Differential effects of ovarian local anaesthesia during pro-oestrus on ovulation by the right or left ovary in normal and hemi-ovariectomized adult rats

R. Domínguez, M. E. Cruz and C. Morán

Research Unit in Biology of Reproduction, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, AP 9-020, CP 15000, México DF, México

The effects on spontaneous ovulation in normal and hemi-ovariectomized cyclic rats of an injection of saline or a local anaesthetic (2-diethylamine-2',6'-acetoxilidide hydrochloride with adrenaline, Xylocaine; administered at 13:00 h) into the bursa ovarica, were studied to investigate the participation of ovarian innervation in the regulation of the response of ovarian follicles to endogenous gonadotrophins on the day of pro-oestrus. The injection of saline or Xylocaine into both ovaries did not affect ovulation by the right ovary. Bilateral injection of Xylocaine did not affect ovulation by the left ovary. However, when saline was injected into the right ovary and Xylocaine into the left ovary, the number of ova shed by the left ovary decreased significantly (0.9 ± 0.7 versus 4.4 ± 0.2 , $P < 0.01$; Mann–Whitney U test). In hemi-ovariectomized animals, when the right ovary was anaesthetized, ovulation was similar to that in saline-treated animals, and when the right ovary was anaesthetized, ovulation was blocked (one of six animals ovulated). The injection of hCG at 14:00 h to hemi-ovariectomized rats did not restore ovulation, whereas all the rats ovulated when anaesthesia occurred at 18:00 h. The present results suggest that on the afternoon of the day of pro-oestrus, before the LH peak, a neural signal modulates the response of the left ovary to gonadotrophins.

Introduction

There is evidence that ovarian innervation plays an important role in the regulation of ovarian functions (Burden, 1985; Ojeda and Aguado, 1985). However, the participation of ovarian innervation in the regulation of ovulation is controversial. For example, ovarian autografts in spayed animals ovulate normally (Domínguez and Riboni, 1971) and ovulation can be induced *in vitro* using perfusion systems (Higuchi and Spey, 1989; Janson *et al.*, 1988). Similarly, the bilateral section of the superior ovarian nerve performed on different days of the oestrous cycle (Chávez *et al.*, 1991) or the freezing of both nerves (Wylie *et al.*, 1985) do not alter ovulation. In addition, denervation of the ovary did not influence ovulation, pregnancy or corpora lutea in rabbits (Weiner *et al.*, 1975a, b, c). On the basis of these and other observations, Espey and Lipner (1994) concluded that "... although one cannot completely rule out the possibility that adrenergic agents might have some modest influence on ovulation, it is, nevertheless, quite apparent that nervous activity is not an essential component of the ovulatory mechanism".

There is evidence suggesting that ovarian innervation plays a definitive role in the regulation of the ovulatory process. Ovarian autograft in hemispayed rats did not present signs of ovulation or follicular growth (Domínguez and Riboni, 1971).

Sequential administration of FSH and hCG induced follicular growth but not ovulation. However, when the same treatment was performed on peripheral sympathetic-denervated animals that had been administered guanethidine, signs of ovulation were present in the graft (Ayala and Domínguez, 1988). The unilateral and bilateral section of the vagus nerve affects spontaneous and gonadotrophin-induced ovulation (Cruz *et al.*, 1986; Chávez *et al.*, 1987a, b). The unilateral section of the superior ovarian nerve affects ovulation by the denervated ovary, while the intact ovary presents a compensatory ovulation (Chávez *et al.*, 1991; Morales *et al.*, 1993) and the denervated ovary does not increase the number of ova shed after the injection of hCG and pregnant mares' serum gonadotrophin, or the sequential administration of both hormones (Morales and Chávez, 1994). Local anaesthesia or the lesion of the cervico-vaginal plexus also affects ovulation (Domínguez, 1990).

The participation of the neural signals in the regulation of ovulation can be explained as modulatory effects that use neurotransmitters (catecholamines, neuropeptides, acetylcholine) released at appropriate times to influence the response of the follicle to gonadotrophins.

Although it is thought, in general, that paired organs are physiologically and pathologically very similar, there is evidence that endocrine paired organs are not identical in their function and control. For example, in genetically female intersex pigs with ovotestes, the higher degree of masculinization

occurs on the right gonad (Hunter *et al.*, 1985). In women, ovulation occurs in the right ovary twice as often as in the left one (Potashnik *et al.*, 1987). A similar difference has been described in monkeys by Morse and Van Wagenen (1936). According to Bernstein (1993) "... endocrine asymmetry is a subset of a broader phenomenon which may be called topoendocrinology (topological endocrinology). Topoendocrinology analyses 'spatial' (topographical) peculiarities of the functioning of the endocrine system."

