SOME PROPERTIES OF A MODEL ASSAY FOR CILIARY CONTRACTILITY

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The response of glycerinated cilia from *Tetrahymena* to the addition of adenosine triphosphate (ATP) can be assayed by measuring the size of the ciliary pellet obtained after brief, low-speed centrifugation in hematocrit tubes (1). Upon the addition of ATP, cilia swell, resulting in pellets up to threefold larger than pellets of control cilia. There are two interdependent hydrodynamic factors which determine pellet size: (a) the density of the pellet, which depends on the packing arrangement as well as particle shape, and (b) the percent of total cilia sedimented, which depends on the rate of sedimentation of the cilia. Concentrations of ATP from 0.1 to 1.0 mM (but not other nucleoside triphosphates) cause an increase in pellet size relative to control cilia both by increasing the percent cilia sedimented and by decreasing the pellet density, apparently by an ATP-induced hydration (1–3). Measurement of the parameters of the assay (per cent cilia sedimented and pellet density) may reveal changes even when the pellet height is unchanged by a particular experimental treatment. In this study, the pellet height assay was employed in conjunction with ATPase activity measurements to investigate certain key features of the interaction of glycerinated cilia with ATP and potential denaturing or uncoupling agents, which have been shown by other workers to alter or inhibit the beating of ciliary models or of cilia in situ. The evidence presented below supports the view that the ATP-induced swelling of cilia measured in the present assay, although not identical to beating, is closely related to ciliary contractile process.

METHODS

Cilia were isolated from *Tetrahymena pyriformis*, strain HSM. The culture of cells and the method of preparing glycerinated cilia have been described in detail (1, 4, 5).

Pellet height assays were performed at 24° by the method previously described (1), except that the buffer was 110 mM imidazole-HCl, pH 7.5, 2.5 mM $MgSO_4$, and 50 mM total Na^+ (Na^+ from ATP or other compounds, plus NaCl), plus test compounds as indicated. To describe the response the "pellet height ratio" was used, defined as the ratio of the height of the pellet in the presence of ATP to the height of the pellet in the absence of ATP; thus, the greater the pellet height ratio, the greater the response to ATP. Pellet heights were read from hematocrit tubes calibrated so that volume could be directly determined from height and adjusted for a total column of liquid of 10.0 cm. The percent of cilia sedimented was determined as the percent of total protein appearing in the pellet, and the pellet density as mg protein/ml pellet.

ATPase assays were carried out under the same conditions as the pellet height assay. Phosphate release from ATP and protein concentrations were determined by the methods of Taussky and Shorr (6) and of Lowry et al. (7), respectively, as described elsewhere in detail (1, 4, 5).

ATP was obtained from General Biochemicals, Chagrin Falls, Ohio, adenosine diphosphate (ADP) from Schwarz Bio Research Inc., Orangeburg, N. Y., and guanosine triphosphate (GTP), uridine triphosphate (UTP) and cytidine triphosphate (CTP) from Sigma Chemical Co., St. Louis, Mo. Thiourea was obtained from Sigma, urea from J. T. Baker Chemical Co., North Phillipsburg, N. J., guanidine sulfate from Eastman Kodak Co., Rochester, N. Y., and imidazole

from Sigma (Grade I). Other reagents were standard analytical grade.

RESULTS

The Effect of Selected Reagents on the Pellet Height Response and ATPase Activity of Glycerinated Cilia

THIOUREA: As shown in Fig. 1A, thiourea in concentrations from 0.1 to 0.2 M significantly inhibited the pellet height response to ATP but slightly activated the ATPase activity. The inhibition reflected not only an inhibition of the ability of cilia to respond to ATP but also an alteration in the state of the ciliary proteins. Thus, in the absence of ATP, thiourea itself increased the height of the ciliary pellets relative to the controls. In the presence of ATP the pellet density was the same with or without thiourea but less protein was sedimented when thiourea was also present. Because of the large decrease in pellet density caused by thiourea itself, this means that the effect of ATP on cilia treated with 0.15 M (or more) thiourea was to increase the pellet density, contrary to the normal situation where ATP decreases pellet density.

The effects of thioruea were also time dependent: 4 min exposure to 0.2 M thiourea caused a 50% inhibition of pellet height response, but 30 min exposure completely inhibited the response. The inhibition caused by short exposures to thiourea could be partially reversed by diluting before centrifuging. Pretreating cilia with ATP did not prevent the inhibition of the pellet height response.

UREA: In concentrations from 0.1 to 0.2 M, urea had only a slight inhibitory effect on the pellet height response and did not significantly increase the ATPase activity (Fig. 1B).

GUANIDINE SULFATE: At concentrations between 0.1 and 0.2 M, guanidine completely inhibited the pellet height response but activated the ATPase activity (Fig. 1C). The decrease in pellet height ratio was due primarily to extensive dissolution of the cilia, which not only prevented the interaction of the cilia with ATP but masked any details of the specific effects of guanidine on the hydrodynamic properties of the cilia.

