Ovulation after sympathetic denervation of the rat ovary produced by freezing its nerve supply

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Summary. The ovaries of cyclic female rats were unilaterally or bilaterally denervated or sham-operated. Denervation was achieved by freezing the ovarian vascular pedicle and suspensory ligament about 1 cm from the ovary; this technique renders the ovary devoid of innervation for up to 10 days. Denervated ovaries from 6 unilaterally and 7 bilaterally operated animals exhibited normal ovulation rates (5.8 ± 1.0 and 5.2 ± 1.2 respectively, mean $\pm$ s.e.m.) compared with intact (5.6 ± 0.4) and sham-operated controls (4.4 ± 0.6 unilaterally sham-operated; 4.1 ± 0.8 bilaterally sham-operated). It is concluded that the mechanism of follicular rupture is unaffected by the absence of ovarian sympathetic innervation.

Introduction

It has not been clearly established whether the ovarian sympathetic nerves play any role in the process of ovulation (Bahr, Kao & Nalbandov, 1974; Mohsin & Pennefather, 1979). Early studies involving abdominal sympathectomy (Sweet & Thorp, 1929; Brooks, 1935) suggested that the ovarian sympathetic nerves were not required for ovulation but results from more recent studies seem inconsistent with these findings. For example, the α-adrenoceptor blocker, dibenzyline, prevents follicular rupture in rabbits when injected into the aorta just above the ovarian artery (Virutamasen, Hickok & Wallach, 1971), and in hens when injected systemically or directly into the follicle wall (Ferrando & Nalbandov, 1969; Kao & Nalbandov, 1972). Also, local chemical denervation of the rat ovary with 6-hydroxydopamine decreases the numbers of gonadotrophin-induced ovulations (Walles, Owman, Sjöberg, Stefenson & Wallach, 1980).

Other early work examining the effects of denervation on ovulation has not necessarily excluded a role for the ovarian sympathetic nerves. For example, ovulation still occurs after transection or complete removal of areas of the spinal cord (Hinsey & Markee, 1932). This procedure however, would be expected to leave sympathetic post-ganglionic fibres that could still play a permissive (e.g. trophic) role in follicular function. Also, transplanted ovaries can sometimes ovulate (Friedman, 1929) but often they do not and instead produce unruptured luteinized follicles (Deanesly, 1956; Dominguez & Riboni, 1971).

In many of these studies, checks were not made to determine whether denervation was complete and that reinnervation had not occurred. This is particularly important if ovulation rate is unaltered because even a few remaining nerves may be enough to maintain normal ovarian function.

The present study was performed to re-examine the effect of ovarian denervation on ovulation rate in the rat. A simple freezing method was used to denervate the ovaries (Gibson, Roche, Summers & Wylie, 1984) and all ovaries were examined thoroughly for the presence or absence of sympathetic nerves. Part of this work has been reported in abstract form (Wylie, Roche & Gibson, 1983).

Materials and Methods

Animals. Cyclic female Sprague–Dawley rats between 50 and 60 days of age were caged in groups of 4 under controlled conditions of light (lights on 07:00–19:00 h) and temperature (20°C). Food and water were freely available. Oestrous cycles were monitored before and during the experiment by taking daily vaginal smears. Only those rats exhibiting a regular 4- or 5-day cycle pattern before the experiment were used.

Denervation. Ovaries were denervated by freezing the ovarian vascular pedicle and ovarian suspensory ligament with their accompanying nerves for 20 ± 5 sec, using a metal probe which had been chilled in liquid nitrogen (Gibson *et al.*, 1984). The vessels were frozen immediately proximal to the point where the uterine branch of the ovarian artery originates, about 1 cm from the ovary. The ovarian suspensory ligament was also frozen about 1 cm from the ovary. This procedure produces complete adrenergic denervation in 50% of ovaries judged by the absence of nerves detectable by fluorescence microscopy and of noradrenaline measured by high-performance liquid chromatography (Gibson *et al.*, 1984).

Sham operations were performed using identical procedures except that the probe was not chilled.