There is evidence that the neuroendocrine mechanisms regulating ovulation in hemi-ovariectomized animals are different from those occurring in normal animals, and that the mechanisms participating are different for the right and left ovary and depend on the ovarian innervation (Chávez *et al.*, 1987c; Domínguez *et al.*, 1989). For example, the unilateral section of the left vagus nerve affected ovulation in rats with one or both ovaries in different ways (Cruz *et al.*, 1986; Chávez *et al.*, 1987a, b). The unilateral section of the superior ovarian nerve, which affects ovulation when the animal has both ovaries, had no effect when the animal was hemi-ovariectomized and the results depended on the day of the oestrous cycle when the treatment was performed (Chávez and Domínguez, 1994).

The rationale for the present study was that on the day of pro-oestrus, the effects of gonadotrophins on the ovulatory process are modulated by neural information reaching the ovary, which would be different for the right and left ovary, and also in intact and hemi-ovariectomized animals. The effects on ovulation of a local anaesthetic, 2-diethylamine-2',6'-acetoxilidide hydrochloride with adrenaline, injected into the bursa ovarica at the beginning of pro-oestrus in the afternoon, were analysed to test this hypothesis.

Materials and Methods

Adult virgin female rats of the C-II-Z-V strain from the authors' stock were kept under lighting controlled conditions (lights on from 05:00 to 19:00 h) with food and tap water available *ad libitum*.

The oestrous cycle was monitored by daily vaginal smears, and only those rats with three consecutive 4 day cycles were used. At 13:00 h on the day of pro-oestrus, the animals were anaesthetized with ether and allotted at random to one of the following experiments, and killed 20 h later.

Experiment 1: effects on ovulation after the unilateral injection of Xylocaine into the ovaries or unilateral injection of saline in normal rats

Rats were anaesthetized and laparotomies were performed; the right or left ovary were exposed, and saline or lidocaine (20 mg 2-diethylamine-2',20. 6'-acetoxilidide clorhidrate ml⁻¹) with 0.01 mg adrenaline (Xylocaine) ml⁻¹ (Astra Chemicals, Naucalpan, México) was injected into the bursa. The bursa ovarica was distended by the fluid injected. The abdominal wall and the skin were sutured. Two groups of rats were anaesthetized and laparotomies were performed, and had the right or left ovary exposed and returned to the abdominal cavity and the wound was sutured (unilateral manipulation of the ovary

Table 1. Treatments used in Experiment 2

	Group 1	Group 2	Group 3	Group 4
Right ovary	Saline	Xylocaine	Xylocaine	Saline
Left ovary	Saline	Xylocaine	Saline	Xylocaine

groups) for comparison. In addition, an untreated control group was killed on the morning of the day of oestrus, after three consecutive 4 day cycles.

Experiment 2: comparison of ovulation by the right and left ovary to the effects of the bilateral injection of saline or Xylocaine

A group of cyclic rats was ether anaesthetized, laparotomies were performed and animals were allotted at random to the following experimental groups (Table 1): group 1: the left and right ovary were exposed, and with the aid of a 1 ml syringe and a 25 needle, 0.1 ml of saline was injected into the bursa ovarica of the right and left ovary; group 2: Xylocaine was injected into the bursa ovarica of the right and left ovary; group 3: saline was injected into the bursa of the right ovary, while Xylocaine was injected into the bursa of the left ovary; and group 4: saline was injected into the left ovary and Xylocaine into the right ovary.

The bursa ovarica was distended by the fluid injected. The abdominal wall and the skin were sutured.

For comparison, a group of rats was anaesthetized, laparotomies were performed and animals had both ovaries exposed and returned to the abdominal cavity and the wound sutured (bilateral manipulation of the ovary group).

Experiment 3: effects on ovulation after the unilateral injection of Xylocaine into the ovaries or unilateral injection of saline to hemi-ovariectomized rats

Rats were anaesthetized and laparotomies were performed, and the right or left ovary was exposed and saline or Xylocaine was injected into the bursa. Two minutes after the injection of saline or Xylocaine, the untreated ovary was exposed and extirpated.