$NiCl_2$: At 5 mM, $NiCl_2$ inhibited the ATPase activity by 25% and almost completely inhibited the pellet height response (Fig. 1D). When $NiCl_2$ was present, pellet height both in the presence and in the absence of ATP were comparable to those of ATP-treated control pellets. This increased pellet height did not represent swelling but, instead, resulted from an increase both in percent of cilia sedimented and in pellet density. The effects of $NiCl_2$ appeared to be independent of the presence of ATP, and in some experiments the typical changes in pellet density or amount of cilia sedimented could be seen (to a small extent) superimposed on the effects due to the $NiCl_2$.

DISCUSSION

Studies on the effects of various reagents on the pellet height response show a correlation between inhibition of this response and inhibition of ciliary beating. Thiourea, for example, inhibits the contraction of *Mytilis* muscle by direct action on the contractile machinery and inhibits the ATP-induced contraction of isolated actomyosin (8). Brokaw found that thiourea reduced the beat frequency of live and of glycerinated sperm (9). Brokaw and Benedict (10) recently reported that the movement dependent dephosphorylation of ATP appeared to be proportional to the beat frequency of glycerinated sea urchin spermatozoa and was specifically inhibited by thiourea. In both glycerinated *Tetrahymena* cilia and sea urchin sperm thiourea increased the ATPase activity, suggesting that one effect of thiourea was to uncouple ATP hydrolysis from the contractile function. The observation that thiourea itself changed the pellet density may provide a tool for further study of the mode of action of thiourea on cilia.

Urea, although closely related to thiourea, has little effect on contractile systems at comparable concentrations. It produced little inhibition of the *Mytilis* actomyosin system (8), scarcely affected the beat frequency of live sperm or of reactivated glycerinated sperm (9), and did not alter the pellet height response or the ATPase activity of glycerinated cilia. Guanidine, unlike thiourea or urea, is charged at pH 7.5; thus both its powerful inhibition of the pellet height response and the concomitant activation of the ATPase are probably due primarily to solubilization of the cilia and release of latent ATPase activity (4).

In suspensions of swimming paramecia, 5 mM $NiCl_2$, by action specific to ciliary beating and separate from membrane polarization effects, causes complete cessation of beating within 3 min (11). Similarly, 5 mM $NiCl_2$ causes almost complete inhibition of the pellet height response. Contrary to the effect of thiourea, the effect of $NiCl_2$ ap-

pears to be independent of the presence or absence of ATP. Also, unlike thiourea, $NiCl_2$ inhibits the ATPase activity of 14 S and 30 S dyneins (12) as well as of glycerinated cilia, although this is probably not the cause of the inhibition of the pellet height response.

Exposure of cells to ethanol is widely used as a method of cilia isolation. Cilia isolated by the ethanol-calcium method possess normal ATPase activities but cannot be reactivated to beat unless treated with glycerol immediately after isolation (13). Ethanol activated the ATPase activity of

