Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4413–4416, September 1978
Cell Biology

Polycationic macromolecules inhibit cilia-mediated ovum transport in the rabbit oviduct

[cilia/poly(L-lysine)]

J. T. NORWOOD*, C. E. HEIN*, S. A. HALBERT†, AND R. G. W. ANDERSON*

* Department of Cell Biology, The University of Texas, Health Science Center at Dallas, Dallas, Texas 75235; and † Department of Biological Structure, University of Washington School of Medicine, Seattle, Washington 98195

Communicated by Saul Roseman, July 5, 1978

ABSTRACT **In both *in vitro* and *in vivo* experiments, polycationic macromolecules, such as poly(L-lysine), inhibited the transport of either surrogate or freshly ovulated cumulus masses across the oviduct epithelial surface without affecting the ciliary beat. Whereas transport across the fimbria *in vivo* was completely inhibited, transport down the ampulla was 3 to 7 times slower than normal. The effects of these polycations suggest that cilia-mediated ovum transport may involve the formation of transient adhesive bonds between the tip of the cilium and elements of the cumulus mass during each beat cycle of the cilium.**

Numerous studies have shown that once the ovum with its surrounding cumulus mass is discharged from the ovary, it is rapidly transported over the fimbrial surface of the oviduct and down the ampulla to the ampullar–isthmic junction (1). The ovum remains at the junction for several hours where the cumulus mass is removed; eventually, the denuded ovum travels through the isthmus to the uterus. Until recently, it has been generally accepted that the cilia of the epithelial cells that line the oviduct are responsible for movement of the cumulus over the fimbria into the ampulla and that the contractile activity of the smooth muscle cells in the wall of the ampulla is responsible for transporting the cumulus down the oviduct to the ampullar–isthmic junction (1–4). However, recent experiments by Halbert *et al.* (5, 6) have shown that pharmacological inhibition of smooth muscle contractility does not impair cumulus transport in the ampulla, which suggests that smooth muscle contractility may not be the primary propulsive force in this region of the oviduct. The effects of muscle activity alone on ovum transport in the ampulla have not been determined because of the unavailability of a method specifically to inhibit cilia activity.

The coordinate beating of cilia creates fluid currents over the oviduct epithelial surface, and these fluid movements have been proposed to be involved in moving the ovum (7, 8). If this is the mechanism of ovum transport, then the cumulus is a passive agent that is carried in a "stream" of fluid over the epithelial surface. The only way to interfere with this process would be to alter the beating of the cilia severely enough to eliminate the surface fluid currents.

Recently we reported that the portion of the rabbit oviduct ciliary membrane that covers the tip of the cilium has a high density of negative charges (9). By using polycationic ferritin as a specific electron-dense probe for anionic sites, we have shown that the probe binds to the tips of the cilia and that this binding is probably due to the presence of sialic acid-containing proteins at this site on the membrane. Evidence from other workers suggests that this might be a general property of ciliary membranes (10).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

The existence of negative charges on a portion of the ciliary membrane that would most likely interact with elements of the cumulus mass during ovum transport suggests that some type of electrostatic interaction between the cilia and the cumulus might be required for ovum transport. If there is such a requirement, regardless of the basic mechanism involved, treating the epithelial surface with agents that block the negative charge on the cilia membrane (e.g., cationic molecules that bind to the negative sites and thus reverse the charge) should impair ovum transport.

In this communication, we report on experiments designed to determine whether various cationic macromolecules retard or inhibit ovum transport. The effects of these agents were tested under both *in vitro* and *in vivo* conditions. Furthermore, in all trials, the effect of these agents on cilia beating was determined. The results of these experiments indicate that cationic macromolecules, which bind to anionic sites on the cilia surface membrane, inhibit ovum transport without detectably affecting the ciliary beat.