On the basis of the results obtained, a group of rats was injected with Xylocaine into the bursa ovarica of the left ovary and right hemi-ovariectomized 2 min later (left ovary *in situ*), and then injected 1 h later (14:00 h of pro-oestrus) with 10 iu hCG (Sigma Chemical Co., St Louis MO), subcutaneously. In another experiment, the treatment (Xylocaine injection into the bursa of the left ovary, and extirpation of the right one) was performed at 18:00 h on the day of pro-oestrus. Both groups of animals were autopsied on the morning of the next day.

In another experiment, the left or right ovary was injected with Xylocaine at 13:00 h, and 2 min later the 'anaesthetized' ovary was extirpated.

Animals were killed by bleeding under ether anaesthesia, on the next morning (on the expected day of oestrus). At autopsy, the oviducts were dissected and the ova were counted with the aid of a dissecting microscope. The ovaries of non-ovulating

Table 2. Ovulation rate of and number of ova shed by (means $\pm$ SEM) the right and left ovaries of untreated control rats and pro-oestrous rats injected with saline or Xylocaine into the right or left ovary and autopsied the next day

Treatment	Ovulation rate of right ovary	Number of ova shed by right ovary	Ovulation rate of left ovary	Number of ova shed by left ovary
Control	8/8	5.6 $\pm$ 0.3	8/8	5.5 $\pm$ 0.8
Saline right ovary	6/10	4.4 $\pm$ 0.8	8/10	5.5 $\pm$ 1.5
Xylocaine right ovary	8/11	5.5 $\pm$ 0.9	9/11	4.4 $\pm$ 0.7
Saline left ovary	4/4	5.5 $\pm$ 1.0	3/4	3.7 $\pm$ 1.2
Xylocaine left ovary	9/11	5.7 $\pm$ 0.8	9/11	4.1 $\pm$ 0.9

Table 3. Ovulation rate of and the number of ova shed by (means $\pm$ SEM) the ovulating ovary of untreated control rats and pro-oestrous rats injected with saline or Xylocaine and autopsied the next day

Treatment on right ovary	Treatment on left ovary	Ovulation rate of right ovary	Number of ova shed by right ovary	Ovulation rate of left ovary	Number of ova shed by left ovary
Control	Control	8/8	5.6 $\pm$ 0.3	8/8	5.5 $\pm$ 0.8
Saline	Saline	8/9	4.6 $\pm$ 0.5	6/9	5.4 $\pm$ 0.7
Xylocaine	Xylocaine	7/9	4.4 $\pm$ 0.7	8/9	4.7 $\pm$ 1.0
Xylocaine	Saline	4/6	4.0 $\pm$ 1.1	6/6	6.0 $\pm$ 0.9
Saline	Xylocaine	7/11	2.9 $\pm$ 0.4*	9/11	1.5 $\pm$ 0.3 [†]

* $P < 0.01$ compared with control group; Kruskal–Wallis test followed by Mann–Whitney U test.

[†] $P < 0.01$ compared with control and Xylocaine–Xylocaine, and saline–saline treated groups; Kruskal–Wallis test followed by Mann–Whitney U test.

animals or those with a small number of ova in the bursa were fixed in Bouin's fluid, embedded in paraffin wax, serially sectioned at 10 μ m, stained with haematoxylin–eosin, and examined under a light microscope for fresh corpora lutea as a sign of ovulation, or the presence of fresh corpora lutea with a trapped oocyte.

The animals were considered to have ovulated when ova were present in the oviduct or when fresh corpora lutea without trapped ova were present in the ovaries. The ovulation rate (number of rats ovulating: total number of rats treated) was analysed by Fisher's exact probability test. The number of ova recovered, or the number of fresh corpora lutea counted in ovaries, where values differed, were analysed using the Kruskal–Wallis test followed by the Mann–Whitney U test ($P < 0.05$ was considered significant).

Results

The ovulation rate (number of ovulating rats: number of treated rats) was not modified by the unilateral or bilateral manipulation of the ovaries (12/12 versus 8/8, not significant). The number of ova shed by ovulating animals decreased in bilaterally manipulated animals (7.8 $\pm$ 0.7 versus 11.1 $\pm$ 0.7, $P < 0.05$ Mann–Whitney U test) and was not affected when the manipulation was performed on the right or left ovary (9.8 $\pm$ 0.8 and 10.0 $\pm$ 0.3 versus 11.1 $\pm$ 0.7, respectively, not significant).

