THE EFFECTS OF ADRENERGIC DRUGS ON OVUM TRANSPORT THROUGH THE RABBIT OVIDUCT

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Summary. The effect of adrenergic drugs on ovum transport through the oviduct was studied at 24, 36, 48, 60, 72 and 84 hr *post coitum* (p.c.) in 240 mature, female Dutch-belted rabbits.

In untreated rabbits, ova were located in the area of the ampullary-isthmic junction at 24 hr p.c.; most ova entered the uterus between 72 and 84 hr p.c. Epinephrine increased ($P < 0.01$) the rate of ovum transport at 60 and 72 hr p.c., and most of the ova were found in the uterus. Phenoxybenzamine (α -blocker) inhibited ovum transport at 48 hr p.c. ($P < 0.05$), while propranolol (β -blocker) was ineffective in altering ovum transport in any of the time periods.

Regulation of ovum transport through the rabbit oviduct appeared to be governed in part, by a neural-endocrine interrelationship; this relationship could be altered by adrenergic stimulants and blocking agents.

INTRODUCTION

Sympathetic innervation to the smooth musculature of the female genital tract is extensive; the physiological and pharmacological significance of this innervation has been extensively reviewed (Brundin, 1965, 1969; Pauerstein, Woodruff & Zachary, 1968; Marshall, 1970).

By histochemical (Falck, 1962) and biochemical (Haggendal, 1963) methods for norepinephrine, the degree of adrenergic innervation has been shown to increase from the ovarian to the uterine end of the rabbit oviduct (Brundin, 1964c, 1965; Brundin & Wirsén, 1964; Owman, Rosengren & Sjöberg, 1966). In the rabbit, the rich adrenergic innervation has been correlated to a sphincter-like activity (Brundin, 1964a, b, 1965).

Longley, Black & Currie (1968a, b) have shown that autonomic drugs can alter ovum transport at 36 hr *post coitum* (p.c.) and that the effect can be potentiated by oestrogen or progesterone. Phenoxybenzamine inhibits ovum transport; this effect can be potentiated by oestrogen. Epinephrine and propranolol are ineffective at 36 hr p.c. unless accompanied by exogenous oestrogen. Longley *et al.* (1968b) concluded that the rabbit oviduct possesses α - and

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β -adrenoceptors and that the α -receptors are most influential in ovum transport.

The present study evaluates the effect of adrenergic drugs on ovum transport from 24 to 84 hr p.c.

MATERIALS AND METHODS

A total of 240 mature, female Dutch-belted rabbits, obtained from our colony or a local rabbitry, were used in this investigation. The rabbits were allocated to four groups of sixty does each, which were individually isolated for 1 week or more and were allowed free access to food and water.

The effects of epinephrine and its antagonists, phenoxybenzamine (an α -adrenoceptor blocker) and propranolol (a β -adrenoceptor blocker) on ovum transport were studied separately in three experimental groups. The fourth group served as a control.

All drugs were injected subcutaneously in aqueous solution or suspension. Epinephrine was purchased in solution; phenoxybenzamine and propranolol were prepared daily in sterile physiological saline (0.9%). The drugs were administered as follows: epinephrine—500 μ g/kg body weight, repeated at 6-hr intervals; phenoxybenzamine—3 mg/kg body weight, repeated at 4-hr intervals; propranolol—7 mg/kg body weight, repeated at 3-hr intervals.

The doses selected were previously used in this laboratory and were known to keep the animal under the influence of the drug between injections (Longley *et al.*, 1968a, b).

Animals in each group were mated to one or two fertile males. Following mating and an intravenous injection of 2.5 mg LH (Kennelly & Foote, 1965), ten animals from each group were killed at either 24, 36, 48, 60, 72 or 84 hr p.c., after having received one of the drugs for a 12-hr period preceding death. The rabbits were killed with an overdose of sodium pentobarbital, the abdomen was opened and the reproductive tract was immediately frozen with isopentane cooled to the temperature of liquid nitrogen (Longley & Black, 1968). After thawing, the oviducts and uteri were removed and trimmed of their supporting mesentery. They were then fixed and cleared according to the method of Orsini (1962). The oviducts were stored individually in tubes of benzyl benzoate. The cleared reproductive tracts were placed in a glass boat with a millimeter scale etched on the bottom and examined with a dissecting microscope to determine the exact position of each ovum, which was then expressed as a percentage of the total oviduct length (infundibulum to uterotubal junction). Ova recovered from the uterus were considered to be 100% transported.

The number of CL on each ovary was recorded. The percentage recovery of ova was determined for each group by comparing the number of CL with the number of ova observed in the cleared oviducts and uteri.

A fourth group of sixty untreated rabbits served as controls and they were examined in the same manner as the experimental animals.

Statistical comparisons between treatments were determined by analysis of variance for a split-plot design. A program for this analysis (BMDO8V) was obtained from a Biomedical Computer Programs Manual (BMD), the

data tabulated, punched on IBM cards and subsequently run on a CDC 3600 computer (University of Massachusetts Computer Center). Following the analysis of variance, the F test and Duncan's New Multiple Range Test were utilized to test and locate significance (Steel & Torrie, 1960).

