PH 53

ANALYSIS OF FLAGELLAR ARREST RESPONSE BY Ca^{2+}-IONTOPHORESIS.

Jun Katada, Chikako Shingyoji and Keiichi Takahashi. Zool. Inst., Fac. of Sci., Univ. of Tokyo, Tokyo.

Local application of Ca^{2+} to the basal region of a reactivated sperm flagellum induces a flagellar arrest response. To study the mechanism underlying this response, the changes in waveform during iontophoretic applications of Ca^{2+} were analysed. When a small amount of Ca^{2+} (as a pulse of about 2×10^{-7} coulombs) was applied to a reactivated flagellum of sea urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*, the flagellum gradually changed its waveform: the beat axis tilted toward the reverse-bend side with respect to the longitudinal axis of the sperm head, and the development of a new reverse bend at the base was inhibited. The arrest occurred just after the formation of a new reverse bend was completely blocked. A local application of Ca^{2+} to a reactivated flagellum beating at 1 Hz in a solution containing 10μM ATP induced the arrest response. If Ca^{2+} was applied while a principal bend was developing at the base, the principal bend continued to grow without the initiation of a new reverse bend until the flagellum was arrested in a characteristic cane-shaped bend. These results suggest that Ca^{2+} acts on the mechanism which triggers the initiation of a new reverse bend.

PH 54

REACTIVATION OF TRITON X-100-EXTRACTED TENTACLE OF NOCTILUCA MILIARIS BY H^+ ION

K. Oami[1], T. Sibaoka[2] and Y. Naitoh[1]. [1] Inst. of Biol. Sci. Univ. of Tsukuba, Ibaraki. [2] Biol. Lab. Kyoritsu Woman's Univ., Hachioji.

In order to understand the mechanisms controlling the tentacular movement in a marine dinoflagellate Noctiluca miliaris, Triton X-100-extracted models of the tentacles were examined with various reactivation media. The extracted tentacle (200-300 μm in length) held at the tip of a holding pipette showed flexion when pH of the reactivation medium was lowered. Degree of the flexion was higher at lower pH ranging from 8.5 to 4.0. The degree declined at pH 3. The tentacle extended when the pH was raised. The pH-controlled flexion-extension cycle could be repeated many times. The flexion of the tentacle occurred most conspicuously at the region 1/3-1/4 length of the tentacle distant from its base. This was consistent with the flexion in live specimens. ATP was not needed for the flexion-extension cycle. An increase in $CaCl_2$ or $MgCl_2$ concentration by more than several mM produced a flexion of the tentacle much less than the pH-induced flexion. KCl concentration also affected the degree of the flexion. It is assumed that the extension-flexion in the tentacle of Noctiluca is under the control of intratentacular pH.

PH 55

A REORIENTATION RESPONSE INDUCED BY TRYPSIN DIGESTION ON TRITON-GLYCEROL-EXTRACTED PARAMECIUM CAUDATUM.

M. Noguchi
Dept. of Biol., Fac. of Sci., Toyama Univ., Toyama.

In order to examine the effects of trypsin digestion on the reorientation response of cilia, Triton-glycerol-extracted models of Paramecium were prepared by brief extraction with 1% Triton X-100 instead of original concentration (0.01%). This modification produced improvement in permeability and reactivity of the cell models. The cell models were placed in a narrow space between a slide and a cover slip. When the solution containning Mg, ATP, EGTA and trypsin was perfused, the cilia pointed towards the rear with a small amplitude beating at first. After a few minutes the cilia beated irregularly for a few second and finally pointed towards the front. cAMP and cGMP did not inhibit the trypsin induced anterior response. High concentration KCl (above 0.2 M) inhibited the trypsin induced anterior responce as well as usual anterior and posterior responses. The similar responses were observed in the cilia on cell fragments obtained by sonicating the models. These results might suggest that the Ca^{2+}-dependent regulating mechanism for ciliary beating direction is selectively digested by trypsin, and that the mechanism is essential for keeping the direction of the effective stroke in normal beating.

PH 56

PHYSIOLOGICAL MANIPULATION OF EXCITABLE MEMBRANE BY QUANTITATIVE MICROINJECTION OF AN AMPHIPHILIC SMALL PEPTIDE, MASTOPARAN.

N.Haga[1] and Y.Kuroda[2]. [1]Institute of Applied Biochemistry, Gifu. [2]Department of Neurochemistry, Tokyo Metropolitan Institute for Neurosciences, Tokyo.

Quantitative microinjection has been utilized to find the domain responsible for the membrane excitation in Paramecium. The swimming behavior of Paramecium is a convenient indicator of the excitation of surface membrane. When mastoparan, a potent calmodulin antagonist from Vespid wasp, was applied on either outside or inside of the cells, the behavioral response was decreased. The effect of mastoparan on the behavior was time-dependent and reversible. The median effective dose in these treatments were nearly equal, that is about 10^{-7} M. When the mixture of hepta-peptides of mastoparan, MP1-7 and MP8-14, was applied on Paramecium with twice higher in hydrophobic contents but presumably lower in helical contents than those of MP, no significant decrease was observed in the behavioral response. These data showed that the structural requirement of mastoparan to decrease in the behavioral response was consistent with the structural requierment to bind to the domain in calmodulin. Electrophysiological studies showed that the resting potential was altered by microinjection of mastoparan. These results suggest the presence of mastoparan-binding domain that might be involved in the fundamental process of ion permeation.