress because propanolol did not cause the reversion of deciduomata when injected on day 6 of pseudopregnancy. The inhibitory effect of propanolol on day 4 is not surprising in light of previous work (Rosenfeld and O'Malley 1970) which showed that this β-blocking agent inhibited some catecholamine-mediated oestrogenic effects that are now considered important for the initiation of implantation.

In conclusion, the results suggest that the embryonic signal responsible for the induction of the decidual cell reaction in mice involves surface interactions between the embryo and uterine luminal epithelium resulting in a stimulation of the uterus via glycoprotein receptors. Glycoproteins containing α-D-glucopyranosyl, α-D-mannopyranosyl, *N*-acetyl-D-glucosaminyl or *N*-acetyl-D-galactosaminyl units are implicated in these interactions because the three lectins employed in this study specifically bind these residues (Goldstein and Hayes 1978) and induced a decidual reaction in pseudopregnant mice. Hicks and Guzmán-González (1979) also concluded that, in mice 'the egg must recognize certain molecules of the endometrial surface (α-D-mannopyranose and α-D-glycopyranose) in order to implant'. They found that the masking of these sites with Con A prevented implantation during pregnancy. Although attempts were made to test the proposal that PGI_2 acts as an intercellular messenger between the stimulated luminal epithelium and stromal cells to induce decidualization, no conclusive results could be obtained to support or invalidate this argument. However, the evidence does indicate a role for calcium ions in this phenomenon.

Acknowledgment

The work was supported by a grant from the University of Newcastle Internal Research Assessment Committee.

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Manuscript received 14 August 1981, accepted 12 October 1981