MATERIALS AND METHODS

***In Vitro* Experiments.** Both oviducts were removed from a one-litter doe that had been injected with a lethal dose of sodium pentobarbital (Abbott Laboratories, Chicago, IL). Each oviduct was placed immediately in oxygenated Earle's balanced salt solution (EBSS) (Grand Island Biological Co., Grand Island, NY) buffered with 0.02 M 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (Hepes) (Sigma Chemical, St. Louis, MO), pH 7.3, and maintained at 37°. Each oviduct to be tested was trimmed of excess fat and the wall was incised longitudinally to expose the epithelial surface. The oviduct was then pinned (epithelial surface up) to the bottom of a dental wax-coated dish by placing tungsten pins in the surrounding mesenteries. Thus, the ciliated epithelium of the entire fimbrial and ampullar portions of the oviduct was exposed for transport studies.

For ovum transport studies, boluses of chicken egg white stained with 5% methylene blue in EBSS and rinsed in 0.1 M Na phosphate buffer (pH 7.3) were used as surrogate cumulus masses. Studies by Halbert (S. A. Halbert, unpublished observations) have established that egg white is a suitable analogue of a normal cumulus mass. Pinacyanole-stained lycopodium spores were used, by the method of Gaddum-Rossé and Blandau (8), as an indicator of cilia-mediated fluid movements. To quantitate both surrogate cumulus mass movement and lycopodium movement, the stained material was gently placed on the surface of the oviduct, which was covered by buffered EBSS, and the time for the material to move a fixed distance was measured under a stereomicroscope. After the measure-

Abbreviations: EBSS, Earle's balanced salt solution; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

ment of the initial transport rate, the oviduct was washed with fresh EBSS and then immersed in EBSS that contained either poly(L-lysine) (M_r 80,000), poly(L-arginine), poly(L-glutamic acid) (all from Sigma Chemical, St. Louis, MO), native ferritin (electron microscopy grade, Polyscience, Warrington, PA), or polycationic ferritin (Miles Laboratories, Elkhart, IN). Transport rates were then measured as before.

The effect of poly(L-lysine) on ciliary beat frequency was measured by using the optical sensor technique of Blandau *et al.* (11). The beat frequency at 37° was measured on a randomly selected area of a 2 mm × 3 mm piece of oviduct placed in a rose chamber that contained modified Eagle's medium (Grand Island Biological Co., Grand Island, NY). The medium was then replaced with Eagle's medium that contained 0.1 mg of poly(L-lysine) per ml and the beat frequency was recorded from the same area.

A similar procedure was used to measure the contractile activity of segments of ampullar muscle in the presence and absence of poly(L-lysine) (0.1 mg/ml). These measurements were made with the technique of Blandau *et al.* (11).

***In Vivo* Experiments.** Freshly ovulated ova, each with a surrounding cumulus mass, were collected from the oviducts of donor animals 12 hr after the injection of 100 μg of luteinizing hormone (kindly provided by The National Institute of Arthritis, Metabolism and Digestive Diseases). These cumulus masses were stained in a weak solution of methylene blue in buffered EBSS. Rabbits for transport studies, which had received 100 μg of luteinizing hormone 12 hr before surgery, were anesthetized with methoxyflurane and prepared according to the technique of Blandau (4).

To measure fimbrial transport, we immersed the oviduct in buffered EBSS maintained at 37° and we placed the stained and washed cumulus mass on the fimbrial surface with a pasteur pipette. Transport rates were measured as described for the *in vitro* experiments. After the initial measurements, the EBSS was replaced with EBSS that contained 0.1 mg of poly(L-lysine) per ml, and the transport of a fresh cumulus mass was monitored.

We measured normal cumulus mass transport rates down the ampulla by determining the time it took for a cumulus to move from the ostium to the ampullar–isthmic junction in oviducts that were immersed in buffered EBSS. We used two procedures to measure the effects of poly(L-lysine) on ampullar transport. In one procedure, the oviducts were exposed to EBSS that contained 0.1 mg of poly(L-lysine) per ml, the cumulus mass was injected into the ostium, and the transport time to the ampullar–isthmic junction was measured. In the second procedure, the cumulus mass was allowed to move to the ampullar–isthmic junction in the presence of EBSS. Then with a syringe, which contained either EBSS or EBSS plus poly(L-lysine), attached to a 26 gauge needle, the oviduct was injected just below the ampullar–isthmic junction, and the cumulus was flushed back to the ostium portion of the ampulla. The needle was then removed from the oviduct and the transport time of the stained cumulus was measured.

