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Control of ciliary movement in mammalian oviductal ciliated cells

Control del movimiento ciliar en células ciliadas del oviducto de mamíferos

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In vertebrates, ciliary-driven flow plays an important role in the clearance of the airways, and in the transport of gametes in the oviduct. However, in spite of the importance of the cilium as a mechanical effector, the chemical signals that control ciliary movement remain vastly unknown. In this paper we review our work and current knowledge of the hormonal signals and intracellular mechanisms that control ciliary movement in mammalian ciliated cells. Our observations indicate that catecholamines can directly stimulate oviductal ciliated cells, and ovarian steroids can modulate the responses of ciliated cells. We have also shown that ciliated cells respond to ATP and prostaglandins and that changes in intracellular calcium play a role in the coupling of hormonal stimulation in oviductal ciliated cells.

INTRODUCTION

Understanding tubal functions is important for the prevention and management of upper genital tract infections, ectopic pregnancy and tubal infertility and possibly for the establishment of new contraceptive alternatives. However, in spite of more than a century of research, most of our current knowledge of the oviduct is, if not empirical, at least largely phenomenological and, certainly, not sufficient to predict the function of the Fallopian tube in any single species (Verdugo, 1985).

The function of the oviduct arises from a complex and poorly understood interaction between several cellular effectors, namely: Smooth muscle of the tubal wall, and stromal, secretory and ciliated cells of the tubal mucosa. Although there is evidence indicating that ciliary action in the oviduct is probably one of the most important components of a highly redundant mechanism that secures the transport of gametes (Verdugo *et al.*, 1980a; Verdugo, 1982) the functional control of oviductal ciliated cells in mammals remain vastly unknown. Here we review our current knowledge of the hormonal signals, and intracellular mechanisms that control ciliary movement in mammalian cells.

REDUNDANCY AND SPECIES VARIATION IN TUBAL FUNCTION

Does cilia play a role in tubal transport? Perhaps the most significant implication of the results of the microsurgical approach to understand tubal function is the strong reaffirmation of the idea that gamete transport in the tube must be a redundant system of high order, where two, three or more functionally convergent subsystems are hierarchically stacked together to virtually guarantee a high reliability of operation (Verdugo *et al.*, 1980a). A direct corollary of this principle is that different effectors, or different mechanisms of tubal transport, or else, different segments of the tube, may be sufficient yet not necessary for successful tubal function. Thus, the observation that reproduction can still exist in oviducts with paralyzed cilia cannot necessarily be interpreted as evidence that cilia do not have a role in ovum transport. (Afzelius *et al.*, 1978). Conversely, the observation

that in oviducts with arrested muscle contractions, ciliary action can still carry the egg across the ampulla, cannot be interpreted as evidence that muscle contraction has only a secondary role in ampullary transport (Halbert *et al.*, 1976). Even the recent observation that some segments of the oviduct can be microsurgically excised without detriment to reproduction cannot be interpreted as proof of the lack of a functional role of any particular segment in tubal physiology (Winston, 1980). Therefore in spite of the uncertainties on how muscle contractions and ciliary and secretory activity are integrated to move the gametes, the neurohormonal mechanisms that regulate tubal effectors remain a most important issue in tubal physiology.

Variations in tubal function among species can arise from slight differences in the timing of the hormone control systems that modulate effector cells and from differences in the type and number of receptors found in homologous effector cells. Therefore, any attempt to develop a strategy to investigate tubal function in mammals must take into consideration that despite a similar anatomy of the tubes and the presence of the same mechanical effectors across the species, the particular effector response and timing of hormonal release are critically adapted to the reproductive plan of each species. Thus clear differences in the physiologic programming and/or effector responses that control tubal gamete transport exist among the mammalia (Verdugo, 1985).

