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MODIFICATION OF CILIARY BEATING IN SEA URCHIN LARVAE INDUCED BY NEUROTRANSMITTERS: BEAT-PLANE ROTATION AND CONTROL OF FREQUENCY FLUCTUATION

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Summary

The modification of ciliary beating by neurotransmitters in sea urchin larvae at the four-armed pluteus stage was analyzed in terms of the direction of beating and fluctuation in the beat period. Application of dopamine to *Pseudocentrotus depressus* causes the cilia to turn their beat plane but retain its characteristic planar feature up to the complete 'reversal' of the beat direction. This new type of response was termed the 'beat-plane turning response'.

It was also found that neurotransmitters, especially dopamine and serotonin, can modify the length of the beating cycle in *P. depressus* and *Hemicentrotus pulcherrimus*. Dopamine decreased and serotonin increased the beat frequency averaged over the ciliated epithelium with the standard deviation from the mean increasing in the presence of dopamine and decreasing with serotonin. The beat-period fluctuation and its modification suggested by this observation was confirmed from measurements of the beating of individual cilia in the presence or absence of these neurotransmitters. Further analysis of the correlation between angular velocity and beat period indicates that variation in the beat period is not controlled by the same processes as those that modulate angular velocity.

These findings in sea urchin larvae suggest that both the stability and the direction of ciliary beating is under nervous control.

Key words: sea urchin, ciliary beating, single cilium, neurotransmitter, dopamine, serotonin, beat-period fluctuation, beat-plane rotation.

Introduction

The growth and development of sea urchin larvae is accompanied by an increase in the complexity of their swimming behaviour and it has been suggested that this involves alterations in the mode of ciliary responses (Mogami *et al.* 1988, 1991). Up to the gastrula stage, electrical stimulation induces the cilia to stop beating briefly in the middle of the effective stroke (Baba, 1975; Baba and Mogami, 1987). This response, which has been called the primitive response, may reduce the speed or alter the orientation of freely swimming organisms. After the prism or pluteus stage (stages before pluteus will be referred to as embryonic and after pluteus as larval), cilia are able to show ciliary reversal either spontaneously or in response to electrical stimulation. It has been suggested that this response may enable the larva to swim backwards, helping it to avoid obstacles or escape from predators (Baba, 1975; Baba and Mogami, 1987).

Five states of movement have been reported in the epaulette cilia of the eight-armed pluteus (Mogami *et al.* 1991): normal beating for forward (arm-leading) locomotion; reversed beating for backward movement; an intermediate position during the change from reversed to normal beating; and two inactive states with upright and inclined ciliary positions. These well-differentiated ciliary responses, combined with the development of nervous control, may enable optimal regulation of larval swimming and feeding behaviour (Lacalli and Gilmor, 1990; Mogami *et al.* 1992).

In previous studies, the role of nervous control of ciliary motility was examined in terms of the swimming behaviour of sea urchin larvae in a medium containing neurotransmitters. Among the neurotransmitters tested, serotonin and dopamine were found to induce changes in larval swimming (Mogami *et al.* 1992). Serotonin increased the swimming speed of pluteus larvae in a concentration-dependent manner in both *Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus*, although to a smaller extent in the latter species. In contrast, dopamine reduced the swimming speed in both species. Dopamine also had the effect of inducing prolonged backward swimming in the pluteus of *P. depressus*, but did not produce this effect in *H. pulcherrimus*. These neurotransmitter-mediated changes in locomotory activity suggest that ciliary motility may be controlled by neurotransmitters whose presence and activity depends upon the development of the nervous system.

Although the swimming behaviour of ciliated

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microorganisms reflects the locomotory activity of their cilia, it only provides information about the integrated activity of all the cilia covering the body surface. The aim of the present paper is to clarify the effects of neurotransmitters on the motility of individual cilia in sea urchin larvae. The highly planar bending configuration of the cilia as well as the sparse ciliation enable analysis of a whole cycle of beating from images recorded within a single focal plane at the light-microscope level (Baba, 1975; Baba and Mogami, 1987; Mogami *et al.* 1993). The present paper describes the effects of neurotransmitters both on the bending and cyclic activity of individual cilia of sea urchin plutei, with particular reference to changes in swimming behaviour induced by dopamine and serotonin.

The backwardly directed swimming of plutei of *P. depressus* induced by dopamine has been explained by the change in the direction of beating during ciliary reversal, which has been shown to occur in correlation with the electrical activity of the cell (Mackie *et al.* 1969; Baba, 1975; Mogami *et al.* 1992). We report here a completely new type of ciliary response in *P. depressus*, which we term the beat-plane turning response (BPTR), and which may also result in backwardly directed swimming. This response involves a gradual rotation of the beat plane, which enables the cilia to reverse the beat direction.

We also investigated the effects of neurotransmitters on the modulation of the cyclic activity of ciliary movement, which may lead to changes in the swimming speed of the larvae. These effects were analyzed in terms of long-term changes in the beat frequency as well as short-term fluctuations in successive individual beats.

Materials and methods

Larvae of the sea urchins *Hemicentrotus pulcherrimus* (Agassiz) and *Pseudocentrotus depressus* (Agassiz) were grown in the laboratory at 17 °C (Degawa *et al.* 1986). Pluteus larvae (55–72 h after insemination) were placed in a perfusion chamber made from a slide and a coverslip separated by two strips of thin adhesive plastic tape (30–60 μm thick). For observation and recording of the motile response of an individual cilium to the bath-application of neurotransmitters, larvae were tethered in the chamber by screwing a thin metal plate on the coverslip to the basement plate. The medium around the tethered larva was replaced continuously through one of the openings of the chamber while excess fluid was drained from the opposite side. The medium was supplied through a fine stainless-steel tube attached *via* silicone tubing to a syringe using a linear slider driven by a stepping motor at a rate of $9\,\mu l\,min^{-1}$ to depress the syringe plunger. All experiments were performed at room temperature (19–26 °C).

Throughout the experiment, the basic medium was artificial sea water (ASW), which consisted of (in $mmol\,l^{-1}$): 450 NaCl, 10 KCl, 10 $CaCl_2$, 25 $MgCl_2$, 28 $MgSO_4$, 10 Tris (pH 8.0). Dopamine (10^{-6}–$10^{-5}\,mol\,l^{-1}$, dopamine hydrochloride), serotonin ($10^{-5}\,mol\,l^{-1}$, 5-hydroxytryptamine creatinine sulphate salt), adrenaline ($10^{-5}\,mol\,l^{-1}$, epinephrine hydrochloride) and carbamylcholine ($10^{-5}\,mol\,l^{-1}$, carbamylcholine chloride) were added to ASW.