Experiment 1

The ovulation rate for the groups of unilateral treated rats was similar to that of untouched control animals (Table 2; 8/10, 9/11, 4/4 and 9/11 versus 8/8, not significant, Fisher's exact probability test). In ovulating animals, neither the ovulation rate for the right or left ovary nor the number of ova shed by ovulating animals was affected by unilateral treatment (Table 2).

Experiment 2

The ovulation rate for the groups of bilaterally treated rats was similar to that for untreated control animals (Table 3; 8/9, 8/9, 6/6, and 9/11 versus 8/8, not significant, Fisher's exact probability test). Ovulation by the right or left ovary (number of ovulating animals, and number of ova shed per animal) was not modified by the bilateral injection of saline or Xylocaine. The injection of Xylocaine into the right ovary and saline into the left ovary did not affect ovulation by the right ovary. Injection of saline into the right ovary and Xylocaine into the left ovary did not affect ovulation rate but decreased the number of ova in the right as well as in the left ovary (Table 3).

Experiment 3

In hemi-ovariectomized animals, injection of saline into the bursa ovarica of the left ovary modified neither the number of

Table 4. Ovulation rate of and the number of ova shed (means $\pm$ SEM) by *in situ* ovaries of pro-oestrous-hemi-ovariectomized rats injected with saline or Xylocaine into the bursa ovarica 2 min before hemi-ovariectomy and autopsied the next day

<i>In situ</i> ovary	Treatment	<i>n</i>	Ovulation rate	Number of ova shed
Right	Saline	5	5/5	4.8 $\pm$ 0.4
Right	Xylocaine	8	8/8	4.4 $\pm$ 0.6
Left	Saline	6	5/6	5.5 $\pm$ 0.9
Left	Xylocaine	6	1/6*	1 corpus luteum

* $P < 0.05$ in comparison with saline-treated groups; Fisher's exact probability test.

ovulating animals nor the number of ova recovered. Anaesthesia of the left ovary by Xylocaine injection resulted in the diminution of ovulation in almost all animals treated. One fresh corpus luteum was observed in the ovary of one animal and, in another three ovaries, one, one and two corpora lutea with trapped ova were observed. Taken together, the number of fresh corpora lutea and the number of luteinized follicles with trapped ova in those animals with Xylocaine injected into the left ovary and right hemi-ovariectomized were significantly lower than in those animals injected with saline into the left ovary and right hemi-ovariectomized (1.3 ± 0.25 versus 4.8 ± 0.4 , $P < 0.01$, Mann-Whitney U test). In the left ovary, several preovulatory follicles were present, and all presented signs of atresia.

The injection of saline or Xylocaine into the bursa of the right ovary affected neither the number of ovulating animals nor the number of ova recovered per ovulating animal (Table 4).

The injection of hCG at 14:00 h into rats treated with Xylocaine on the left ovary and then hemi-ovariectomized, partially restored ovulation (4/5 animals presented fresh corpora lutea, and the mean number was 1.8 ± 0.8). In only two animals were ova recovered in the oviduct (mean: 2.5 ova). In the ovary of two animals, fresh corpora lutea with trapped ova were observed. When injection of Xylocaine into the left ovary and extirpation of the right ovary were performed at 18:00 h, all the animals ovulated normally (6/6 ovulated, and the mean number of ova shed was 5.2 ± 0.8).

The ovulation rates (left ovary *in situ* 5/7; right ovary *in situ* 4/5) and number of ova shed (left ovary *in situ* 4.0 ± 0.8 ; right ovary *in situ* 5.3 ± 0.5) were not modified in those animals in which Xylocaine was injected into one ovary and the same ovary was extirpated 2 min later.

Discussion

The results of the present study suggest that during the afternoon of pro-oestrus, the left and right ovaries exchange some form of neural information necessary for the release of LH by the pituitary and for the normal response of the ovaries to LH.

This interpretation is based on the following findings. In the intact animal, the manipulation of one ovary did not affect ovulation. The injection of Xylocaine in the left ovary and the injection of saline in the right ovary results in a decrease in the number of ova shed by both ovaries. Because this effect was not observed when the right ovary was injected with Xylocaine and the left ovary with saline, we propose that the initial signal originates in the left ovary. When both ovaries were injected with Xylocaine or saline, ovulation was normal in both ovaries, suggesting that other mechanisms are able to close the circuit necessary for normal ovulation.