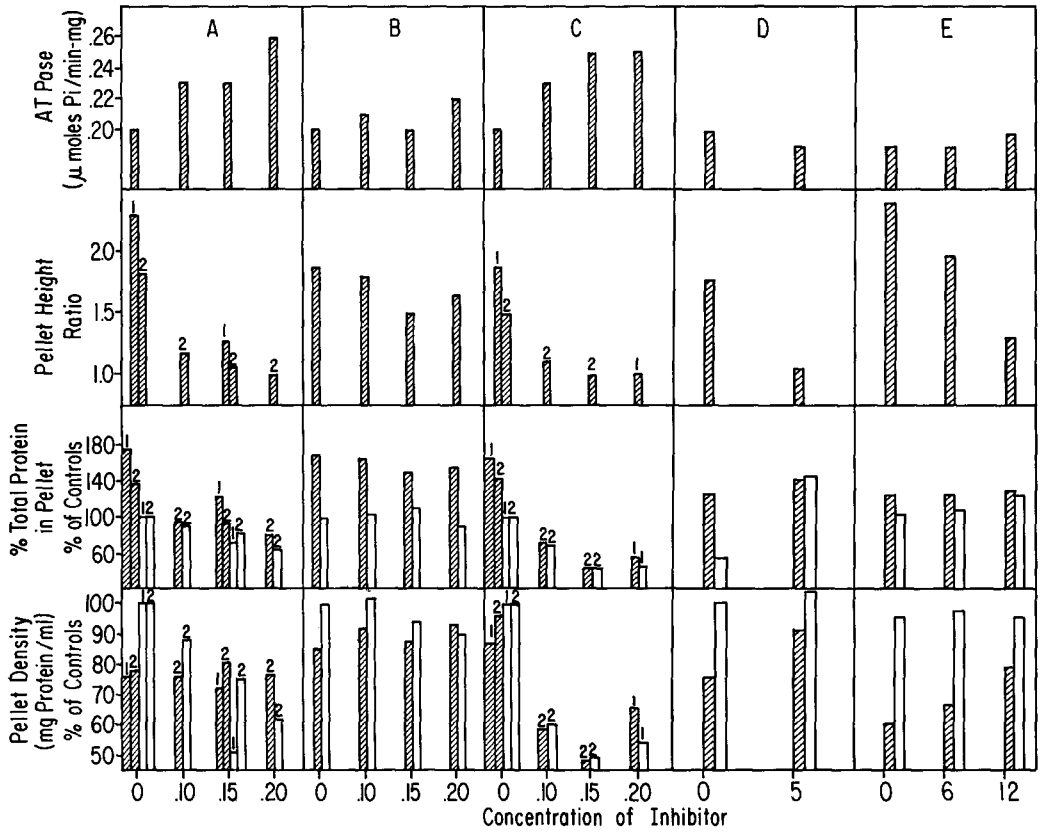

FIGURE 1 *The Effect of Several Modifiers on the Pellet Height Response and ATPase Activity of Glycerinated Cilia*

The conditions of the assays were as described in Methods, with the test compounds included at the concentrations indicated on the abscissae. Concentration units are given below for each experiment. Pellet height assays included 10 min of incubation at 24° followed by 5 min of centrifugation at room temperature. Other conditions were as follows: Modifiers used and the concentration units of the abscissae were: experiment A, thiourea, M; experiment B, urea, M; experiment C, guanidine sulfate, M; experiment D, $NiCl_2$, mM; experiment E, ethanol, % ethanol (v/v). ATP concentrations during the ATPase assays were: 0.5 mM for experiment E; 1.0 mM for all other experiments. Protein concentrations during the ATPase assays were (mg/ml): experiment A_1, 0.3; A_2, 0.5; B, 0.3; C_1, 0.3; C_2, 0.3; D, 0.25; E, 0.14. ATP concentrations during the pellet height assays were (mM): A_1, 0.25; A_2, 0.5; B, 0.25; C, 0.25; D, 0.5; E, 0.5. Protein concentrations during the pellet height assays were (mg/ml): A_1, 1.4; A_2, 1.2; B, 1.5; C_1, 1.5; C_2, 1.4; D, 1.2; E, 0.62.

For the parameters of the pellet height assay the shaded bars indicate the presence of ATP, and the unshaded bars, the absence of ATP. The values of controls (100%) in the absence of ATP for percent of protein in the pellet were: A_1, 34%; A_2, 52%; B, 35%; C_1, 37%; C_2, 40%; D, 55%; E, 55%. The values of controls (100%) for pellet density were (mg/ml): A_1, 8.8; A_2, 11.0; B, 8.6; C_1, 9.3; C_2, 7.6; D, 11.0; E, 8.0.

glycerinated cilia but inhibited the pellet height response. It thus appears that ethanol may alter the contractile properties of cilia, and that the glycerol isolation procedure may be preferable to the ethanol-calcium isolation procedure for certain purposes.

It is believed that the contractile properties of cilia depend on an interaction between at least two proteins (4, 14, 15), and recently it has been suggested that ciliary motility depends on a sliding filament mechanism (16). Three agents, thiourea, ethanol, and heating at 40°C for 10 min (1), inhibit the pellet height response but slightly activate the ATPase activity of glycerinated cilia. It would, however, be premature to identify the increased ATPase as the result of an uncoupling of the dynein ATPase from the rest of the contractile machinery especially because of the presence of some nondynein ATPase activity in whole cilia. From the similarities of the effects of reagents such as thiourea, urea, and $NiCl_2$ on ciliary beating and on the pellet height response, it appears that the pellet height response is related to some aspect of the mechanism responsible for ciliary beating. However, it is not identical to beating, as is evident from the fact that beating is a cyclic process whereas the pellet height response to ATP appears to be a one-step process. Secondly, whereas Ca^{++} alone is sufficient for the pellet height response (4), Mg^{++} is required for beating.

The increase in amount of cilia sedimented and the decrease in pellet density upon addition of ATP might seem to be simply a reflection of the ATP-induced hydration (1, 2, 3); that is, the cilia imbibe water and thus sediment more rapidly and, simultaneously, being more swollen, they pack into less dense protein pellets. The process appears to be more complex, however, since several treatments [e.g. ethanol, thiourea, Ca^{++} versus Mg^{++} (4)] alter the two parameters independently, and since in different preparations the two changes occur to different degrees.

It is not known whether the pellet height response represent part of the beating process per se (e.g. contraction or relaxation) or is the result of a beating-related change. In either case, however, the pellet height response appears to represent an aspect of ciliary contractility related to the beating mechanism, and as such, should prove useful in studying some aspects of ciliary motility.

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