Before applying neurotransmitters, tethered larvae were left for more than 10 min to adapt while the chamber was perfused with ASW. The perfusing tubing and syringe were then replaced with others containing the test solution, with minimum interruption of the continuous flow at the rate specified above. This process ensured a rapid and smooth exchange of perfusing solutions. After bathing the larvae for 10 min in the test solutions, perfusion with ASW was reinstated.

The beating of an individual cilium was observed and recorded using video-microscopy with phase-contrast optics (40× objective, Splan 40 Olympus, Tokyo, Japan). For video-tape recording (VTR), we used stroboscopic illumination with a xenon flash tube (FX193, EG&G), which was triggered by vertical syncronization pulses (60 Hz for NTSC standard) generated by a CCD camera (XC-77RR, Sony, Tokyo, Japan). For electrical stimulation, a square-pulse current (intensity 200 V, duration 150 ms) was applied through two platinum electrodes placed at the two openings of the perfusion chamber, and the moment of stimulus was recorded on the VTR by superimposition.

The recordings were analyzed field by field using a video motion analyzer (SVM-1110, Sony). To analyze quantitatively the bending patterns of the ciliary shaft, bend configurations traced from a video monitor were digitized; the shear angles (tangential angles to the shaft with respect to a line perpendicular to the surface of the larva) at a given distance from the base were then calculated following the method described previously (Mogami *et al.* 1993).

We used two methods to analyze the beat frequency, designated as long-term and short-term analyses.

Long-term analysis

The mean beat periods of individual cilia were determined from the duration of successive 10 beat cycles selected at 30 s intervals. The beat frequency, i.e. the reciprocal of the mean beat period as a function of time, was normalized to the mean value obtained for the 2 min period prior to neurotransmitter application, and was averaged from recordings from several cilia obtained using identical procedures.

Short-term analysis

The individual periods of successive beats and the angular velocities at the middle of the effective stroke were measured beat by beat for 1000 cycles from a selected cilium both in the absence and in the presence of neurotransmitters. The period was defined as the interval between successive 'zero-cross' times, at which the shear angle of the ciliary shaft at the middle of its length changes its sign during the effective stroke, i.e. when the cilium passes through a line vertical to the body surface. To improve the time resolution, the zero-cross time was determined from field-by-field measurements of the shear angle by means of linear interpolation, and was verified by a high-time-resolution analysis, which showed that the shear

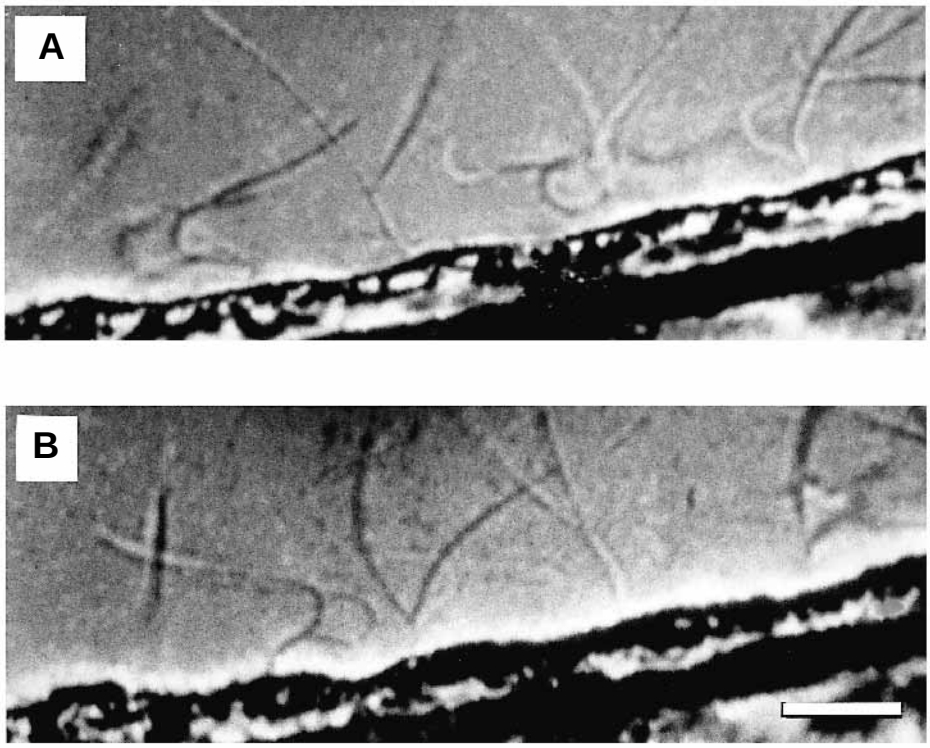

Fig. 1. Photographs of cilia of a *Pseudocentrotus depressus* pluteus larva. Four successive fields taken from a video-tape recording were superimposed and then enhanced by image processing. (A) Normal beating with the effective stroke to the right (posteriad). (B) Turned beating during the beat-plane turning response induced by 10^{-5} mol l^{-1} dopamine. The direction of the effective stroke is reversed (anteriad). Scale bar: 10 μm.

angle for most of the length of the cilium changed almost linearly during the effective stroke (Baba and Mogami, 1987). The angular velocity was obtained from the difference between the two shear angles used for the interpolation described above divided by the interval between the video fields.