RESULTS

The ovum recovery rate for the total experiment was 96%. The rate of ovum transport in the control groups is presented in Table 1. By 24 hr p.c., the ova had traversed over 50% of the total oviduct length and were situated in the vicinity of the ampullary-isthmus junction. Ovum transport through the

Table 1. Distance of ovum transport in cleared oviducts and uteri of rabbits

Treatment	Hours p.c.					
	24	36	48	60	72	84
Control	54.1 (1.53)	62.0 (1.44)	69.0 (1.40)	71.3 (1.14)	79.3 (1.17)	95.5 (2.63)
Epinephrine	51.6 (2.53)	61.9 (2.50)	71.3 (1.99)	82.3 (3.10)	95.4 (1.90)	98.9 (1.10)
Phenoxybenzamine	48.9 (1.63)	60.5 (1.58)	62.9 (2.20)	68.8 (1.50)	77.4 (2.00)	96.8 (1.63)
Propranolol	50.6 (2.37)	60.0 (1.73)	65.4 (1.40)	74.9 (3.90)	76.8 (1.16)	95.1 (1.90)

Values expressed as mean % distance transported with S.E. in parentheses.

Table 2. Distance of transport for rabbit ova remaining in oviducts at 24, 36, 48, 60, 72 and 84 hr p.c.

Treatment	Hours p.c.					
	24	36	48	60	72	84
Control	54.1	62.0	69.0	71.3	78.4	77.0 (3)
Epinephrine	51.6	61.9	71.3	76.0 (8)*	81.1 (5)	81.5 (1)
Phenoxybenzamine	48.9	60.5	62.9	68.8	76.8	82.2 (5)
Propranolol	50.6	60.0	64.7	72.5	76.8	81.2 (5)

Values expressed as mean % distance transported.

* Group values representing fewer than ten animals with some ova remaining in oviducts.

isthmus was much slower, requiring about 2½ days. At 48 and 60 hr p.c., ova were located in about the same position. The majority of ova reach the uterus by 84 hr p.c. In one animal, however, some ova had reached the uterus by 72 hr p.c. As shown in Table 2, the position of control ova which had not entered the uterus by 84 hr p.c. was similar to that found at 72 hr.

Epinephrine did not significantly alter ovum transport at 24, 36 or 48 hr p.c. Epinephrine increased ($P < 0.01$) the rate of transport at 60 and 72 hr p.c. when most ova were found in the uterus. The position of those remaining in the oviduct was similar to that found in controls (Table 2).

Phenoxybenzamine inhibited ovum transport at 48 hr p.c. ($P < 0.05$), but did not alter ovum transport at 24, 36, 60, 72 or 84 hr p.c. Ovum transport

was similar in the phenoxybenzamine-treated groups at 36, 48 and 60 hr p.c. The positions of ova at other time periods were significantly different.

Propranolol did not alter ovum transport during any of the time periods investigated. Ovum transport in propranolol-treated animals at 60 and 72 hr p.c. was similar, while all other within-treatment comparisons differed with time. Although propranolol did not significantly alter the mean transport rate when compared to controls, some ova were transported to the uterus as early as 48 and 60 hr p.c.

Table 3. Results of Duncan's New Multiple Range Test on the four treatment means for rabbits killed at 24, 36, 48, 60, 72 and 84 hr p.c.

<i>Hours after coitus</i>	<i>Treatment means</i>			
24	(Ph)* 48.9	(Pr) 50.6	(E) 51.6	(C) 54.1
36	(Pr) 60.0	(Ph) 60.5	(C) 61.9	(E) 62.0
48	(Ph) 62.9	(Pr) 65.4	(C) 69.0	(E) 71.3
60	(Ph) 68.8	(C) 71.3	(Pr) 74.9	(E) 82.3
72	(Pr) 76.8	(Ph) 77.4	(C) 79.3	(E) 95.4
84	(Pr) 95.1	(C) 95.5	(Ph) 96.8	(E) 98.9

All means underscored by the same line are not significantly different from one another.

* (Ph) = phenoxybenzamine; (Pr) = propranolol; (E) = epinephrine; (C) = control.

Table 4. Number of corpora lutea and oviduct length for the right and left ovaries and oviducts of rabbits in each of the treatments

<i>Treatment</i>	<i>Mean number of corpora lutea</i>		<i>Mean oviduct length (cm)</i>	
	<i>Right</i>	<i>Left</i>	<i>Right</i>	<i>Left</i>
Control	4.25	4.07	12.93**	11.91
Epinephrine	4.57*	3.90	11.27**	10.29
Phenoxybenzamine	4.73	4.40	12.15**	11.26
Propranolol	5.00*	4.32	12.29**	11.45
Overall mean	4.64**	4.17	12.16**	11.23

* $P < 0.05$. ** $P < 0.01$.

Statistical comparisons of all four treatment means within each time period are shown in Table 3.

