brief communication

Stimulus-response coupling in mammalian ciliated cells

Demonstration of two mechanisms of control for cytosolic [Ca^{2+}]

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ABSTRACT Changes of cytosolic [Ca^{2+}] have been proposed to couple stimulation of ciliary movement, however, quantitative measurements of fluctuations of intracellular free [Ca^{2+}] associated with stimulation of ciliated cells have not been investigated.

In primary cultures of rabbit oviductal ciliated cells, the stimulation of ciliary activity produced by micromolar concentrations of adenosine triphosphate (ATP) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was associated with a transient increase of intracellular [Ca^{2+}]. Whereas the increase of cytosolic [Ca^{2+}] and beat frequency produced by ATP were inhibited by the Ca-channel blocker $LaCl_3$, the rise of cytosolic [Ca^{2+}] and frequency of ciliary beat produced by $PGF_{2\alpha}$ was not affected by $LaCl_3$.

These results are the first direct demonstration that fluctuations of cytosolic [Ca^{2+}] are associated with increased ciliary beat frequency in mammalian epithelial cells. The present findings suggest two different calcium-dependent mechanisms for stimulus-coupling in ciliary epithelium: ATP acting via purinergic receptor coupled to transmembrane influx of Ca^{2+}, and $PGF_{2\alpha}$ acting via receptor-mediated release of intracellular sequestered Ca.

INTRODUCTION

Direct evidence to validate the role of calcium in the coupling of physiologic stimulation in ciliated cells, other than the avoidance response in *Paramecium caudatum* (Eckert and Brehm, 1979), has been only preliminarily investigated (Villalón et al., 1988; Sanderson and Dirksen, 1988). Epitheliary ciliated cells of the rabbit oviduct provide an excellent model to study stimulus-response coupling as they are known to react to hormonal and chemical transmitters, including prostaglandins, β-adrenergic agonists, and ATP (Verdugo et al., 1980; Villalón and Verdugo, 1982; Villalón et al., 1988). In the present study, exogenous ATP and prostaglandin were used to investigate two different stimulus-response coupling mechanisms in which fluctuations of cytosolic [Ca^{2+}] appear to provide the message that initiates stimulation of beat frequency.

MATERIALS AND METHODS

Primary cultures of mucus-free ciliated cells were grown in monolayers for 5–7 d in Rose chambers containing Eagle's medium with 10% horse serum (Verdugo et al., 1980). The frequency of ciliary beat was monitored and recorded by dynamic laser scattering spectroscopy (Lee and Verdugo, 1976).

Rose chambers containing the cultured cells were set in an inverted microscope (Reichert Scientific Instruments, Buffalo, NY) adapted for laser spectroscopy. The beam of a 2-mW He-Ne laser was attenuated and collimated to illuminate an area of 10^4 μm^2 containing ~5 × 10^3 cilia. The Doppler-shifted light scattered by the moving cilia was collected by the objective lens aperture of the microscope at an angle of 30° and detected with a photomultiplier tube. A detailed description of the instrumentation has been published elsewhere (Verdugo et al., 1980; Villalón and Verdugo, 1982; Verdugo and Golborne, 1988). The spectral structure of the scattering fluctuations was processed on line by a fast Fourier transform (FFT) digital spectrum analyzer designed in our laboratory. The power spectrum of the beat frequency was recorded on an *x-y* plotter (Fig. 3, *inset*).

The effect of 100 μM ATP and 14 μM $PGF_{2\alpha}$ on intracellular [Ca^{2+}] and beat frequency was investigated in 26 tissue cultures. The Ca-channel blocker $LaCl_3$ (0.6 mM) was used in 10 tissue cultures to ascertain the involvement of Ca channels in the observed variations of beat frequency and intracellular [Ca^{2+}].

Ciliated cells were first equilibrated in Hanks' solution at 37°C for a control period of 15 min while ciliary activity was continuously monitored. Ciliary rate and cytosolic [Ca^{2+}] were monitored for 15 min after infusion of the agonists into the Rose chamber. The preparation was then washed to study the reversibility of the stimulatory effect.

Measurements of cytosolic [Ca^{2+}] were performed using Fura-2 after the method described by Grynkiewicz et al. (1985). Ciliated cells were loaded with Fura-2 by incubating the cultures for 1 h at 37°C in Hanks' solution containing 1 μM Fura-2AM. The preparation was excited with 347- and 380-nm wavelengths, and changes in fluorescence emission were monitored at 500 nm before and after stimulation with the agonists. A 470-nm cut-off filter was used to reduce reflected light.