Results

A novel type of dopamine-induced ciliary response in the pluteus of Pseudocentrotus depressus

Because the cilia of sea urchin embryos beat in a highly planar configuration, we could select an individual cilium for study which beat within a single focal plane throughout the whole cycle of beating, as shown in Fig. 1. Upon application of dopamine to plutei of *P. depressus*, a cilium began to rotate its beat plane around the normal to the cell surface with a latency of 2–3 min. During the course of rotation, the profile of beating gradually appeared to narrow, with portions of it moving out of focus, until beating occurred approximately in line as shown in Fig. 2 (middle diagram); the profile then broadened again to show all beat forms in focus. This observation may indicate that the beating is planar throughout the rotation. The gradual change in the beat-plane orientation finally resulted in an anti-parallel beat within 3 min of the start of rotation, with the effective stroke being in the opposite direction to that observed in the absence of dopamine (Figs 1,

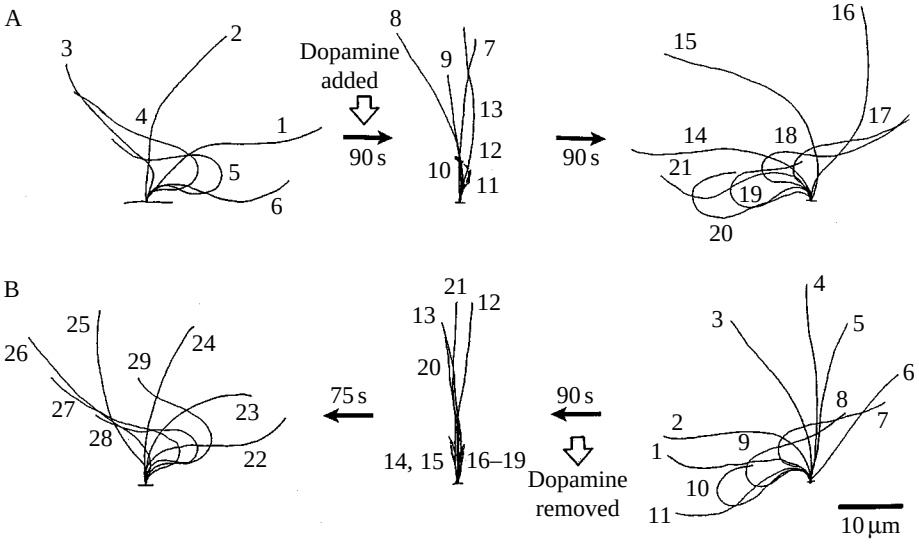

Fig. 2. Reconstructed beating patterns from video recordings of a single cilium of a *Pseudocentrotus depressus* pluteus larva. (A) After addition of dopamine (10^{-6} mol l^{-1}), the cilium with the effective stroke (1–3) to the left of the diagram (left) gradually rotates its beating plane (middle), and finally beats with the effective stroke to the right (right). (B) After removal of dopamine, the cilium rotates back again to restore the original direction of beating as indicated by the solid arrows. Numbers in the diagram indicate the sequence of video fields (60 fields s^{-1}). The length of time elapsed between each set of superimposed tracings is indicated.

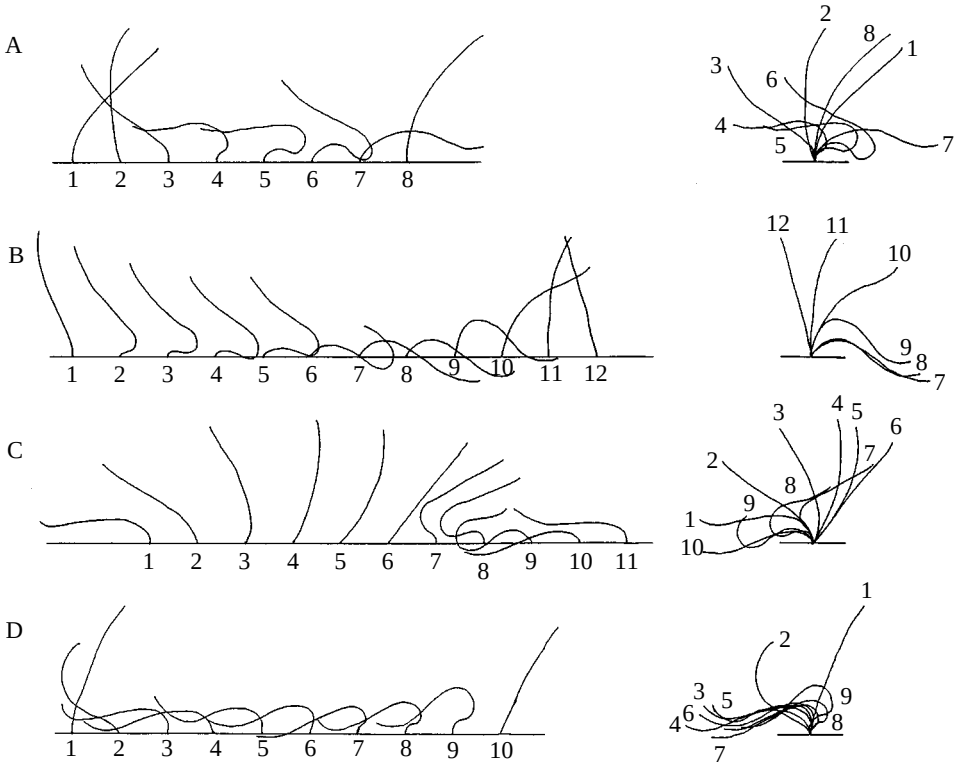

Fig. 3. Sequential and superimposed tracings of the beat configuration of a single cilium of a *Pseudocentrotus depressus* pluteus larva. (A) Normal beating. (B) Spontaneous reversed beating occurring during normal beating. (C) Turned beating. (D) Spontaneous reversed beating during turned beating. Numbers indicate video-field sequence (60 fields s^{-1}). Selected images are shown in superimposition for clarity.

2A). The resultant 'reversal' of beating, which was usually attained several minutes after the application of dopamine, was stable without further changes in the beat direction during prolonged dopamine application (>10 min). When dopamine was removed, the cilium regained its original beating posture by a reversal of beat-plane rotation (Fig. 2B).

Rotation of the beat plane and the resultant changes in beat direction observed in the pluteus of *P. depressus* are quite different from features of the reversal response previously described for sea urchin embryo cilia (Strathmann *et al.* 1972; Baba, 1975; Baba and Mogami, 1987; Mogami *et al.* 1993). Thus, we will term this new type of response the 'beat-plane turning response' (BPTR) and the resultant beating in the reversed direction as a 'turned beat', in order to distinguish it from the known type of reversed beating. The BPTR was observed mainly in cilia on the surface of the arms of plutei. The frequency of the observation was not high (approximately 10 %) and we sometimes found a cilium that failed to respond to dopamine situated next to a cilium showing turned beating. Application of serotonin to *P. depressus* did not change the beating direction or the bending patterns.