HORMONAL CONTROL OF CILIARY MOVEMENT IN THE OVIDUCT

Although the physiology and pharmacology of oviductal smooth muscle have received a great deal of attention in the past (for review see Verdugo, 1985), the study of oviductal cilia on the other hand, has been rather limited. A few investigations have addresses the problem of ciliary current by studying the direction of mucociliary flow in the slit open oviduct of a variety of species, but without regard to hormonal regulation of ciliary function. Several very revealing investigations have focused on the electronmicroscopy of tubal epithelia and the effect of hormonal influences on the density of ciliated cells. However, evidence on the functional control of oviductal ciliated cells in mammals is rather limited. Various densities of ciliated cells have been found in the mucosa that lines the convoluted internal surface of the oviduct in all vertebrate animals. It is now well established that, with some few species variations, estrogen influences the presence and density of cilia in the mucosa of the oviduct. In some species such as the rabbit, castration can lead to complete deciliation that can be reversed by exogenous estrogen (Frenko, 1954). The same trophic effect of estrogen can be observed in the oviduct of immature rhesus monkeys, where estrogen can strongly increase the density of cilia (Allen, 1928).

Although in some primates there are cyclic variations in the density of oviductal cilia in relation to their estrous cycle (Joachimovits, 1935; Brenner, 1967), in humans the observed changes seem to affect more the height of the ciliated epithelium than the number of cilia. The oviductal epithelium is low (10 μm) in periods of progesterone domination; its height doubles during estrogen domination; and it shows signs of atrophy in late menopausal state (Westman, 1930; Fredericsson, 1959; Andrews, 1951; Gaddum-Rosse *et al.*, 1975).

The involvement of oviductal cilia in gamete transport was postulated very early (Lim and Chao, 1927); yet it continued to be a highly speculative and controversial issue until recently when direct evidence indicated that, in the absence of muscle contractions, ciliary activity is sufficient to drive the ovum throughout the ampulla of the rabbit (Halbert *et al.*, 1976). Although ciliary action is sufficient to power tubal transport, it certainly does not seem to be essential since individuals suffering from Immotile Cilia Syndrome have been shown to undergo normal reproduction (Afzelius *et al.*, 1978). These findings have been subjected to controversial interpretations due to the

preconceived idea that the role of muscle and ciliary actions in the oviduct are mutually exclusive. This apparent paradox can be explained on the basis of the existence of redundancy in oviductal function, i.e., muscle contractions and ciliary movement might not be both necessary yet each one alone could be sufficient to drive the transport of gametes (Verdugo *et al.*, 1980a).

In primates, as in most other mammalian species, cilia beat in a pro-uterine direction throughout the different segments of the tube (Gaddum-Rosse and Blandau, 1976). However, the present understanding of the physiology and pharmacology of oviductal ciliated cells is meager and the significance of many of the available data is uncertain. The application of yet unvalidated methods to detect ciliary movements and the presence of mucus-secreting cells in the oviductal mucosa make it difficult to ascertain unequivocally the significance of much of the published material. For instance, photoelectric methods that have been used in the past to detect ciliary movements (Mercke *et al.*, 1974; Westrom *et al.*, 1977; Dalham and Rylander, 1962) have not yet been objectively validated, and in some instances have been shown to be inaccurate (Naitoh and Kaneko, 1973). On the other hand, hormonal actions on ciliated cells can not be unequivocally established by measuring ciliary movements in the oviduct, since hormones can affect ciliary activity and/or secretory activity producing changes in the rheological properties of the secreted mucus and thereby changing indirectly ciliary movements due to variations on the viscous load on the ciliated cells. For example, the frequency of ciliary beat in various sections of the human and rabbit oviduct varies during different periods of the sexual cycle (Borell *et al.*, 1957; Westrom *et al.*, 1977). The interpretation of these data with regard to the site of hormonal action can be equivocal since hormonal effects taking place in either or both, ciliated or secretory cells, can result in a change of ciliary activity.

High speed cinematography was for many years the only reliable method to measure ciliary movement. However, the large investment of time and money involved in this method was discouraging. Also, in order to have reasonable resolution to measure the motion of cilia by this method, it is necessary to use rather large microscopic magnifications, which limits severely the number of cells that can be interrogated in any particular experiment and thereby weakened the statistical significance of experimental results. Other methods to measure ciliary movements, i.e., stroboscopic techniques and Rylander's photometric monitoring, can be unreliable when cilia beating is asynchronous (Naitoh and Kaneko, 1973; Dalham and Rylander, 1962). The more recent application of laser light as the carrier wave in Doppler spectroscopy provides a simple accurate, and reproducible way to measure ciliary movement both *in vitro* an *in vivo* (Lee and Verdugo, 1976; Verdugo and Golborne, 1988).