RESULTS AND DISCUSSION

The time course of the effect of 100 μM ATP on intracellular [Ca^{2+}] and on the frequency of ciliary beat

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are illustrated Fig. 1. Whereas the increase in intracellular [Ca^{2+}] persists only for ~150 s, the stimulation of beat frequency lasts for as long as the cells remain exposed to exogenous ATP. Stimulation ceased as soon as ATP was washed off the preparation.

Fig. 2 illustrates the corresponding effects of 14 μM $PGF_{2\alpha}$ on intracellular [Ca^{2+}] and on beat rate. Here again, after a brief transient increase in cytosolic [Ca^{2+}] the agonist produced a prolonged increase of beat frequency. The effect of $PGF_{2\alpha}$ was also reversible but only after 30–40 min after its removal from the bathing medium.

Variations of intracellular [Ca^{2+}] enable a broad range of cellular responses including excitation-contraction coupling, excitation-secretion coupling, and the characteristic changes associated with fertilization in eggs (Rasmussen et al., 1984; Rubin, 1974; Szent-Gyorgyi, 1976; Jaffe, 1983). In ciliated cells, experimental manipulations of intracellular and extracellular [Ca^{2+}] can produce changes in ciliary and flagellar motion in several species ranging from protozoa to vertebrates (Naitoh and Kaneko, 1973; Murakami and Takahashi, 1975; Satir, 1975; Lee and Verdugo, 1976; Lee et al., 1976; Verdugo et al., 1977; Eckert and Brehm, 1979; Brokaw, 1979; Verdugo, 1980; Gibbons and Gibbons, 1980; Tamm and Tamm, 1981; Tash and Means, 1982; Hard and Rieder, 1983; Stommel and Stephens, 1985).

Also, when demembranated-axonemal-models of epitheliary cilia, are reactivated with ATP, the beat frequency can be modulated by nanomolar changes of [Ca^{2+}] if calmodulin is present in the bathing solution (Verdugo et al., 1983).

Changes of fura-2 fluorescence emission spread over neighboring cells after mechanical stimulation of tracheal

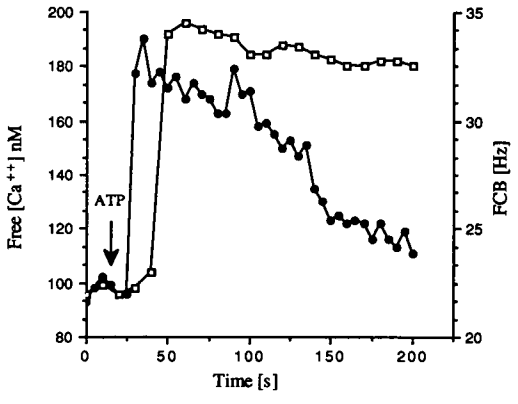

FIGURE 1 Time course of the effect of 100 μM ATP on frequency of ciliary beat (FCB, *open squares*) and cytosolic [Ca^{2+}] (*solid circles*) in tissue-cultured monolayers of epitheliary ciliated cells of the rabbit oviduct. Measurements of FCB and fluorescence were conducted every 10 and 5 s, respectively.

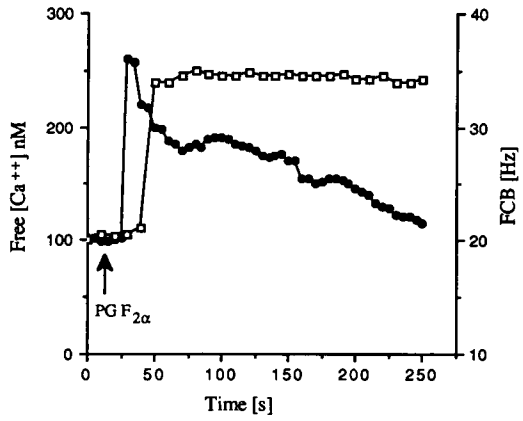

FIGURE 2 Time course of the effect of 14 μM $PGF_{2\alpha}$ on the frequency of ciliary beat (FCB, *open squares*) and intracellular [Ca^{2+}] (*solid circles*) in tissue-cultured monolayers of epitheliary ciliated cells of the rabbit oviduct. Measurements of FCB and fluorescence were conducted every 10 and 5 s, respectively.

ciliated cells (Sanderson and Dirksen, 1988). However, quantitative measurements of variations of cytosolic [Ca^{2+}] associated to physiologic stimulation of ciliary movement had been only preliminarily investigated (Villalón et al., 1988).