Experimental procedure. All operations were performed at metoestrus under general anaesthesia produced by an intraperitoneal injection of a mixture of methohexitone sodium (Brietal Sodium: Eli Lilly, Sydney, Australia), 50 mg/kg, and amobarbital sodium (Amytal Sodium: Eli Lilly, Indianapolis, U.S.A.), 83 mg/kg. Rats were bilaterally denervated (Group B) or sham-operated (Group SB), unilaterally denervated (Group U) or sham-operated (Group SU), or unoperated (Group C). Males were introduced into the cages 3–5 days after surgery so that the time of oestrus in the females could be determined. When spermatozoa were detected in the vaginal smear, females were killed and their ovaries and oviducts removed. All females were killed between 4 and 10 days after surgery, since this is the period in which no adrenergic nerve fibres are found in ovaries successfully denervated by this method (Gibson *et al.*, 1984).

Ovaries from 19 rats in Group U, 14 in Group B and 48 in Groups SU, SB and C were placed in embedding medium (O.C.T. compound, Tissue-Tek, Miles Labs, IL., U.S.A.), frozen in a cryostat and then sectioned at 14 μm. Five sections from equally spaced regions across each ovary were prepared using fluorescence histochemistry (de la Torre & Surgeon, 1976). These sections were examined under a fluorescence microscope (Leitz Wetzlar, Germany; × 25 water immersion objective and × 10 eyepiece) to determine the amount of adrenergic innervation present in each. Only those ovaries with no adrenergic nerve fibres in any of their 5 representative sections were considered to be completely denervated.

Oviducts were subjected to routine histological procedures, serially sectioned at 12 μm and stained with haematoxylin and eosin. The ovulation rate for each ovary was determined by counting the number of oocytes in the corresponding oviduct.

The ovaries from other rats in Groups U and B were also subjected to routine histological procedures and serially sectioned at 12 μm. The numbers of fresh corpora lutea (CL) were counted and compared with the corresponding tubal oocyte count. This permitted a check on the accuracy of oocyte counts as indicators of numbers of ovulations.

Results

All control ovaries (from rats in Groups SU, SB and C and from the unoperated sides of rats in Group U) had moderate to abundant numbers of adrenergic nerve fibres (∼ 200–500 per section) in at least 3 of their 5 representative sections. Of the 19 ovaries from the operated side of rats in Group U, 6 had no nerve fibres in any of their 5 sections; in 7 denervation was nearly complete (1–25

adrenergic nerve fibres per 5 sections); and the remaining 6 had sparse to moderate numbers of fibres ($\sim$ 26–200 fibres). There were no nerve fibres in any of the sections from 18 of the 28 ovaries from the rats in Group B: 7 of these rats were completely bilaterally denervated.

Ovulation

The completely denervated ovaries from rats in Groups U and B ovulated normal numbers (mean $\pm$ s.e.m.) of oocytes (5·8 $\pm$ 1·0 and 5·2 $\pm$ 1·2, in Groups U (N = 6) and B (N = 14) respectively) compared with those from Group C (5·6 $\pm$ 0·4, N = 32), Group SU (4·4 $\pm$ 0·6, N = 16) and Group SB (4·1 $\pm$ 0·8, N = 16) ($P > 0.05$, unpaired t tests). In addition, the numbers of oocytes ovulated from completely denervated ovaries in rats in Group U were similar to those from the unoperated contralateral ovaries (5·8 $\pm$ 1·0 and 5·0 $\pm$ 1·0, respectively, $P > 0.05$, paired t test).

Agreement of CL and tubal oocyte counts

In 7 out of 8 rats in Group U, and in 2 Group-B rats for which counts were obtained, the number of fresh CL in the ovary and the number of oocytes in the corresponding oviduct were in agreement. In the 8th rat in Group U, there was agreement for the operated side but not for the unoperated side (7 CL, 9 oocytes).