RESULTS

***In Vitro* Trials.** We carried out a number of different trials to test the effects of polycationic molecules on ovum transport. At the beginning of each trial, the rate of transport for both the surrogate cumulus masses and the lycopodium was quantitated. In five experiments that involved five separate oviducts, the average initial surrogate cumulus transport rate was 0.10 ± 0.03 mm/sec, whereas the average lycopodium transport rate was 0.14 ± 0.04 mm/sec. However, upon the addition of any polycationic macromolecule, the transport of the cumulus masses was completely inhibited, but lycopodium transport was normal (Tables 1 and 2).

Table 1. A typical experiment that demonstrates the effects of poly(L-lysine) on surrogate cumulus mass transport and lycopodium transport

	Transport, mm/sec	
	Lycopodium	Surrogate ovum
Untreated oviduct	0.1	0.09
	0.08	0.09
	0.08	0.11
	0.075	0.08
	0.075	0.08
Poly(L-lysine) treated oviduct	0.075	0
	0.08	0
	0.13	0
	0.08	0
	0.09	0

All studies were carried out on freshly removed oviducts that were placed in oxygenated EBSS buffered with 0.02 M Hepes buffer, pH 7.3, at 37°. After the determination of initial transport rates, the EBSS was removed and fresh EBSS containing 0.1 mg of poly(L-lysine) per ml was added. We then determined transport rates for both lycopodium and surrogate cumulus masses (boluses of egg white stained with 5% methylene blue in EBSS and rinsed in 0.1 M Na phosphate buffer, pH 7.3) by measuring the time it took the objects to move a fixed distance. The five measurements represent trials to determine transport rates in five different regions of the oviduct (isthmus excluded). Fresh surrogate cumulus masses and lycopodium were used for each measurement.

Table 1 shows the results of a typical experiment to test the effects of poly(L-lysine) on the transport of both surrogate cumulus masses and lycopodium. Clearly, poly(L-lysine) (80,000 M_r) was a potent inhibitor of surrogate transport. Likewise, other polycationic macromolecules such as poly(L-arginine) and polycationic ferritin had similar inhibitory effects on surrogate transport (Table 2). On the other hand, neither poly(L-glutamic acid) nor native ferritin, which are anionic macromolecules, inhibited transport (Table 2). These results suggest that the polymer must have a positive charge to be inhibitory.

Lycopodium transport was normal in the presence of all the macromolecules tested; therefore, cilia beating was not affected by the polycations. To substantiate these observations, the ef-

Table 2. Effects of various macromolecules on surrogate cumulus mass transport and lycopodium transport

Treatment	Concentration, mg/ml	Index of Surrogate transport	Index of Lycopodium transport
Balanced salt solution	—	1.0	1.0
Poly(L-arginine)	0.1	0	NM
Poly(L-lysine)	0.1	0	1.21
Poly(L-glutamic acid)	0.1	1.17	1.16
Native ferritin	0.32	0.88	0.95
Polycationic ferritin	0.32	0	0.93

The transport rate for both surrogate cumulus masses and lycopodium was determined in freshly isolated oviducts prepared in oxygenated EBSS buffered with 0.02 M Hepes, pH 7.3 at 37°. The buffered EBSS was then replaced with buffered EBSS that contained the macromolecule. An average of 7 measurements for surrogate cumulus transport and 15 measurements for lycopodium transport was made for each treatment. Data are expressed as the ratio of the transport rate of treated oviducts to the transport rate of untreated oviducts. NM, not measured.

fects of poly(L-lysine) (80,000 M_r) on ciliary beat frequency were tested. Taking the recordings from the same area of oviduct before and after treatment with poly(L-lysine), the beat frequency averaged 20 Hz and 19 Hz, respectively.

