Effects of restraint stress on luteal function in rats during mid-pregnancy

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This study was undertaken to investigate the relationship between the inhibitory effect of restraint stress and the protective effect of the feto-placental luteotrophic factors on luteal function during mid-pregnancy in rats. The number of conceptuses was adjusted to one (1C group) or more than ten (FC group) on day 7 of pregnancy, and each rat received restraint stress from day 12 to day 17 of pregnancy. Restraint stress consisted of placing a rat individually in a small plastic holder three times a day for 1 h each time. Restraint stress significantly decreased serum progesterone concentration on day 17 of pregnancy in the 1C group, but not in the FC group. Restraint stress also decreased serum progesterone concentration on day 17 of pregnancy in the 1C group which received bilateral adrenalectomy on day 12 of pregnancy. The number of animals with fetal resorption in this group of rats (10 out of 14 animals) was significantly greater than in any other group of rats. The number of animals with fetal resorption in the adrenalectomized 1C group was significantly lower after daily injections of 4 mg progesterone from day 12 to day 17 of pregnancy. In the FC group of rats, even in adrenalectomized rats, restraint stress did not cause any changes in serum progesterone concentration or fetal loss. These data indicate that restraint stress is luteolytic and causes fetal loss during mid-pregnancy; this effect can be blocked by some factors from conceptuses, as occurred in the FC group.

Introduction

It has been reported that environmental stress influences reproductive events, for example increased intrauterine mortality, impaired fetal development and behavioural changes, or low fertility in offspring (Ward, 1972; Euker and Riegler, 1973; Lorraine, 1979; Pollard and Dyer, 1985; Wiebold *et al.*, 1986). Wiebold *et al.* (1986) suggested that the high incidence of fetal loss in mice caused by restraint stress in early pregnancy was due to a decrease in progesterone production by the corpus luteum. However, Ward and Weiz (1984) reported that in rats restraint stress given from day 14 to day 21 of pregnancy did not affect the concentration of progesterone in serum. There appear to be conflicting findings concerning the effect of stress on luteal function. Kato *et al.* (1988) and Sugino *et al.* (1991) reported that ACTH and corticosterone, well known as stress-related hormones, inhibited progesterone secretion in rats during mid-pregnancy; this effect was blocked by placental hormones. The luteolytic effects of ACTH or corticosterone were apparent in rats carrying a single conceptus, but not in those rats with many conceptuses. It is, therefore, possible that the feto-placental unit has a role in the effect of stress on luteal function. The present study was undertaken to test the possibility that the influence of stress on luteal function

depends on the status of feto-placental function in rats during mid-pregnancy.

Materials and Methods

Animals

Sprague-Dawley rats, weighing 220–270 g, were housed under controlled conditions (lights on from 05:00 to 19:00 h) with free access to standard rat chow and water. Pro-oestrous rats were housed with males overnight. Day 1 of pregnancy was determined as the day on which spermatozoa were found in the vaginal smear. All rats underwent laparotomy on day 7 of pregnancy under a light ether anaesthesia. Rats with more than ten conceptuses were divided into two groups: 1C group: those in which all conceptuses except the one nearest to the ovary were removed by aspiration (as described previously by Kato *et al.*, 1979) and FC group: those in which aspiration was not carried out.

Experiments

Restraint stress was applied by placing the animals individually into a 188 mm × 43 mm × 85 mm plastic holder for 1 h. Each rat received the stress three times a day, at 10:00, 13:00

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Table 1. Effects of restraint stress on serum progesterone concentration and organ masses in rats during mid-pregnancy

Treatment	n	Serum progesterone (nmol l ⁻¹)			Organ mass (mg)						
		Day 12	Day 15	Day 17	CL	NL	Fetus	Placenta	Adrenal	Pituitary	
Intact rats											
IC no stress	8	147 ± 4	204 ± 5	244 ± 18	2.51 ± 0.10	17.7 ± 0.5	522.5 ± 13.0	454.9 ± 15.2	36.9 ± 1.7	10.6 ± 0.5	
IC stressed	9	152 ± 9	199 ± 7	181 ± 9*	2.84 ± 0.16	17.4 ± 0.7	531.8 ± 18.1	446.9 ± 16.0	48.0 ± 1.7**	11.9 ± 0.4	
FC no stress	8	168 ± 8	292 ± 11	294 ± 10	4.53 ± 0.12	18.6 ± 0.9	439.8 ± 30.8	299.9 ± 21.4	39.6 ± 0.8	11.5 ± 0.5	
FC stressed	9	167 ± 10	280 ± 16	263 ± 19	4.50 ± 0.12	18.4 ± 0.9	486.3 ± 11.9	287.4 ± 6.6	54.7 ± 1.2**	11.3 ± 0.6	
Adrenalectomized rats											
IC no stress	4	163 ± 20	208 ± 26	214 ± 29	3.39 ± 0.23	18.2 ± 1.3	448.7 ± 15.0	436.4 ± 17.6		12.1 ± 0.7	
IC stressed	4	202 ± 31	164 ± 31	120 ± 6†	3.36 ± 0.27	17.8 ± 1.2	459.7 ± 29.5	435.5 ± 16.0		11.2 ± 0.2	
FC no stress	5	185 ± 23	293 ± 15	204 ± 16	4.84 ± 0.11	20.6 ± 1.1	446.1 ± 7.8	270.0 ± 9.3		12.9 ± 0.7	
FC stressed	5	136 ± 6	253 ± 22	179 ± 17	4.59 ± 0.30	16.5 ± 0.4	415.8 ± 18.7	269.6 ± 33.1		12.0 ± 0.7	