An analysis of variance incorporating all treatments and time periods was used to compare ovum transport in the left oviduct with that in the right. Ovum transport on the left side was faster ($P<0.01$) than on the right; this difference was apparent in most time periods for all four treatments.

The mean number of ovulations per rabbit was 8.8 (Table 4). The number of CL on the right ovary significantly ($P<0.05$) exceeded that on the left in the epinephrine- and propranolol-treated groups. There was no significant difference between the mean number of CL on the right and left ovaries in the control and phenoxybenzamine-treated groups. When all treatment means were combined, the numbers of CL for the right and left sides were 4.64 and 4.17, respectively. This difference was highly significant ($P<0.01$).

As can be seen from Table 4, the mean length of the right oviduct (12.2 cm) was significantly ($P<0.01$) greater than that of the left (11.2 cm).

DISCUSSION

The mean rate of ovum transport in the left oviduct exceeded that of the right in most of the time periods tested within the four treatments ($P<0.01$). This is in agreement with the data of Longley *et al.* (1968b), who reported similar findings utilizing the same clearing technique. The fact that the mean length of the right oviduct was 1.0 cm longer than the left might account for the difference.

It is apparent that the normal rate of ovum transport in the ampulla differs from that of the isthmus. This is in agreement with the findings of earlier studies (Harper, 1961) that the rate of movement is not uniform over the entire length of the oviduct. Ova traverse over 50% of the oviduct length by 24 hr p.c. (see Blandau, 1969 for complete reference).

It is generally believed that rabbit ova are retained in the vicinity of the ampullary-isthmic junction until about 48 hr p.c. and then enter the isthmus (Greenwald, 1959, 1961). Recently, the clearing method has been used and found to be a more accurate method for determination of ovum position (Longley & Black, 1968; Howe, 1970). In the present study, most ova had entered the ovarian end of the isthmus by 36 hr p.c.

Transport through the isthmus is extremely slow and appears to be dependent upon muscle contraction (Black & Asdell, 1958). Ovum transport was relatively linear until 48 to 60 hr p.c. when the ova were similarly located at 70% of the tubal length.

Transport through the uterotubal junction is rapid; only one ovum was found in this area. This is in agreement with the findings of Howe (1970) who, also, did not recover ova in this area. Although some ova were found in the uterus at 72 hr p.c., most of them entered between 72 and 84 hr p.c. Since the location of the few ova remaining in the oviduct at 84 hr was similar to the position of ova at 72 hr p.c., the majority of ova must traverse the last 20% of the tube rapidly. It appears, therefore, that the normal rate of ovum transport is regulated by the isthmus and uterotubal junction.

Adrenergic control of the rate of movement of ova through the oviduct is closely related to the morphology of the tube. Ovum transport is rapid in the ampulla where the innervation is least (Brundin & Wirsén, 1964; Brundin, 1965; Owman *et al.*, 1966).

Increased musculature and innervation of the isthmus is undoubtedly involved in slower transport of ova through this segment (Brundin, 1965). The results of our study agree with those of Longley *et al.* (1968b), Pauerstein, Fremming & Martin (1970) and Bodkhe & Harper (1971) who concluded that functional adrenergic innervation is modified by the endocrine state of the animal. Changes in the concentration of circulating steroid hormone following ovulation might explain the variable response to adrenergic drugs on ovum transport observed in this study. Since epinephrine retards ovum transport only when administered with oestrogen (Longley *et al.*, 1968b) and oestrogen-induced arrest of ova at the ampullary-isthmic junction can be antagonized with phenoxybenzamine (Pauerstein *et al.*, 1970), it appears that oestrogen increases the activity of α -adrenoreceptors preventing the ova from entering the isthmus. Norepinephrine, in the oviduct, is lower when ova are in the upper isthmus of the rabbit (Bodkhe & Harper, 1971). Oestrogen production is also reduced during gamete transport (Eaton & Hilliard, 1971).

The most noticeable neuroendocrine effect in the present study was the epinephrine-induced increase in ovum transport at 60 and 72 hr p.c. Epinephrine was ineffective at earlier time periods. Eaton & Hilliard (1971) reported that ovum transport occurs during an acute withdrawal of ovarian steroids, and that both oestrogen and progesterone levels begin to rise toward the end of transport and before implantation. Since the response of the oviduct to catecholamines appears to be enhanced by oestrogens (Longley *et al.*, 1968b), the suspected increase in oestrogen and progesterone toward the end of transport may account for the epinephrine effects at 60 and 72 hr p.c.

In the present study, phenoxybenzamine inhibited ovum transport at 48 hr p.c. Longley *et al.* (1968b) noted the same effect at 36 hr.

Although the stimulatory effect of the catecholamine is generally attributed to their α -activity (Brundin, 1965; Longley *et al.*, 1968b), it is not known why phenoxybenzamine (α -blocker) was ineffective in lowering the rate of ovum transport substantially below the controls of 60 and 72 hr p.c. The failure of propranolol to alter transport significantly does not necessarily mean that the β -activity noted in the rabbit oviduct by Martin, Ware, Crosby & Pauerstein (1970) may not contribute to the regulation of gamete transport.

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