CLEAVAGE FURROW ESTABLISHMENT IN CYLINDRICAL SAND DOLLAR EGGS

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In animal cells the mitotic apparatus (MA) accomplishes both chromosome distribution and establishment of the physical mechanism which carries out cytokinesis. In spherical cells, the cytokinetic mechanism is generally considered to be irreversibly established in the cell surface by anaphase when the achromatic portion of the MA achieves maximum development. However, the time of appearance of the furrow can be delayed or advanced by altering the cell's geometry by methods which do not affect the schedule of mitotic events. This apparent dissociation between mitotic timing and the initiation of division implies that cytokinesis is not associated with a specific phase in the chain of mitotic events and suggests that the custom of relating the events of cytokinesis to those of mitosis may not be useful. The principal goal of this investigation was to determine when the position of the cleavage furrow is determined in real time, rather than in relation to mitosis.

The geometrical relations of cell components and the orientation of the division plane were standardized and controlled by reshaping the cells into cylinders. E. parma eggs were denuded by glycine treatment and confined in capillary tubes (approximately 300 μm long by 85 μm i.d.) immediately before completion of first cleavage. The cell usually oriented so that its long axis coincided with that of the capillary. After completion of the first cleavage and before the second, the mitotic apparatus of the two blastomeres oriented parallel to the capillary axis and division subsequently occurred simultaneously in the cells whether or not their volumes were equal provided the distance between astral centers was equal. In this circumstance, events in one blastomere can serve as a time control for the other. Temperature was maintained at 16°.

The time when the furrow position is determined was ascertained by aspirating the MA from one cell and measuring the time between the operation and the appearance of the furrow in the control cell. When the interval between MA removal and control furrowing was four minutes or less the furrow always developed in the operated cell. When the interval was five minutes or more, furrows never developed. The position of the furrow is apparently irreversibly established four minutes before it becomes visible. When one aster is removed more than four minutes before the control furrow appears, no furrow develops in the operated cell, indicating that both asters are necessary for this activity of the mitotic apparatus. Removal, sequestration or continuous stirring of the polar portion of one aster during the period of furrow establishment does not prevent division. When the mitotic apparatus is skewered with a glass needle through its long central axis and the needle is moved back and forth in the polar direction continually during the period of furrow establishment, division ensues even though the normal radiate appearance of the apparatus is absent in the central axis and at the poles of the cell. This investigation was supported by NSF Grant PCM-7902624.

RENAL AMMONIAGENESIS IN THE DOGFISH (SQUALUS ACANTHIAS): ROLES OF GLUTAMINASE AND GLUTAMINE SYNTHETASE

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An increase in renal ammoniagenesis is well documented as part of the kidney's response to acidosis in mammalian species. Glutamine is the major source of urinary ammonia in these animals but the regulation of ammonia production, while well studied, is still unclear (Goldstein, Int. Rev. Physiol., Vol. 11, 283-316, 1976). In comparison, the role of the kidney in the acid-base balance of fish has received little attention. Previously, we have shown that in dogfish, although the absolute urinary ammonia concentration is low, the kidney does respond to acid loading by significantly increasing renal ammonia excretion (King and Goldstein, Bull. MDIBL. 19:77-80, 1979). The current study was

designed to investigate the major amino acid precursor(s) of renal ammonia and the enzymatic control of ammonia formation in the dogfish. In addition we extended parts of this study to four other marine fishes.

Methods: The major substrates for renal ammonia were investigated by measuring ammonia production by dogfish kidney slices incubated in 3.0 ml of Forster's elasmobranch saline which included 10 mM amino acid (gln, asp, ala, gly, or glu). Dogfish were killed by transection of the spinal cord and the kidneys quickly removed and placed on ice. Kidney slices were prepared by hand as previously described (King et al. J. Exp. Zool. 212: 69-77, 1980). Incubations were performed at 15° C for 1 hour (gas phase 99% O_2/1% CO_2) and terminated by the addition of 0.5 ml 2 N perchloric acid. The contents of the flask were then homogenized, centrifuged, and an aliquot of the supernatant taken for ammonia analysis (Chaney and Marbach, Clin. Chem. 8: 130-132, 1962).