The inhibitory effects of poly(L-lysine) (80,000 M_r) were not reversible even after repeated washings with fresh EBSS. However, if low molecular weight poly(L-lysine) (3700 M_r) was used, the inhibitory effects could be partially reversed (data not shown).

In other trials, the effect of trypsin, an enzyme that removes both the negative charge and the glycocalyx from the tips of the cilia (9), on surrogate cumulus mass was tested. This enzyme caused a 60% reduction in the transport rate without having any effect on the transport of lycopodium. These tests represent an independent method for assessing the role of the ciliary tip glycocalyx in cumulus transport.

***In Vivo* Trials.** Having established that polycationic macromolecules inhibit the transport of surrogate cumulus masses *in vitro*, we next carried out similar experiments on oviducts *in vivo* using normal cumulus masses from a donor animal. Once the oviduct was prepared, it was immersed in buffered EBSS and maintained at 37°. When the cumulus masses were placed on the fimbrial surface, they adhered tenaciously and movement toward the ostium began immediately. The transport rate averaged 0.06 ± 0.03 mm/sec (Table 3). However, when the EBSS was replaced with balanced salt solution that contained poly(L-lysine) (80,000 M_r), the cumulus masses would no longer adhere to the fimbrial surface and transport across the fimbria was completely abolished. Similarly, polycationic ferritin also inhibited fimbrial transport (data not shown). On the other hand, cumulus masses that were pretreated with poly(L-lysine) (0.1 mg/ml) were transported normally by fimbriae that had not been exposed to the polycationic molecule.

In contrast to the complete inhibition of fimbrial transport of normal cumulus masses, poly(L-lysine) did not abolish transport down the ampulla. However, this cation did cause a decrease to one-third to one-seventh in the rate of transport (Table 3). Poly(L-lysine) did not significantly alter the contractile rate of the ampullar musculature [10–15 contractions/min in the absence and presence of poly(L-lysine) at 0.1 mg/ml]; therefore, the decreased transport rate was probably related to its effect on cilia-mediated movement of the cumulus.

Table 3. Effects of poly(L-lysine) on cumulus mass transport rates across the fimbria and down the ampulla

	Rate of ovum transport, mm/sec	
	Across the fimbria	Down the ampulla
Untreated	0.11 ± 0.01	0.13 ± 0.02
	0.04 ± 0.01	0.06 ± 0.03
	0.03 ± 0.003	
Poly(L-lysine)	0	0.02 ± 0.004
	0	0.02 ± 0.01
	0	

Oviducts of animals treated with luteinizing hormone 12 hr before surgery were prepared according to the method of Blandau (4). Normal transport rates (an average of three measurements per experiment) were determined in oviducts that were immersed in EBSS. However, the effects of poly(L-lysine) were determined after the immersion of the oviduct in a solution of EBSS that contained 0.1 mg of poly(L-lysine) per ml (an average of three measurements per experiment). ± indicates SD. For effect of treatment, $P < 0.05$.

DISCUSSION

These experiments demonstrate that cilia-mediated transport of either a surrogate cumulus mass or a normal ovum with cumulus mass can be dissociated from the normal beating activity of the cilia. One possible explanation of these results is that polycations form crosslinks between negative charges on the tips of the cilia and negative charges on the surface of the cumulus that result in the cumulus being bound so tightly to the oviduct surface that the cilia can no longer move the mass. Two observations suggest that this is not the case: (*i*) Pretreatment of the oviduct with poly(L-lysine) leads to inhibition of ovum transport, but pretreatment of the normal cumulus mass has no effect. One would expect that pretreatment of either surface with the polycation would have similar effects if the molecules were crosslinking the two surfaces. (*ii*) A more important observation is that the polycations reduced the adhesive interactions between the two surfaces as measured by the ability of the normal cumulus to stick to the fimbrial surface. The latter observation is consistent with the idea that adhesive interactions between the tips of the cilia and the gelatinous matrix of the cumulus are necessary for transport to occur. Because only polycations were effective inhibitors, it is possible that the high density of negative charges on the tips of ciliary membrane must be present to form these adhesive bonds. On the other hand, when these macromolecules bind to the ciliary tips, they may mask membrane receptors that ordinarily function to bind specific molecular constituents of the cumulus and form adhesive bonds. The present data do not distinguish between these two possibilities.