Reversed beating showing the characteristic features of ciliary reversal during normal beating (Strathmann *et al.* 1972; Baba and Mogami, 1987; Mogami *et al.* 1993): short latency and a lower curvature in the recovery stroke compared with a high curvature in the effective stroke, occurred spontaneously (Fig. 3) or was induced by electrical stimulation (Fig. 4) during the BPTR. It therefore appears that cilia can show ciliary reversal in the turned-beat plane. These findings indicate that a common mechanism may work both in the ciliary reversals

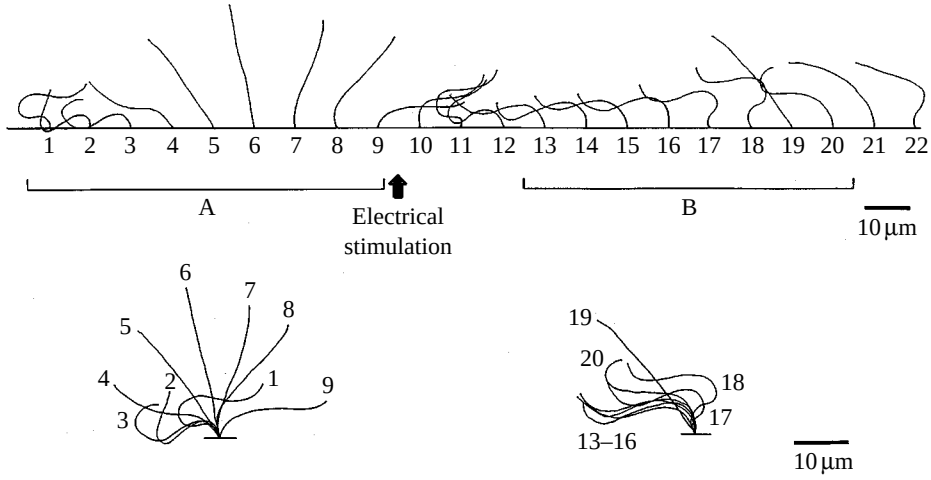

Fig. 4. An electrically elicited reversal response during the turned beating of a single cilium of a *Pseudocentrotus depressus* pluteus larva. Sequential tracings from video recordings of the turned beating before stimulation followed by induced reversed beating. Superimposed tracings are shown in the lower half of the figure for the sequences indicated by bars A and B.

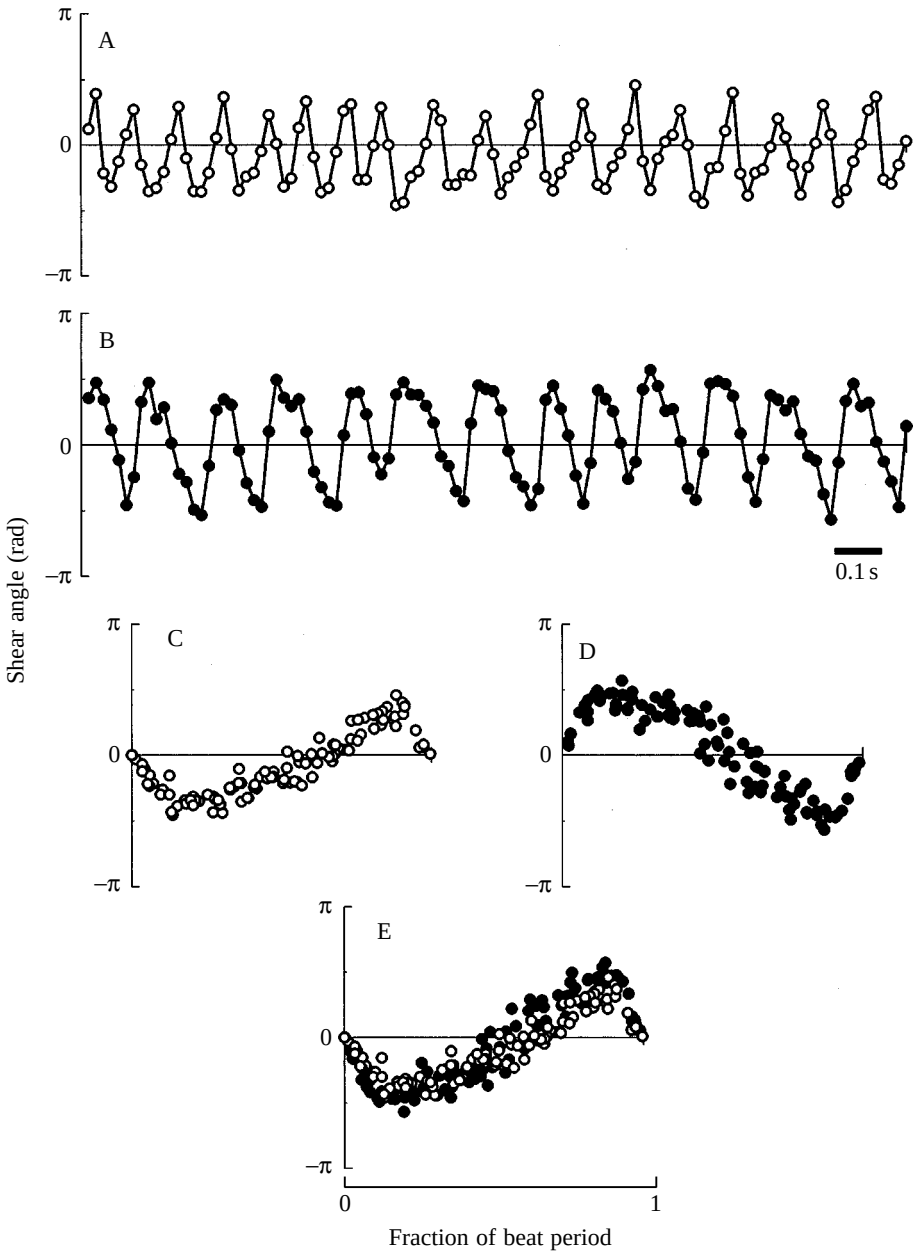

Fig. 5. Time course of the shear angle at the proximal portion of a single cilium of a *P. depressus* pluteus larva. (A) Normal beating. (B) Turned beating. (C) Superimposition of the shear angle after normalization of the time between zero-crossing points in A for the beat period. (D) Normalized superimposition for B. (E) C and D after inversion of D showing that the plots are now closely superimposed.

that occur during normal beating and during BPTR, and that the BPTR could be distinguished from ciliary reversal. Thus, sea urchin larvae have two means of reversing the water flow around the body, which could be used under different situations, e.g. when swimming and when feeding.