Since in hemispayed animals, ovulation was affected only in those animals with the left ovary injected with Xylocaine, and the right ovary extirpated, it seems that such a signal needs two steps: the first step in the left ovary and a second step in the right ovary. The hypothesis that the blockade of innervation affects the response of the ovary to LH is supported by the incomplete ovulation observed in those hemi-ovariectomized animals treated with hCG. The neural information provided at a given time in relation to the secretion of LH is supported by the results obtained in those animals in which hemi-ovariectomy and anaesthesia were performed at 18:00 h.

Engeland *et al.* (1985), Engeland and Dallman (1976) and Holzwarth *et al.* (1987) observed that in the adrenal, innervation plays a role in the regulation of the response of endocrine organs to pituitary hormones. However, another possibility is that Xylocaine anaesthesia or saline injection affects ovarian vascularity. Zackrisson *et al.* (1996) have shown that the acute ligation of the ovarian artery of the ovarian branch of the uterine artery reduces the number of ova shed by the ovulating rats and that, in prepubertal rats primed with equine chorionic gonadotrophin (eCG) injected with hCG, sham operation to the arteries does not affect ovulation rate. However, a vascular interpretation does not explain the present results, since Xylocaine anaesthesia or saline injection affected ovulation only when the left ovary was injected with Xylocaine and the right ovary with saline. There is also evidence indicating differences in venous drainage between the right and left ovary (Reynolds, 1973). In addition, there is evidence that the sympathetic adrenal denervation modifies the adrenal blood flow without altering the increase in cortisol plasma concentration that is the response to haemorrhage (Engeland *et al.*, 1985).

When a similar study was carried out in hemi-ovariectomized animals, the differences between the left and right ovaries were more evident. As was noted in previous studies, the left ovary seems more 'dependent' on neural signals when the right ovary is absent. The hypothesis that some neural information modulates the response of follicles to LH is supported by the finding that injection of hCG 1 h after Xylocaine administration to the left ovary did not induce ovulation, and that fresh corpora lutea, with and without trapped ova, were observed. The dose of hCG used in the present study is enough to induce ovulation in pro-oestrous rats treated with sodium pentobarbital at 13:00 h (R. Domínguez, unpublished). The temporality of the neural signal for the regulation of follicular sensitivity to LH is supported by the normal ovulation observed in those animals when Xylocaine was injected into the left ovary at 18:00 h.

The finding that normal ovulation occurred in rats in which anaesthesia of the left ovary was followed by extirpation of the 'anaesthetized' ovary supports the proposed existence of a neural signal, elicited in these animals by the extirpation of the left ovary, between the two ovaries.

On the basis of the results of the present study and those published earlier by the present authors and others, our hypothesis is that during the oestrous cycle, and particularly on the afternoon of pro-oestrus, the left ovary sends a signal to the right ovary that, in turn, relays a neural signal to the central nervous system arriving at the hypothalamus (Cruz *et al.*, 1990a, b; Morán *et al.*, 1994), the mesencephalic central grey matter (Kawakami and Ando, 1981; Bergen and Leung, 1987a, b; Domínguez-González and Chávez, 1993, 1994), the A1 region (Kawakami *et al.*, 1981; Kaba *et al.*, 1983; Tanaka *et al.*, 1985), the dorsal raphe nucleus (Ayala *et al.*, 1994) or the amygdala (Sánchez and Domínguez, 1995), originating another neural signal directed to the left and right ovaries via the superior ovarian nerve, which modulates the response of the ovaries to LH that results in ovulation.

In conclusion, the present results suggest that on the afternoon of the day of pro-oestrus, before the LH peak, a neural signal modulates the response of the left ovary to gonadotrophins.

Supported by DGAPA: grants IN208996, IN210893; CONACYT: grant IN1719 and Programa Universitario de Investigación en Salud, UNAM.