Values are means ± SEM for the number of animals (n) which maintained pregnancy.

IC: Number of conceptuses adjusted to one on day 7 of pregnancy; FC: more than ten conceptuses present on day 7 of pregnancy.

CL: corpora lutea; NL: non-luteal ovarian tissue.

*P < 0.01 compared with no stress group in the IC group (Duncan's new multiple range test); **P < 0.01 compared with no stress group (Student's t test); †P < 0.05 compared with the adrenalectomized IC + no stress group (Duncan's new multiple range test).

and 16:00 h, from day 12 to day 17 of pregnancy. The rats in the control group were left undisturbed in their own cages.

Effect of restraint stress on luteal function. The influence of stress on luteal function was studied by subjecting several rats from the 1C and FC groups to the stress-exposure experiments described above.

Effect of restraint stress and adrenalectomy on luteal function. The effect of stress on luteal function in adrenalectomized rats was studied by bilaterally adrenalectomizing several rats from the 1C and FC groups on day 12 of pregnancy. The rats were then subjected to the stress-exposure experiments described above. Animals with bilateral adrenalectomy were maintained with 0.9% NaCl added to their drinking water after surgery. A group of adrenalectomized rats from the 1C group also received s.c. injections of 4.0 mg progesterone (Sigma Chemical Co., St Louis, MO), suspended in 0.25 ml of sesame oil, once a day from day 12 to day 17 of pregnancy, and were then subjected to the stress-exposure experiment described above. Serum progesterone concentrations of rats injected with 4.0 mg progesterone were $380 \pm 38 \text{ nmol l}^{-1}$ (mean $\pm$ SEM).

Blood samples were obtained by jugular venepuncture under light ether anaesthesia before treatments on day 12 and on day 15 of pregnancy. All rats were killed on day 17 of pregnancy, and blood samples were obtained from the inferior vena cava. Serum samples were stored at -20°C for progesterone assay. The weights of the corpora lutea, non-luteal ovarian tissue, fetuses, placentas, adrenal glands and pituitary glands were recorded. The ovaries were removed and cleaned of adhering tissue in a watch-glass. All corpora lutea were removed from each pair of ovaries, separated from the rest of the ovary, and weighed immediately as a group, from which the mean corpora lutea mass was calculated. The remaining ovaries were weighed and the mean mass calculated as non-luteal weight. Six or seven placentas and fetuses were weighed from each rat in the FC group and the mean masses were calculated. The group mean was then calculated.

Progesterone assay

Concentrations of progesterone in serum were determined by the specific radioimmunoassay reported by Kato *et al.* (1982). The sensitivity of the assay was 300 pmol per tube, and the intra- and interassay coefficients of variations were 7.0% and 14.4%, respectively.

Statistical analysis

Data were analysed by analysis of variance and the Duncan's new multiple range test. Where appropriate, Student's *t* test and χ^2 test were used. Differences were considered to be significant if $P < 0.05$.

Results

Restraint stress significantly decreased serum progesterone concentration on day 17 of pregnancy in intact rats in the 1C group (Table 1), and one of ten rats of this group showed

Table 2. Effects of progesterone treatment on the effect of restraint stress in adrenalectomized pregnant rats in which only one conceptus remained

Treatment	n	Fetus	
		Alive	Resorbed
ADX, not stressed	4	4	0
ADX plus stress	14	4	10
ADX plus P ^a , not stressed	4	4	0
ADX plus P ^a plus stress	12	9	3*

Values represent the number of animals. ADX: adrenalectomy.

^aProgesterone (P) 4 mg was injected daily from day 12 to day 17 of pregnancy.