It has been demonstrated that the time sequences of angular changes at the proximal region of the cilia for both normal and reversed beating are almost indistinguishable, although the bending wave constructions in these two types of beating are apparently different (Mogami *et al.* 1993). Fig. 5 shows the time courses of the shear angle at the proximal region (5 μm from the base) obtained during normal and turned beating of a single cilium. The plots of these time courses could not be made to fit with each other by any simple linear translation of the data (as used in Fig. 6 in Mogami *et al.* 1993). A better fit for the two plots was obtained by reversing the sign of the angle of turned beating; i.e. by inversion of the plot (Fig. 5E).

The BPTR could not be induced in plutei of *H. pulcherrimus* by the catecholaminergic agents dopamine and adrenaline. Among the neurotransmitters tested in this study, carbamylcholine was effective in changing the bending patterns in *H. pulcherrimus*. In response to carbamylcholine, the cilia frequently stopped at the middle of the effective stroke, which was then followed by an incomplete recovery stroke. This is similar to the primitive response observed in the blastula and gastrula stages (Baba, 1975; Baba and Mogami, 1987). This response was reversible.

Effects of neurotransmitters on the cyclic activity

During an extensive period of recording, the cilia changed

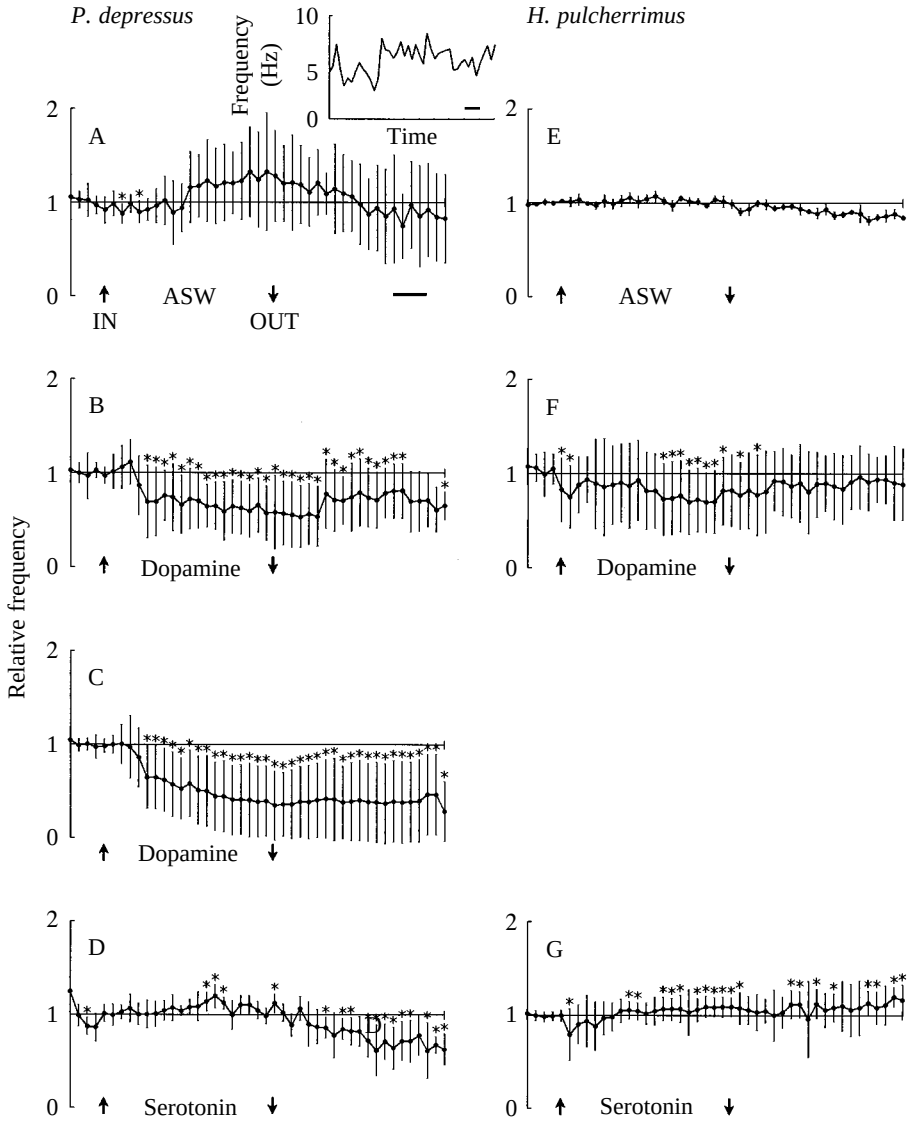

Fig. 6. Effects of dopamine and serotonin on the ciliary beat frequency in pluteus larvae (A-D: *Pseudocentrotus depressus*, E-G: *Hemicentrotus pulcherrimus*). The mean beat frequencies of a number of cilia, each of which varies as shown in the inset of A, are plotted. The mean values relative to that before application of reagents (control) (with S.D. bars) are shown for 4–23 cilia. (A,E) Artificial sea water; (B,C,F) 10^{-5} mol l^{-1} dopamine; (D,G) 10^{-5} mol l^{-1} serotonin. Neurotransmitters were applied between the arrows. (A,B,D,E,F,G) Normal beating; (C) turned beating. Asterisks indicate values that are significantly different (P<0.05) from the control value. The ordinate is given in relative values that are normalized to the mean obtained for the 2 min period prior to the application of experimental solutions. Note that the ordinate of the inset of A is given in Hz. Scale bars: 2 min.

their beat frequency to a variable extent. The frequency of a single cilium varied by up to 50 % of the mean value during the recording time, for example, that shown in the inset of Fig. 6A varies within the range 2.97–8.96 Hz with a mean of 6.14 Hz (N=45).