Oestrous cycles

Denervation of the ovary did not affect the length of the oestrous cycle in rats in Groups U or B; there was no significant difference from values in the other groups. However, the operative procedure itself did lengthen the cycle since the mean lengths after the operation were greater than those before the operation in all groups. The mean ($\pm$ s.e.m.) length rose from 4·5 $\pm$ 0·2 days before operation to 4·9 $\pm$ 0·2 days after operation in Group SU ($P < 0.02$, paired t test), from 4·3 $\pm$ 0·2 to 5·2 $\pm$ 0·2 days in Group SB ($P < 0.01$), from 4·8 $\pm$ 0·3 to 5·0 $\pm$ 0·4 days in Group U (not significant); and from 4·3 $\pm$ 0·3 to 4·7 $\pm$ 0·2 days in Group B (not significant).

Discussion

The results clearly indicate that, in rats, ovulation can still occur at a normal rate after complete sympathetic denervation of the ovary. It seems therefore that the mechanism of follicular rupture is independent of any influence from the ovarian sympathetic innervation. These results agree with those from early studies on sympathectomized rats (Sweet & Thorp, 1929) and rabbits (Brooks, 1935) and with those of Weiner, Wright & Wallach (1975) who found that completely sympathetically denervated rabbit ovaries ovulate normal numbers of oocytes. They also agree with the results from several studies showing that isolated ovaries will ovulate normal numbers of oocytes in response to exogenous gonadotrophins (mice: Fritz, Cho & Biggers, 1965; rabbits: Lambertson, Greenbaum, Wright & Wallach, 1976; Janson *et al.*, 1982). In contrast, follicular rupture can be prevented in rabbits and hens by systemic administration of the α-adrenoceptor blocker, dibenzyline (Ferrando & Nalbandov, 1969; Virutamusen *et al.*, 1971). This effect is also produced in hens when dibenzyline is injected directly into the follicle wall (Kao & Nalbandov, 1972).

It is not clear why a discrepancy exists between the effects of surgical denervation and of pharmacological agents. Surgical denervation is known to induce supersensitivity in smooth muscle (Westfall, 1981). Ojeda, Aguado & Smith (White) (1983) report that this also occurs in ovarian granulosa cells and we therefore cannot rule out the possibility that low concentrations of circulating catecholamines may bind to the adrenoceptors in the ovary and mimic the actions of the neurotransmitter. However, studies in which drugs are used to block the adrenoceptors exclude this

possibility since the actions of circulating catecholamines, as well as those of the neurotransmitter, would be prevented. Alternatively, pharmacological agents may have additional non-specific actions on the ovary.

It is not clear why oestrous cycle length was increased by the operative procedure. Barbiturate anaesthesia administered to rats on the afternoon of pro-oestrus will block the ovulatory surge of luteinizing hormone and delay ovulation by 24 h (van der Schoot, 1978). However, this cannot explain the present finding since all operations in this study were performed on the day of metoestrus.

Since the denervation procedure used in this study also denervates the oviduct (Gibson *et al.*, 1984), it was necessary to determine whether the number of tubal oocytes reliably represented ovulation rate. Although completeness of denervation was checked in all other rats, it could not be checked in this part of our work since the ovaries were treated only by conventional histology in order to count the newly formed corpora lutea. In all but one case, the number of fresh corpora lutea in the ovary agreed with the number of oocytes in the corresponding oviduct. It seems, therefore, that oocyte transport to the oviduct is not dependent on ovarian or oviducal innervation. This also appears to be true for mice since Johns, Chlumecky, Cottle & Paton (1975) found that the fertility of mice was unchanged after nearly complete denervation of the ovaries and oviducts.

The time course of denervation resulting from the procedure used in this study (Gibson *et al.*, 1984) meant that ovaries were denervated for 2–8 days before ovulation occurred. According to Hirshfield & Midgley (1978) it takes a small antral follicle in the rat 3–4 days to grow to ovulatory size. Our study therefore indicates that normal numbers of follicles grew through from the small antral stage in the absence of any adrenergic innervation. In conclusion then, it seems that the final stages of follicular growth, as well as the mechanism of follicular rupture, are unaffected by the absence of ovarian sympathetic innervation.

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