Prostaglandins in concentrations normally found in the fallopian tube can strongly stimulate ciliary activity in oviductal ciliated cells (Verdugo et al., 1980), and indirect evidence suggests that their action may be coupled by intracellular release of Ca^{2+} (Verdugo, 1980). Stimulation of ciliated cells produced by extracellular ATP is probably mediated by a P_2 receptor (Burnstock, 1981) because the same effect can be produced by the nonhydrolyzable ATP analogue AMP-PCP (Villalón et al., 1988). However, the mechanism that couples physiological stimulation in epithelial ciliated cells has not been established.

The evidence presented here is the first objective indication that an increase in cytosolic [CA^{2+}] initiates the stimulation of epitheliary ciliated cells by both ATP and $PGF_{2\alpha}$. The increase from 100 to 200 nM intracellular [Ca^{2+}] that follows the application of either agonist is well within the range of [Ca^{2+}] that produces a calmodulin-dependent stimulation of ciliary movement in demembranated axonemal models of epitheliary cilia (Verdugo et al., 1983). The present results further indicate that cytosolic [Ca^{2+}] increases only transiently after exposure of the cells to ATP or $PGF_{2\alpha}$. A similar pattern of response has been observed after the stimulation of vascular smooth muscle with angiotensin II (Morgan and Morgan, 1982). It has been thought that in myocytes a transitory increase of [Ca^{2+}] might couple the contractile response by activation of protein kinase-C (Rasmussen et

al., 1984). However, the stimulation of ciliated cells further requires the presence of the agonist in the medium, suggesting that in addition to the brief increase of cytosolic [Ca^{2+}] the receptors must remain occupied for stimulation to be maintained.

It is possible that in ciliated cells there is a delayed receptor-gated influx of Ca^{2+} which, though not resolved by the fluorescence detection method, may explain the need for continuous occupancy of the P_2 receptor. This is further supported by the observation that addition of $LaCl_3$ to ATP-stimulated cells can reverse stimulation of ciliated cells (data not shown).

The blockage of Ca channels by $LaCl_3$ has no effect upon spontaneous ciliary activity, nor on the agonistic actions of $PGF_{2\alpha}$, but it can suppress both the increase in intracellular [Ca^{2+}] and the stimulation of ciliated cells produced by ATP (Fig. 3). Hence, whereas ATP must modulate intracellular [Ca^{2+}] via receptor-coupled gating of Ca channels, $PGF_{2\alpha}$ appears to regulate cytosolic [Ca^{2+}] by controlling the release of this cation from intracellular stores. The gating of Ca channels by ATP receptors has also been demonstrated in vascular smooth muscle (Benham and Tsien, 1987). Likewise, the existence of two sources for the regulation of cytosolic Ca^{2+} has also been described in myocytes (Weis et al., 1987). Nevertheless, irrespective of the source of Ca^{2+}, the net result is that in ciliated cells, as in other tissues, an increase of cytosolic [Ca^{2+}] couples the stimulus received by membrane receptors to the effector organelle, which in this case is the axonemal engine.

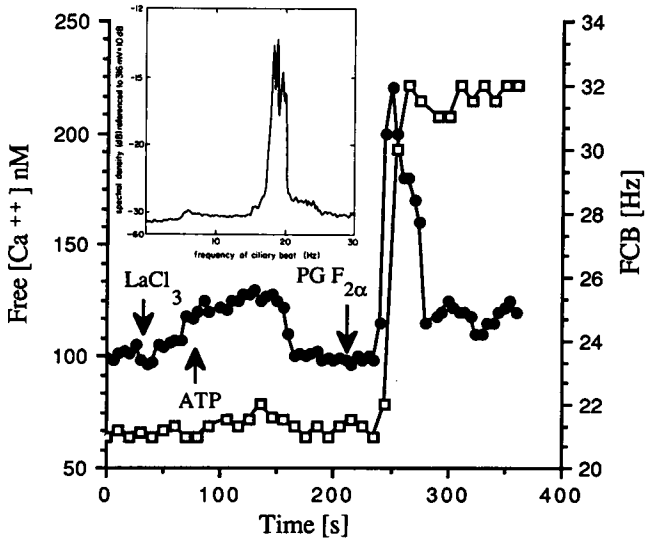

FIGURE 3 Time course of the effect of 100 μM ATP and 14 μM $PGF_{2\alpha}$ on frequency of ciliary beat (FCB, *open squares*), and intracellular [Ca^{2+}] (*solid circles*) in tissue-cultured monolayers of ciliated cells of the rabbit oviduct that have been preincubated with the Ca-channel blocker $LaCl_3$ (0.6 mM). Measurements of FCB and fluorescence were conducted every 10 and 5 s, respectively. Inset is the power spectrum of beat frequency as detected by laser-Doppler spectroscopy.

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