Fig. 6 shows long-term changes in the beat frequency in the presence of dopamine and serotonin, measured from plutei of *P. depressus* and *H. pulcherrimus*. In both species, application of dopamine reduced the beat frequency, irrespective of whether the BPTR was induced. The reduction was more acute and larger during both normal (Fig. 6B) and turned beating (Fig. 6C) in *P. depressus* than during normal beating in *H. pulcherrimus* (Fig. 6F). The effect of dopamine tended to last even after the removal of the reagent (Fig. 6B,C,F), although a quick recovery of cyclic activity concomitant with the recovery of the beating pattern was observed in some specimens (results not shown). Serotonin, in contrast, affected the beat frequency to a smaller extent than did dopamine. It increased the beat frequency slightly but significantly in both *H. pulcherrimus* and *P. depressus*.

Carbamylcholine significantly reduced the beat frequency in *H. pulcherrimus* in the same manner as did dopamine in *P. depressus*, but the reduction due to carbamylcholine was mostly caused by the intermittent beating described above. Adrenaline, which did not affect the beating pattern, had no significant effect on the beat frequency (data not shown).

Ciliary beating is composed of alternate strokes in opposite directions (effective and recovery strokes) with an intervening pause not only during but also between strokes (Baba, 1979; Baba and Mogami, 1987). To examine the effects of neurotransmitters on the composition of beating, we further analyzed the motility of individual cilia during cyclic motion (the short-term analysis described in Materials and methods). The beats that were found to be significantly affected by these neurotransmitters in the preceding analysis were selected for this analysis. Fig. 7 shows the beat period measured from 1000 successive cycles of an individual cilium before and after the application of dopamine to *P. depressus* (Fig. 7A) and serotonin to *H. pulcherrimus* (Fig. 7B).

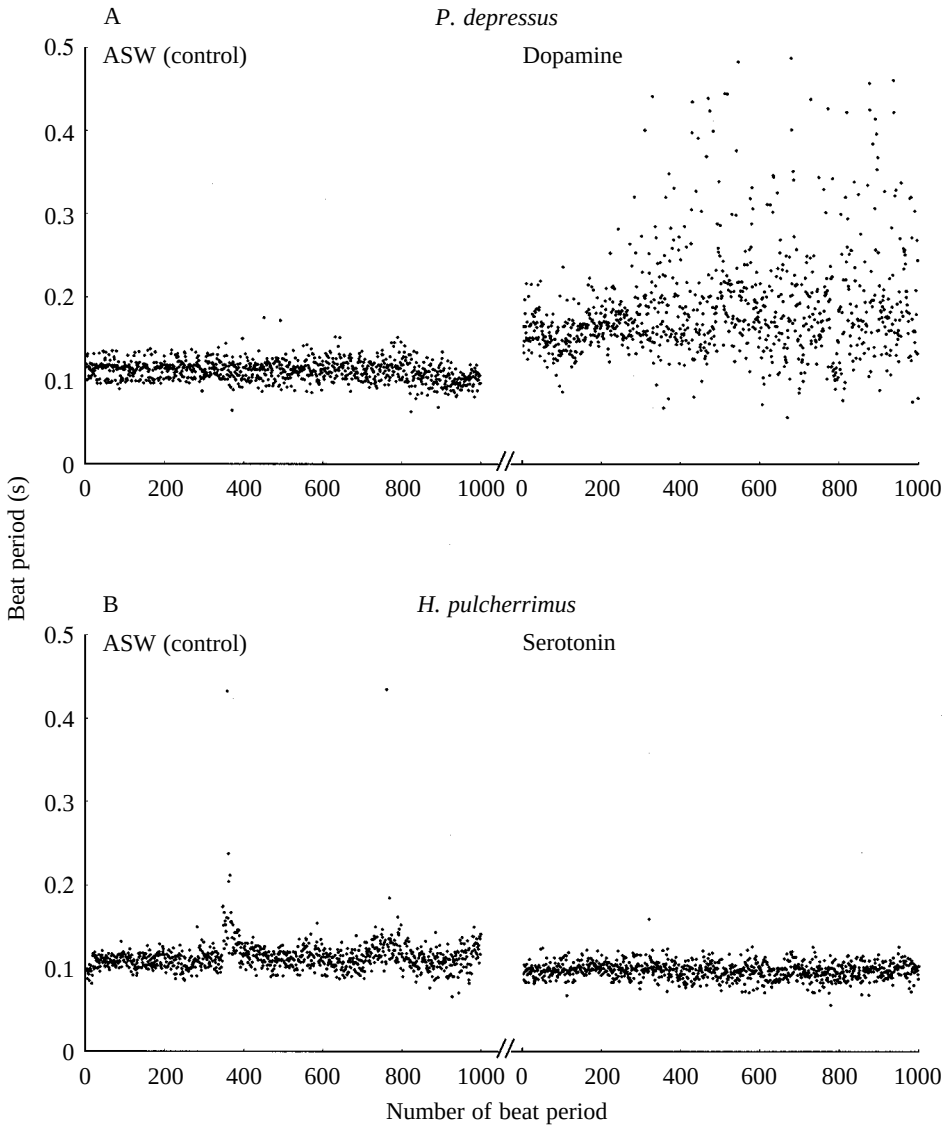

Fig. 7. Plots of successive beat periods of a single cilium of pluteus larvae. (A) Treatment with 10^{-5} mol l^{-1} dopamine after perfusion with artificial sea water (*Pseudocentrotus depressus*); (B) treatment with 10^{-5} mol l^{-1} serotonin after perfusion with artificial sea water (*Hemicentrotus pulcherrimus*). The break in the time axis represents 5.3 min in A and 4 min in B.

In *P. depressus*, the beat period varied beat by beat, successive measurements from a single cilium gave a beat period of 0.112±0.012 s (mean ± S.D., *N*=999), which, when plotted gave a cluster of points around the fluctuating local mean as shown in Fig. 7A (control). After application of dopamine, the beat period became longer, for example, one series of measurements gave a value of 0.203±0.128 s (mean ± S.D., *N*=999), with increasing fluctuation as shown in Fig. 7A (dopamine) (in this case, the cilium showed BPTR). The scatter of points was broadened because of the increased fluctuation. A close examination revealed a dense region of values with shorter periods with more widely dispersed points in the upper region of the plot. This feature seems to be consistent with the observation that the beat period often lengthened quite suddenly and then returned quickly to its original level. The rate of angular change measured at the middle of the effective stroke also fluctuated beat by beat in ASW, giving a value of 47.2±14.9 rad s^{-1} (mean ± S.D., *N*=1000) in a typical series of measurements. After the application of dopamine, the rate of angular change was reduced, producing, in one series of measurements, a value of 25.8±8.48 rad s^{-1} (mean ± S.D., *N*=1000), responsible for the increase in the beat period. This indicates that dopamine elongates the beat period by reducing the sliding velocity of tubules. However, dopamine did not have as great an effect on fluctuations in angular velocity as it did on beat period. We found a low correlation between the beat period and the angular velocity; *r*=0.025 before application of dopamine and *r*=–0.048 after application. Thus, fluctuation in the beat period may involve some processes independent of those causing the reduction in angular velocity, i.e. steps in the dynein cycle that initiate pauses are more variable than those responsible for the swing speed and are more sensitive to dopamine.