References

- Ayala ME and Domínguez R (1988) Ovulation response to the sequential administration of the follicle stimulating hormone and human chorionic gonadotropin by autografted ovary in unilaterally ovariectomized adult rat with peripheral denervation induced by guanethidine treatment *La revista de Investigación Clínica (Mexico)* **40** 149–155
- Ayala ME, Rosas P and Domínguez R (1994) Different effects of unilateral and bilateral lesions of the dorsal raphe nucleus on puberty and first ovulation *Brain Research Bulletin* **34** 27–30
- Bergen H and Leung PCK (1987a) Electrical stimulation of ventral versus dorsal mesencephalic tegmental areas in the conscious rat: effects on luteinizing hormone release *Neuroendocrinology* **46** 117–124
- Bergen H and Leung PCK (1987b) Electrical stimulation of ascending noradrenergic tract in the midbrain: suppression of steroid-induced luteinizing hormone release *Endocrinology* **122** 899–905
- Bernstein LM (1993) Topoendocrinology *Journal of Endocrinology* **137** 163–166
- Burden HW (1985) The adrenergic innervation of mammalian ovary. In *Catecholamines as Hormone Regulators* pp 261–278 Eds N Ben-Jonathan, JM Bahr and RI Weiner. Raven Press, New York
- Chávez R and Domínguez R (1994) Participation of the superior ovarian nerve in the regulation of compensatory ovarian hypertrophy: the effects of its section performed on each day of the oestrous cycle *Journal of Endocrinology* **140** 197–201
- Chávez R, Cruz ME and Domínguez R (1987a) Modifications on the ovarian response to gonadotrophins induced by catecholamine depletion in vagotomized adult rats *La Revista de Investigación Clínica (Mexico)* **39** 149–153
- Chávez R, Cruz ME and Domínguez R (1987b) Ovulation and compensatory ovarian hypertrophy (COH) to gonadotrophins depends on the vagus nerve and catecholaminergic ovarian innervation *Medical Science Research* **15** 1529–1530
- Chávez R, Cruz ME and Domínguez R (1987c) Differences in the ovulation rate of the right and left ovary in unilaterally ovariectomized rats: effects of ipsi and contralateral section of the vagus nerves on the remaining ovary *Journal of Endocrinology* **113** 397–401
- Chávez R, Carrizosa L and Domínguez R (1991) Effects of superior ovarian nerve section on spontaneous and induced ovulation in the adult rat *Medical Science Research* **19** 41–42
- Cruz ME, Chávez R and Domínguez R (1986) Ovulation, follicular growth and ovarian reactivity to exogenous gonadotrophins in adult rats with unilateral or bilateral section of the vagi nerves *La Revista de Investigación Clínica (Mexico)* **38** 167–171
- Cruz ME, Morán JL, Jaramillo LP and Domínguez R (1990a) Differential effects of unilateral hypothalamic lesion on ovulation and compensatory ovarian hypertrophy in hemispayed adult rats *Journal of Endocrinology* **112** 37–41
- Cruz ME, Morán JL, Jaramillo LP and Domínguez R (1990b) Differences in spontaneous ovulation in rats with unilateral lesion of the hypothalamus *Brain Research Bulletin* **24** 739–742
- Domínguez R (1990) Different ovulatory response of the right and left ovary to unilateral lesion and anaesthesia of the cervico-vaginal plexus *Journal of Endocrinology* **124** 43–45
- Domínguez R and Riboni L (1971) Failure of ovulation in autografted ovary in hemispayed rat *Neuroendocrinology* **7**, 164–170
- Domínguez R, Cruz ME and Chávez R (1989) Differences in the ovulatory ability between the right and left ovary related to ovary innervation. In *Growth Factors and the Ovary* pp 321–325 Ed. AN Hirshfield. Plenum Press, New York and London
- Domínguez-González A and Chávez R (1993) Asymmetric effects of unilateral lesions in the midbrain central gray matter on ovulation in the adult female rat *Medical Science Research* **21** 513–514
- Domínguez-González A and Chávez R (1994) Evidence of the participation of catecholaminergic system on the midbrain central gray matter on ovulation *Medical Science Research* **22** 311–312
- Engeland WC and Dallman MF (1976) Neural mediation of compensatory adrenal growth *Endocrinology* **99** 1659–1662
- Engeland WC, Shinsako J and Dallman MF (1976) Corticoids and ACTH are not required for compensatory adrenal growth *American Journal of Physiology* **229** 1461–1464
- Engeland WC, Lilly MP and Gann DS (1985) Sympathetic adrenal denervation decreases adrenal blood flow without altering the cortisol response to haemorrhage *Endocrinology* **117** 1000–1010
- Espey LL and Lipner H (1994) Ovulation. In *The Physiology of Reproduction* 2nd Edn pp 725–780 Eds E Knobil and JD Neill. Raven Press, New York
- Higuchi Y and Espey LL (1989) Pattern of ovarian steroid secretion during ovulation of *in vitro* perfused rat ovary varying with methods of sampling *Journal of Reproduction and Fertility* **87** 821–828
- Holtzwarth MA, Cunningham LA and Kleitman N (1987) The role of adrenal nerves in the regulation of adrenocortical function *Annals of the New York Academy of Sciences* **512** 449–464
- Hunter RHF, Cook B and Baker TG (1985) Intersexuality in five pigs, with particular reference to oestrous cycle, the ovotestis, steroid hormone secretion and potential fertility *Journal of Endocrinology* **106** 233–242
- Janson PO, Brännström M, Holmes PV and Sogn J (1988) Studies on the mechanisms of ovulation using the model of the isolated ovary *Annals of the New York Academy of Sciences* **541** 22–29
- Kaba H, Saito H, Otsuka K, Seto K and Kawakami M (1983) Effects of estrogen on the excitability of neurons projecting from the noradrenergic A1 region to the preoptic and anterior hypothalamic area *Brain Research* **274** 156–159
- Kawakami M and Ando S (1981) Lateral hypothalamic mediation of midbrain catecholaminergic influences on preovulatory surge of serum gonadotropin and prolactin in female rats *Endocrinology* **108** 66–71
- Kawakami M, Ando S, Nishihara M and Kadokoro Y (1981) Participation of the lower brain stem in induction of preovulatory gonadotropin surges in female rats *Endocrinology Japonica* **28** 809–818
- Morales L and Chávez R (1994) El nervio ovárico superior como modulador de la acción de las gonadotropinas. In *Program of the XVIII Congreso Latinoamericano de Ciencias Fisiológicas Abstract P5/19* Montevideo, Uruguay
- Morales L, Chávez R and Domínguez R (1993) Participation of the superior ovarian nerve in the regulation of ovulation in the prepubertal rat: differential effects of unilateral and bilateral section of the nerve *Medical Science Research* **21** 15–17
- Morán JL, Cruz ME and Domínguez R (1994) Differences in the ovulatory response to unilateral lesion in the preoptic or anterior hypothalamic area performed on each day of the estrous cycle of the adult rat *Brain Research Bulletin* **33** 663–668
- Morse AM and Van Wagenen G (1936) Observations upon ovulation in primates *American Journal of Obstetrics and Gynecology* **32** 823–832