Although we favor the adhesive interaction hypothesis, certainly other explanations must be considered. One possibility is that the polycations in some way weaken the force of the ciliary beat so that, even though a normal beat frequency is maintained, the force generated is not sufficient to move the cumulus. Another possibility under consideration is that the cations and the cumulus act synergistically to inhibit the beating of the cilia.

The effects of poly(L-lysine) on cumulus transport down the ampulla must be interpreted with caution. Based on the *in vitro* experiments, the simplest explanation is that cilia-mediated movement of the cumulus was abolished but that the rhythmic contractions of the muscle were able to transport the mass at a much reduced rate. The reduced transport rate was not due to an inhibitory effect of the poly(L-lysine) on muscle contractility. Because inhibition of muscle contraction alone does not affect the rate of ovum transport (5, 6), it may be that normally the cilia provide the primary propulsive force for ovum movement down the ampulla.

Therefore, in the rabbit, both the cilia and musculature of the ampulla are capable of independently transporting the ovum to the ampullar–isthmic junction. Although interference with cilia-mediated transport would impair the delivery of the ovum to the ampulla if the ovum were to enter the ostium, the musculature would be able to move it down the ampulla, albeit at a much reduced rate. This redundancy in the ovum transport system may explain why female patients with Kartagener syndrome, a genetic disease in which the cilia are immotile, are fertile (12).

If, as the data suggest, cilia-mediated transport of the cumulus involves transient adhesive interactions between the ciliary membrane and the cumulus mass, then we must change our concept of how cilia function in transport processes. Such a mechanism may also be responsible for mucus transport in the respiratory system. We must now consider the possibility that the creation of fluid currents by beating cilia is not sufficient for the transport of various objects across a ciliated epithelium.

We would like to thank Dr. R. J. Blandau for making his laboratory available to teach us the ovum transport techniques. This work was supported by Grants HD 08987, HD 03752, and HD 10988 from the National Institutes of Health.

1. Blandau, R. J. (1973) in *Handbook of Physiology*, eds. Greep, R. O. & Astwood, E. B. (Williams & Wilkins, Baltimore, MD), Vol. 2, Part 2, pp. 153–163.
2. Harper, M. J. K. (1961) *Proc. Int. Congr. Anim. Reprod.* **4**, 375–380.
3. Blandau, R. J. (1969) in *The Mammalian Oviduct*, eds. Hafez, E. S. E. & Blandau, R. J. (University of Chicago Press, Chicago, IL), pp. 129–162.
4. Blandau, R. J. (1971) in *Methods in Mammalian Embryology*, ed. Daniel, J. C., Jr. (Freeman, San Francisco, CA), pp. 1–14.
5. Halbert, S. A., Tam, P. Y. & Blandau, R. J. (1976) *Science* **191**, 1052–1053.
6. Halbert, S. A., Tam, P. Y., Adams, R. J. & Blandau, R. J. (1976) *Gynecol. Invest.* **7**, 306–320.
7. Dirksen, E. R. & Satir, P. (1972) *Tissue Cell* **4**, 389–404.
8. Gaddum-Rossé, P. & Blandau, R. J. (1973) *Am. J. Anat.* **136**, 91–104.
9. Anderson, R. G. W. & Hein, C. E. (1977) *J. Cell Biol.* **72**, 482–492.
10. Kuhn, C. & Engleman, W. (1978) *Cell Tissue Res.* **186**, 491–498.
11. Blandau, R. J., Boling, J. L., Halbert, S. A. & Verdugo, P. (1975) *Gynecol. Invest.* **6**, 123–145.
12. Afzelius, B. A., Camner, P. & Mossberg, B. (1978) *Fertil. Steril.* **29**, 72–74.