DEVELOPMENT OF A MUCUS-FREE TISSUE CULTURE METHOD TO GROW MAMMALIAN EPITHELIAL CILIATED CELLS

Ciliary activity depends on both the fluidic load on the cilia and the regulatory influences controlling the ciliated cell. Therefore, the interpretation of experiments addressing the control of ciliated cells can be equivocal unless the fluidic load on the cilia is known and controlled. Since mucus exerts an unknown and uncontrolled load on cilia, its presence is a major source of uncertainty in experiments investigating the funcional control of oviductal ciliated cells. Because of this reason *in vivo* or *in vitro* studies on the control of ciliary movement using oviductal mucosa which are the vast majority, can be equivocal. The presence of mucus has indeed hampered severely previous attempts to unequivocally identify the hormonal messages that control ciliated cells in mammals.

To overcome this problem we implemented a tissue culture method (Verdugo, *et al.*, 1980b) adapted from a technique originally introduced by Rumery *et al.* (1970) to grow oviductal ciliated cells

in culture. The uniqueness of this preparation is that it yields monolayers rich in ciliated cells and almost completely devoid of mucus secreting cells (Figure 1). The application of both the laser Doppler and tissue culture methods permitted us to rapidly advance our investigations on the role of ions and hormones and their mechanism of action in the control of ciliary movements in mammalian ciliated cells *in vitro*.

Fig. 1: Scanning electron micrograph of ciliated cells from the rabbit oviduct grown *in vitro*. The cells are flat, with 100-150 cilia positioned in one region and the rest of the cell surface is covered with microvilli (Mag. 1300X).

THE EFFECT OF SYMPATHOMIMETIC AGONISTS

A variety of hormonal and pharmacological influences, including catecholamines, can alter the rate of oviductal gamete transport and critically interfere with reproduction (Polidoro *et al.*, 1975; Hodgson and Eddy, 1975; Verdugo, 1985). To understand how hormonal messages are transduced and integrated by the effectors of the tube, we have studied the effect of zinterol, a nonoxidable β-adrenergic agonist (Villalón and Verdugo, 1982) and phenylephrine, an α-adrenergic agonist (Villalón and Verdugo, 1989), on the frequency of ciliary beat in mucus-free tissue culture of ciliated cells from the rabbit oviduct. Our results indicated that oviductal ciliated cells are responsive to α and β adrenergic stimulation. Figures 2 and 3 show a dose response curve for both agonists. The increase in the frequency of ciliary beat takes place at concentrations of α and β agonists that are equivalent to or lower than the concentration of catecholamines found in the rabbit oviduct (Brundin 1965). The observation that the increase in the frequency produced by α and β adrenergic agonists can be prevented by the adrenergic blockers phenoxybenzamine and propanolol respectively, demonstrate the receptor-specificity of these responses.

To appreciate the functional significance of these findings, it is important to consider that in the rabbit, as well as in primates, the oviduct is densely innervated by sympathetic terminals, and its content of catecholamines has been found to vary during the sexual cycle due to estrogen-progesterone control (Brundin, 1965; Owman *et al.*, 1976).

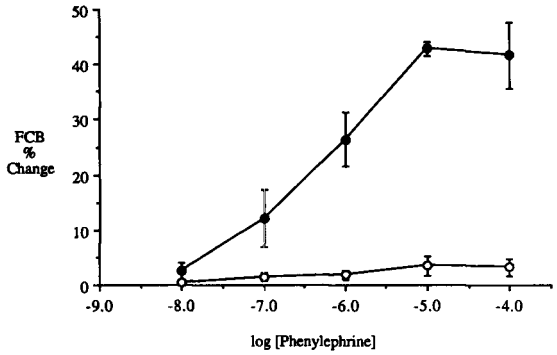

Fig. 2: Dose-response relationship of the effect of the α-adrenergic agonist phenylephrine, on the frequency of ciliary beat (FCB) expressed as percent of change over control values (filled circles). The response to phenylephrine after alpha-blockage with 10 μM of phenoxybenzamine is shown in open circles. Each point represents the average ± SEM of 6 experiments.