The activities of enzymes involved in glutamine metabolism in the kidney, glutaminase and glutamine synthetase, as well as the levels of glutamine in plasma and renal tissue were investigated. Phosphate-dependent glutaminase was assayed in kidney homogenates at 25° C as described by Goldstein (Am. J. Physiol. 210:661-666, 1966). For the assay of glutamine synthetase, 10% homogenates of renal tissue were prepared in 0.9% NaCl and activity levels were measured by the method of Vorhaben et al. (Biochem. J. 135:893-896, 1973). The reaction mixtures were incubated at 25° C for 1 hour. Glutamine and glutamic acid levels were measured by the enzymatic methods of Lund (in Methods of Enzymatic Analysis Vol. 4 1719-1722, 1974). Plasma samples were mixed (1:1) with 1N perchloric acid, centrifuged, and the supernatant neutralized with KOH. Freeze-clamped kidney tissue was homogenized with 0.4 N perchloric acid (5 ml/g tissue), centrifuged, and the supernatant neutralized. The neutralized supernatants were used for analyses.

The subcellular localization of glutaminase and glutamine synthetase in the dogfish renal cell was also examined. The tissue was prepared by homogenization in 0.3 M sucrose and the cell fractions obtained by centrifugation as described by Vorhaben and Campbell (J. Biol. Chem. 247(9): 2763-2767, 1972). The pellets representing the nuclear, heavy and light mitochondrial fractions were resuspended in 0.3 M sucrose and subjected to sonic disruption (75 watt output for 4 minutes, 50% duty time). Activity in each fraction was assayed by the methods referenced above.

Results and Discussion: The major amino acids investigated as renal ammonia sources were glutamine, glutamic acid, glycine, aspartate, and alanine. Their concentrations in dogfish plasma were found to be .078 mM glutamine, .026 mM glutamic acid, .017 mM aspartic acid, .053 mM alanine, .309 mM glycine; amino acid concentrations in the kidney were 3.69 mM glutamine, 3.39 mM glutamic acid, 0.61 mM aspartic acid, 1.58 mM alanine, 1.65 mM glycine. Ammonia production by dogfish kidney slices was greatest in incubations containing glutamine (3.50 $\pm$ 1.46 μmoles NH_3/g·hr, mean $\pm$ S.E., n=4) followed by aspartate (1.30 $\pm$ .55 μmoles NH_3/g·hr, mean $\pm$ S.E., n=4). In incubations with either glycine, alanine, or glutamic acid, less than 1.00 μmole NH_3/g kidney tissue · hr was formed. The high renal glutamine concentration (3.69 mM) relative to the plasma glutamine level (.078 mM) suggests that glutamine is synthesized by dogfish kidney. However, the slice experiments indicate that glutaminase is also active in dogfish renal tissue. This suggests that a substrate cycle between glutamine and glutamate plus NH_3 may exist in the dogfish kidney cell and that the ammoniagenic response to acidosis could be regulated by a modulation in the activities of either glutaminase or glutamine synthetase or both:

$$\text{glutamine} \underset{\text{glutamine synthetase}}{\overset{\text{glutaminase}}{\rightleftharpoons}} \text{glutamic acid} + NH_3$$

Such a strategy has been proposed for the regulation of flux through a 2-way metabolic pathway and is dependent on both enzymes being catalytically active in the control state (Newsholme and Start, Regulation in Metabolism, 1973). Both glutaminase and glutamine synthetase were found to be active in the dogfish kidney (Table 1) at levels of 1.01 μmoles/g·min and 2.41 μmoles/g·min, respectively. The Km values for the two enzymes were similar, 4.48 μmoles

TABLE 1

KINETIC PARAMETERS FOR DOGFISH RENAL GLUTAMINASE AND GLUTAMINE SYNTHETASE

	Glutaminase	Glutamine Synthetase
Activity	1.01 μmoles/g·min	2.42 μmoles/g·min
Km	4.48 μmoles Gln/ml	4.33 μmoles Glu/ml
V_{max}	1.89 μmoles/g·min	3.33 μmoles/g·min
$[Substrate]_{kidney}$	3.69 μmoles Gln/g	3.36 μmoles Glu/g
$[Substrate]_{plasma}$	.078 μmoles Gln/ml	.026 μmoles Glu/ml

glutamine/ml for glutaminase and 4.33 μmoles glutamic acid/ml for glutamine synthetase, and in both cases were close to the in vivo renal concentrations. The subcellular localization of glutamine synthetase and glutaminase in the dogfish kidney cell revealed that both enzymes are located primarily in the heavy mitochondrial fraction. The absence of compartmentation of the two enzymes supports the idea of their simultaneous activity.