- Ojeda SR and Aguado LI (1985) Adrenergic control of the prepubertal ovary: involvement of local innervation and circulation catecholamines. In *Catecholamines as Hormones Regulators* pp 293–310 Eds N Ben-Jonathan, JM Bahr and RJ Weiner. Raven Press, New York
- Potashnik G, Insler V and Meizner J (1987) Frequency, sequence, and side of ovulation in women menstruating normally *British Medical Journal* **294** 219
- Reynolds SRN (1973) Blood and lymph vascular system of the ovary. In *Handbook of Physiology and Endocrinology II, Part I* pp 261–316 Eds RO Greep and EB Astwood. American Physiological Society, Washington DC
- Sánchez MA and Domínguez R (1995) Differential effects of unilateral lesions in the medial amygdala on spontaneous and induced ovulation *Brain Research Bulletin* **38** 313–317
- Tanaka J, Kaba H, Saito H and Seto K (1985) Inputs from the A1 noradrenergic region to the hypothalamic paraventricular neurons in the rat *Brain Research* **335** 368–371
- Weiner S, Whright KH and Wallach EE (1975a) Selective ovarian sympathectomy in the rabbit *Fertility and Sterility* **26** 353–362
- Weiner S, Whright KH and Wallach EE (1975b) Studies on the function of denervated rabbit ovary: human chorionic gonadotropin-induced ovulation *Fertility and Sterility* **26** 363–368
- Weiner S, Whright KH and Wallach EE (1975c) Lack of effect of ovarian denervation on ovulation and pregnancy in the rabbit *Fertility and Sterility* **26** 1083–1087
- Wylie SN, Roche PJ and Gibson WR (1985) Ovulation after sympathectomy denervation of the rat ovary produced by freezing its nerve supply *Journal of Reproduction and Fertility* **26** 369–373
- Zackrisson U, Mikuni M, Makinoda S, Janson PO, Peterson CM and Brännström M (1996) Ovulation rate in the rat is decreased by acute ligation of the ovarian artery of the ovarian branch of the uterine artery *Biology of Reproduction* **54** (Supplement 1) Abstract 46