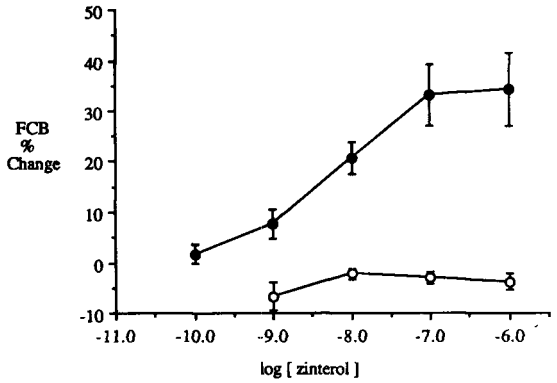

Fig. 3: Effect of the β-adrenergic agonist zinterol on the frequency of ciliary beat (FCB) of oviductal ciliated cells before (filled circles) and after β-blockade (open circles) by 50 μM propanolol. Each point is the average frequency ± SEM of a total of 6 experiments.

The mammalian oviduct contains receptors for estrogen and progesterone, and it is known that these hormones play an important role in the regulation of tubal function, including ovum transport (Pino *et al.*, 1982; Verhage *et al.*, 1980; Harper, 1988; Verdugo, 1985). There is evidence that estrogen and progesterone can regulate the catecholamine content in the oviduct (Helm, 1981), modulate the response of smooth muscle to catecholamines (Moawad *et al.*, 1977), affect the morphology of the tubal epithelium (Brenner, 1973); and influence oviductal secretions (Jansen, 1978, 1980). Preliminary results indicated that steroids can also affect ciliary activity in the oviduct (Villalón and Verdugo, 1983). In our studies we observed that estrogen, progesterone or a combination of both hormones did not show a direct effect on the frequency of ciliary beat, however they did enhance the response to zinterol when either hormone was added to the culture medium. When both hormones were present in the medium the response to zinterol was abolished.

Our observations indicate that steroids might not directly affect the rate of ciliary beating but they can modulate the effect of catecholamines providing valuable information on the signals that control ciliary activity and its role in oviductal transport.

EFFECT OF PROSTAGLANDINS

There is considerable evidence, obtained from several species, that oviductal tissue contains prostaglandins and is sensitive to their actions (Harper, 1988). Prostaglandins, PG $F_{2\alpha}$, PG E_1 and PG E_2 stimulate the frequency of ciliary beat of cells in culture (Verdugo *et al.*, 1980b; Villalón and Verdugo, 1990). Figure 4 shows a dose response curve of the stimulatory effect of PG $F_{2\alpha}$ on cultured ciliated cells from the rabbit oviduct. The stimulatory effect observed at low concentrations (10 nM range) of prostaglandins could have special significance, since similar concentrations have been found in the wall of the oviduct (Saksena and Harper, 1975) and have been implicated in the regulation of ovum transport (Aref *et al.*, 1973).

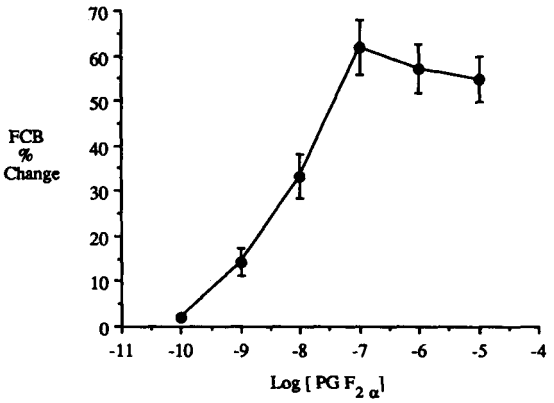

Fig. 4: Dose-response relationship of the stimulatory effect of PG $F_{2\alpha}$ on the frequency of ciliary beat of rabbit oviduct ciliated cells in culture. Notice that a statistically significant increase in the frequency is observed at concentrations as low as 1 nM.