*Significantly different ($P < 0.05$) from ADX plus stress group (χ^2 test).

resorption of uterine content by day 17 of pregnancy. The number of animals in the 1C group with fetal resorption was further increased by adrenalectomy and stress (Table 2). The number of animals with fetal resorption was significantly lower after the progesterone supplement (Table 2). Restraint stress also significantly decreased serum progesterone concentration on day 17 of pregnancy in the adrenalectomized 1C group which could maintain pregnancy (Table 1). Restraint stress did not result in changes in serum progesterone concentration or fetal loss in the FC group, even in adrenalectomized rats. Restraint stress resulted in increased masses of adrenal glands in the 1C and FC groups, but caused no significant changes in the masses of the corpora lutea, non-luteal ovarian tissue, fetuses, placentae or pituitary gland (Table 1).

Discussion

The study reported here demonstrated that restraint stress decreased serum progesterone concentration in the group in which only one conceptus remained. Although the effect of restraint stress was restricted to the 1C group, the results strongly suggest that restraint stress inhibits luteal function during mid-pregnancy in rats. It is likely that the inhibitory effect of restraint stress on luteal function is mediated by an increase in ACTH secretion. The method of restraint stress used in this study activates the pituitary-adrenal axis (Ward and Weiz, 1984); this finding was confirmed by the enlargement of the adrenal glands in the present study. The inhibitory effect of ACTH on the maintenance of pregnancy has been demonstrated in several species (Velardo, 1957; Yang *et al.*, 1969; Ogle, 1974; Kittinger *et al.*, 1980). Ochiai *et al.* (1983) found that the pituitary had a luteolytic effect only during mid-pregnancy in rats when only one conceptus remained. Kato *et al.* (1988) and Sugino *et al.* (1991) also reported that ACTH or corticosterone have a luteolytic effect only during mid-pregnancy in rats when only one conceptus remained. These findings are in agreement with the results reported here that the decrease of serum progesterone concentration by restraint stress was apparent only in the 1C group.

Stress increases the concentration of prolactin in serum (Smith and Gala, 1977; Kant *et al.*, 1983), and prolactin is luteotrophic in early pregnancy (Morishige and Rothchild,

1974). However, in a preliminary study, prolactin had no effect on progesterone secretion during the *in vitro* incubation of corpora lutea from rats during mid-pregnancy. However, Kittinger *et al.* (1980) reported that hyperprolactinaemia results in pregnancy failure in mice. The role of prolactin on luteal function during stress therefore seems controversial.

Fetal resorption caused by restraint stress was particularly apparent in the adrenalectomized IC group but the number of animals with fetal resorption in this group was significantly lower after progesterone administration. The adrenal glands appear to secrete progesterone which blocks the luteolytic effect caused by restraint stress and, therefore, maintains pregnancy. In rats, the adrenal glands secrete progesterone into the peripheral blood (Fajer *et al.*, 1971; Macdonald and Matt, 1984), and this secretion is increased by stress (Plas-Roser and Aron, 1981; Hulse and Coleman, 1983; Denis *et al.*, 1989). Although ACTH secretion is markedly enhanced by stress in adrenalectomized rats (DeSouza and Van Loon, 1985), it cannot stimulate progesterone secretion in adrenalectomized animals. Furthermore, Sugino *et al.* (1991) reported that ACTH exerts its luteolytic effect by directly inhibiting luteotrophin secretion from the placenta. Thus, the luteolytic effect of restraint stress would be more apparent in adrenalectomized rats. Since fetal resorption caused by restraint stress was not completely prevented by progesterone administration, it is possible that restraint stress acts in ways other than via inhibition of progesterone secretion to induce fetal loss. However, further investigations are required to confirm this.

The luteolytic effect of restraint stress was apparent only in the IC group. It therefore appears that the luteolytic effect of restraint stress is eliminated by placental hormones. It may be that placental hormones block the effect of restraint stress at the corpus luteum or at some other site (for example, the hypothalamus–pituitary axis). Since placental luteotrophic hormones, including rat placental lactogen and testosterone (which is aromatized to oestrogen in the corpus luteum) are present in higher concentrations in rats carrying the full complement of conceptuses (Ochiai *et al.*, 1983; Sugino *et al.*, 1991), they may overcome the luteolytic effect of restraint stress. The luteolytic effects of ACTH and corticosterone are blocked by placental hormones (Kato *et al.*, 1988; Sugino *et al.*, 1991). Sugino *et al.* (1991) suggested that the corpora lutea of the FC group, which were sufficiently exposed to placental hormones before day 12 of pregnancy, may not be influenced by the pituitary–adrenal system. In addition, progesterone may inhibit ACTH secretion during stress (Viau and Meaney, 1991). The higher concentration of serum progesterone in the FC group than in the IC group may block the effect of restraint stress by inhibiting ACTH release from the pituitary. These findings indicate that foeto-placental units might protect luteal function from the luteolytic effect of restraint stress in rats during mid-pregnancy. Thus, rats with a full complement of conceptuses are more resistant to environmental stress than are those with a single conceptus. This mechanism would preferentially maintain pregnancies with a large litter size, and tend to abort pregnancies with a small litter size, particularly under stressful environmental conditions.

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