In *H. pulcherrimus*, serotonin reduced the beat period of cilia measured beat by beat so that in one series of measurements (shown in Fig. 7B), it fell from 0.120±0.121 s (mean ± S.D., *N*=1007) to 0.098±0.010 s (*N*=1001), as predicted from the increase in beat frequency measured in the long-term

analysis described above. In the presence of serotonin, the beat-by-beat fluctuation in beat period was less than that found under control conditions, and was also much more stable over a longer time scale (hundreds of beats). In contrast, the fluctuation in beat period of cilia in ASW, as compared with the latter, showed a gradual change with occasional spike-like deviations. While the observed reduction in beat period might suggest an increase in angular velocity in the presence of serotonin, the angular velocity of the effective stroke was actually reduced by serotonin, from $44.4\pm12.0\,\mathrm{rad\,s^{-1}}$ (N=1008) to $42.7\pm15.7\,\mathrm{rad\,s^{-1}}$ (N=1002) in a representative series of measurements. Although the difference in angular velocity was usually small, it was often significant ($P<0.01$, t-test). This suggests that serotonin may shorten pauses as stated above and further reduce the amplitude of beating by an early switching to reverse sliding with a minimal influence on the angular velocity.

Discussion

A previous paper (Mogami *et al.* 1992) showed that dopamine induces long-lasting backwardly directed swimming in plutei of *P. depressus*. The BPTR and the resultant turned beating described in the present paper may explain this dopamine-induced reversal of swimming. The absence of BPTR in *H. pulcherrimus* is in agreement with the much lower ratio of plutei swimming backwards in the presence of dopamine compared with those of *P. depressus*.

If backwardly directed swimming was caused by ciliary reversal, larvae would immediately swim backwards with a reversed angle of roll, because in this case the cilia reverse their beat direction in the same beating plane. In contrast, BPTR will cause gradual changes in swimming direction and rotation. In fact, it was observed that just after being transferred to ASW containing dopamine in a chamber in which the thinness of the ASW layer prevented helical swimming and allowed two-dimensional observation, the larvae of *P. depressus* often pivoted on their arm and then began to swim backwards (data not shown). The pivoting of the larvae seems to reflect the gradual beat-plane rotation that leads to turned beating. Three-dimensional recordings have demonstrated that the angle of roll during helical swimming in a wide chamber remains the same during spontaneous backward swimming (Baba *et al.* 1991). This observation can also be explained by beat-plane turning but not by ciliary reversal, which occurs in a single plane. A spontaneous ciliary reversal, usually reported to last for only several hundred milliseconds (Mogami *et al.* 1993), cannot explain by itself a long-lasting period of backward swimming such as that induced by dopamine. In contrast, turned beating, which continues as long as dopamine is present, could well be responsible for long periods of swimming backwards. Therefore, it seems more reasonable to consider turned beating as a direct cause of long-lasting backward swimming of the plutei of *P. depressus* in the presence of dopamine. If dopamine induces turned beating in only 10 % of cilia in free-swimming larvae, as suggested by the present observations using tethered larvae, it may seem unlikely that turned beating alone can induce backwardly directed swimming. However, the small fraction of cilia showing turned beating in our experiments may reflect adaptation or reduced sensitivity to dopamine under unusual conditions, e.g. tethering, compression by the coverslip and the increased loads caused by the flow of experimental solutions.

BPTR is a new type of response in which the ciliary beat plane is modified. Although turned beating during BPTR results in the same reversal of propulsive force as does reversed beating during ciliary reversal, the two processes can be distinguished in that BPTR involves turning the beat plane without any pronounced changes in bending-pattern formation while ciliary reversal is accompanied by fast and significant changes in the bending pattern without changes in the beat plane. It is known that ciliates such as *Opalina japonica* Sugiyama change swimming direction by a gradual reversal of the ciliary beat direction (Kinosita, 1954; Tamm and Horridge, 1970); which we will call ciliate-type reversal. It has also been demonstrated that some metazoan cilia reverse their beat direction within a single plane (Baba, 1975; Tamm and Tamm, 1981); and this we will call metazoan-type reversal. It is, perhaps, surprising that these two types of ciliary reversal (ciliary reversal and BPTR) can be found in a single system.

The turned beating occurred with a much greater latency and longer transients than reported for reversed beating by Baba and Mogami (1987). After perfusion of the chamber with dopamine it took approximately 2 min for rotation to be initiated. Since the exchange of medium in this study was completed within 1 min, the concentration of dopamine will reach an effective level for the initiation of the BPTR, within some tens of seconds, which is much shorter than the latency described above. This latency and the long transients associated with the BPTR suggest that it uses different intracellular signal transduction pathways from those used in ciliary reversal, where a transient influx of extracellular Ca^{2+} is considered to induce the response (Degawa *et al.* 1986).

Observation of ‘reversed-turned beating’ (Figs 2, 3) indicates that separate mechanisms exist for the BPTR and for ciliary reversal. In response to appropriate stimulation, the latter mechanisms could be functional even during the operation of the former. This ‘piggybacking’ of ciliary reversal on BPTR is reminiscent of the flip-flop motion seen during the inactive state of the epaulette cilia of the eight-armed pluteus (Mogami *et al.* 1991). The epaulette cilia frequently stop beating and become motionless in the upright posture. Even after the cilia lose their cyclic motion, they spontaneously show a slow an inclination towards the beginning of the effective stroke and maintain this inclined posture. The inclined-inactive state is broken by a quick flip back to the former upright inactive state. This flip-back motion has features similar to the transition from normal to reversed beating. It is, therefore, likely that the epaulette cilia maintain their ability to carry out fast transient responses even after they have switched their basic motility from the active to the inactive state.