EFFECT OF EXTRACELLULAR ATP

The presence of purinergic receptors in a variety of cellular effectors has been well documented (Burnstock, 1981). Since Burnstock first postulated the existence of a purinoceptor, two types of purinergic receptors have been proposed: P_1 and P_2 receptors, which are preferentially activated by adenosine and ATP respectively (Burnstock, 1978).

Previous studies have shown that ATP can stimulate ciliary activity in the oviduct of the salamander (Murakami *et al.*, 1978), it can also increase mucociliary transport of the pharyngeal mucosa of the frog (Vorhaus and Deyrup, 1953) and stimulate human nasal cilia (Forrest *et al.*, 1979). However all these effects were observed in intact mucosa and in the presence of mucus and can not be unequivocally interpreted as a direct purinergic action on ciliated cells. 100 μM ATP can strongly increase the frequency of ciliated cells in culture, devoid of mucus secreting cells (Figure 5). The finding that the stable ATP analog AMP-PCP can also stimulate ciliary movement is an indication that the stimulatory effect of ATP is not dependent upon dephosphorylation but probably is an ATP-receptor mediated action.

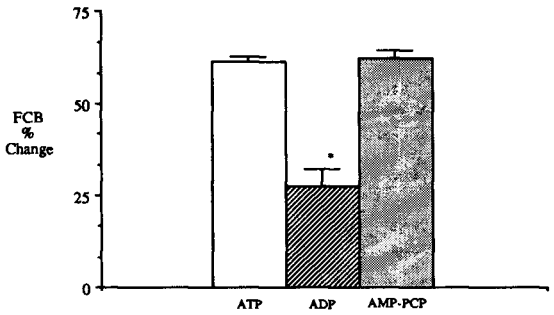

Fig. 5: Effect of 100 μM Adenosine 5'-triphosphate (ATP), Adenosine 5'-diphosphate (ADP), or β, γ-Methyleneadenosine-5' -triphosphate (AMP-PCP) on the frequency of ciliary beat (FCB) expressed as % change over basal values. The increase on the frequency of ciliary beat after the addition of ADP is significantly less ($p < 0.05$) compared to the ATP or AMP-PCP response. No differences are observed between the responses to ATP and AMP-PCP.

THE ROLE OF CALCIUM IN THE REGULATION OF CILIARY ACTIVITY IN MAMMALIAN CILIATED CELLS

It has been shown that calcium is involved in the stimulus-response coupling in a variety of cells including muscle and secretory cells (Rasmussen *et al.*, 1984; Szent-Gyorgyi, 1976; Rubin, 1974). Calcium has been implicated in the control of ciliary motion in different species (Eckert & Brehm, 1979; Satir *et al.*, 1976; Naitoh & Eckert, 1974). In the presence of 2 x 10^{-8} M Calmodulin, demembranated axonemal models of ciliated cells of the rabbit trachea can also exhibit Ca-dependent variations of beat frequency upon reactivations with ATP (Verdugo *et al.*, 1983).

In mammalian ciliated cells the stimulatory effect of prostaglandins has been shown to be independent of extracellular calcium and it was thought to be coupled to the release of intracellular calcium (Verdugo, 1980).

Measurements of intracellular calcium with the fluorescence probe FURA-2 provided the first direct evidence that the stimulatory effects of ATP and PG $F_{2\alpha}$ were mediated by an increase in intracellular calcium (Villalón *et al.*, 1989; Villalón and Verdugo 1990). The finding that the blockage of Ca-channels by $LaCl_3$ can prevent both the increase in intracellular calcium and the ciliostimulation produce by ATP, but not that produced by PG $F_{2\alpha}$, was in agreement with our previous observations (Verdugo, 1980). It suggested that while ATP may act by increasing the influx of Ca^{++} across the cell membrane, PG $F_{2\alpha}$ must induce the release of Ca^{++} from intracellular sources. Whichever the source of Ca^{++} the net result in both cases is that an increase of cytosolic [Ca^{++}] couples the ciliostimulation produced by ATP and PG $F_{2\alpha}$.

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