The urinary ammonia excretion, urine pH, and renal glutaminase and glutamine synthetase activities of several marine fishes are shown in Table 2. At comparable urine pH values, the urinary ammonia concentration was higher

TABLE 2

URINARY AMMONIA EXCRETION AND RENAL GLUTAMINASE AND GLUTAMINE SYNTHETASE ACTIVITIES FOR VARIOUS MARINE FISHES

	urinary NH_3 excretion (μEq/kg·hr)	urine pH (range)	glutaminase activity (μmoles/g·min)	glutamine synthetase activity (μmoles/g·min)	glutaminase / gln synthetase ratio
dogfish *Squalus acanthias*	.027 (16)	5.5-6.1	1.01 (5)	2.41 (2)	0.42
skate *Raja erinacea*	.640 (3)	4.8-5.1	0.39 (2)	7.78 (2)	0.05
sea raven *Hemipterus americanus*	.372 (3)	5.5-6.5	1.42 (5)	0.07 (2)	20.28
eelpout *Macrozoarces americanus*	.271 (3)	5.5-6.2	1.13 (2)	0.16 (2)	7.10
flounder *Pseudopleuronectes americanus*	.222 (2)	6.6	0.27 (3)	0.07 (2)	3.86

Values are means; the number of fish is shown in parentheses.

in the eelpout, flounder, and sea raven than in the dogfish (urine samples for eelpout and sea raven were obtained from Dr. A. Devries). Analysis of kidney enzymes in these species revealed that while glutaminase levels were fairly constant there were large differences in glutamine synthetase activities which correlated with the difference in ammonia excretion between marine teleosts and the dogfish. The skate was found to have a urine pH approximately 1 unit lower than the other fishes. Because acidification of the skate urine occurs primarily in the kidney (Holliday et al., Bull. MDIBL 19:52-53, 1979), a 10-fold increase in ammonia excretion due to nonionic diffusion alone would be expected. When the urinary ammonia excretion is corrected for this difference in pH, the ammonia concentration of the skate urine decreases from .64 μEq NH_3/kg·hr to approximately .06 μEq NH_3/kg·hr. Thus, as in the dogfish, the skate's corrected low renal ammonia production would be coincident with a high glutamine synthetase activity. Comparing the glutaminase and glutamine synthetase activities among the teleost species, it can be seen that an increasing glutaminase/glutamine synthetase ratio is associated with an increasing urinary ammonia excretion and indicates the potential for control of renal ammoniagenesis via the modulation of these two enzymes. Finally, the high glutamine synthetase activities in the dogfish and skate could also be related to the urea retaining characteristics of the elasmobranchs. Anderson (Science 208:291-293, 1980) has proposed that the biosynthesis of urea in the elasmobranch liver occurs via carbamoyl phosphate synthetase III, a form of CPS that utilizes glutamine as the nitrogen-donating substrate and thus requires that the fish have the capacity for adequate glutamine synthesis.

In conclusion, glutamine appears to be an important source of renal ammonia in the dogfish. Our results indicate that the ammonia production depends on the relative activities of glutaminase and glutamine synthetase and that in dogfish a substrate cycle between glutamine and glutamic acid plus ammonia may exist. This cycle most likely does not operate in teleosts due to the low levels of renal glutamine synthetase found for these species. The tissue slice experiments also suggest that aspartate may be an important renal ammonia source for the dogfish and aspartate amino transferase activities in kidney tissue should be investigated. This work was supported by NSF Grant PCM 79-22476.

EXCHANGE OF RADIOLABELLED POLYETHYLENE GLYCOLS BETWEEN BRAIN AND EXTRADURAL FLUID IN SKATES

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The elasmobranch brain is surrounded by extradural fluid (EDF). This fluid differs in ionic composition from cerebrospinal fluid (CSF) and has a protein concentration approaching that of plasma. Whether there is communication between EDF and the underlying central nervous system is unclear. In a previous communication we presented qualitative evidence for exchange of protein tracers between brain and EDF in skates (Cserr et al., The Bulletin 19:21, 1979). We have now extended our studies of brain-EDF exchange to two radiolabelled tracers: ^{3}H-PEG (polyethylene glycol; 900 daltons) and ^{14}C-PEG (4,000 daltons).

To examine the possible distribution of tracer from brain to EDF, an isotonic solution of the two polyethylene glycols was microinjected into the skate telecephalon (Raja erinacea or Raja ocellata) through a guide cannula implanted several days previously (~0.2 μCi ^{3}H and ~0.03 μCi ^{14}C in 0.4 μl). Radioactivity of various fluid and tissue samples was then determined 0, 1, 4 or 24 hrs after injection into brain. Results at all time periods indicate tracer movement from brain to EDF. Values for samples collected 4 hrs after intracerebral tracer injection are summarized in Tables 1 and 2. Tracer concentration in EDF is higher than in plasma showing that PEG did not enter EDF via the plasma. In higher vertebrates, patent perivascular spaces around the cerebral blood vessels provide a pathway for exchange between brain and its surrounding fluid (CSF in mammals). The high concentration of tracer in vessels sampled from the dorsal surface