The rotation of the beat plane and the resultant 'mirror-image' relationship between the bending pattern of turned beating and that of normal beating (Figs 2, 4) suggest the rotation of the whole motile apparatus without changes in its internal motile activities (model R) or, alternatively, modulation of the internal activities without rotation of the whole apparatus (model A). Rotation of the whole motile apparatus might occur at the boundary of ciliated epithelial cells (model R1) or as a rotation of the basal body (model R2). It seems unlikely, however, that the epithelial cell rotates by itself *in situ* because cell junctions, including septate junctions, are present in developing sea urchin embryos (Spiegel and Howard, 1983), so this model (R1) will not be discussed further. In contrast, the rotation of the basal body might be possible if the striated rootlet connecting with the proximal end of the basal body (Immers and Lundgren, 1972) were contractile, as suggested for amoebo-flagellates (Simpson and Dingle, 1971) and for flagellated green algae (Salisbury and Floyd, 1978; McFadden *et al.* 1987), and could rotate the basal body by pulling it. Ultrastructural studies of the basal rootlets of sea urchin larvae, such as three-dimensional reconstruction and measurements of the band width of striation in the presence and absence of dopamine, will allow critical testing of model R2.

Model A can be subdivided by the mode of the sliding pattern change into two models: model A1 with a 'primarily continuous' (i.e. non-synchronous and time-modulated) transfer of active sliding around the axoneme (Sugino and Machemer, 1990), and model A2 with a 'synchronous' (i.e. discontinuous and group) transfer of active sliding at switch-points (Wais-Steider and Satir, 1979; Satir, 1982, 1985). Gradual reversal in the BPTR seems more compatible with the continuous transfer of model A1, while persistent planar beating during the BPTR is consistent with model A2. Thus, a multiple switching-point hypothesis (Satir and Sleigh, 1990) modified from models A1 and A2 may explain these features of the BPTR. In any case, the BPTR shows that different sets of tubules can generate a similar pattern of bending by rotating the switching point of the effective and recovery stroke. The observation that turned beating is not a strict mirror image of normal beating (Fig. 5) may indicate a subtle difference in positioning of the effective stroke switch between tubules. Using demembranated sea urchin spermatozoa, Shingyoji *et al.* (1991) demonstrated that the beat-plane rotation of model A can be induced by forced vibration.

Tamm and Tamm (1981) concluded from electron-microscopic studies of instantaneously fixed ctenophore comb plates that the central pair of tubules does not rotate during metazoan-type ciliary reversal. However, we cannot exclude a rotation of the central pair in the BPTR, since such a rotation has been demonstrated during ciliary reversal in *Opalina japonica* (Tamm and Horridge, 1970). Whether the rotation of the central pair of tubules, either in ciliate-type reversal (BPTR) or in three-dimensional beating (Satir, 1968; Omoto and Kung, 1979) is active or passive remains to be determined (Tamm and Tamm, 1981).

In addition to changes in the swimming direction of larvae, slowing of forward swimming is recognized as another effect of dopamine on the swimming behaviour of plutei of *P. depressus* and *H. pulcherrimus* (Mogami *et al.* 1992). The down-regulation of the ciliary beat frequency by dopamine described above may explain the reduction in swimming speed in both species, although the increase in the fraction of turned-beating cilia may also be responsible for a reduction in apparent forwardly directed propulsive forces.

An up-regulation of beat frequency, which was anticipated from the observation that serotonin induced an increase in the forward swimming speed, was not clearly seen (Fig. 6). There was a slight increase in the beat frequency (<10 %) in 10^{-5} mol l^{-1} serotonin, while the speed was found to increase to a larger extent for the same concentration (approximately 20 % in *P. depressus* and 70 % in *H. pulcherrimus*; data from Fig. 5 in Mogami *et al.* 1992). This quantitative discrepancy may suggest that the regulatory effects of serotonin affect beating parameters other than beat frequency. In *Paramecium caudatum*, hyperpolarization-dependent augmentation of ciliary beating is accompanied by a posterior shift in the beating direction, which increases the efficiency of ciliary power output for forward movement. However, serotonin did not induce any observable changes in the direction of beating. In ciliated microorganisms, a mutual coordination of the motion of individual cilia (e.g. metachronism) may underlie efficient propulsion and serotonin may enhance such coordination between neighboring cilia. However, metachronism was not apparent either in controls (Fig. 1A) or in the presence of serotonin (not shown), probably because of the lack of coordination or because of the sparse ciliation of sea urchin larvae. The increased stability of beating in the presence of serotonin may cause a stable, and hence possibly effective, mechanical coupling of the individually beating cilia.

Dopamine and serotonin both modulated fluctuation in the beat period, with dopamine disrupting and serotonin stabilizing the rhythm of beating. A possible cause of fluctuation in the beat period is a transmitter-induced regulation of angular velocity (rate of shear angle), which is a possible indicator of sliding activity in the axoneme (Brokaw, 1989, 1991). However, the angular velocity showed a poor correlation with the beat period with or without transmitters, when analyzed beat by beat; the angular velocity even decreased when the beat period was decreased by serotonin. It has been suggested that serotonin, which is known to activate ciliary beating in molluscan gill cilia and sperm flagella (Aiello, 1960; Kadam and Koide, 1990), induces cyclic-AMP-dependent phosphorylation of the dynein light chains in cilia (Stephens and Prior, 1992; Hamasaki *et al.* 1991) and of the α heavy chain in flagella (Stephens and Prior, 1992) which may lead to changes in beat frequency. It has been argued that this increase is directly related to increases in the velocity of translocation of microtubules on 22S dynein under conditions in which associated 29 kDa polypeptides are phosphorylated (Hamasaki *et al.* 1991). It has also been demonstrated, using isolated cortical sheets from *Paramecium tetraurelia* that cyclic GMP or cyclic AMP added to the

reactivating medium modifies the beating of individual cilia and gives rise to a stable metachronal wave in otherwise uncoordinated neighboring cilia (Okamoto and Nakaoka, 1994*a*,*b*). The present study suggests that serotonin, and hence dynein phosphorylation, can stabilize ciliary beating, resulting in an increase in beat frequency, through facilitation of steps in the dynein cycle. The question of whether phosphorylation of the axoneme stabilizes ciliary beating remains to be studied.

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