CLEAVAGE OF ARTIFICIALLY CONSTRICTED SAND DOLLAR EGGS

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In certain cells, at certain times in the cell cycle, the centers of the asters of the mitotic apparatus are closer to the poles than they are to the equatorial surface. This observation has been incorporated as an essential component of several hypothetical explanations of the way in which the mitotic apparatus establishes the division mechanism in the cell surface of all animal cells. The purpose of this investigation was to determine whether that geometrical relationship is necessary for sand dollar egg cleavage.

Sand dollar (Echinarachnius parma) gametes were obtained by 0.5 M KCl injection. Eggs were mechanically denuded 4 min. after fertilization. About 30 min. before cleavage, the spherical 142 μm diameter cells were inserted into glass loops (78 μm i.d.) so that they were partly constricted into equal parts, and the mitotic apparatus was maneuvered so that it straddled the constriction. In this circumstance, the length of the mitotic apparatus is not significantly affected, but the cell is reshaped into an elongate dumbbell in which the long axes of the cell and the mitotic apparatus are coincident. Artificial constriction increases the distance from the astral center to the pole and decreases the distance from the astral center to the equatorial surface. In this case, the normal relationship is reversed, because the polar surface is located farther from the astral center than the equatorial surface. These cells divide normally.

The geometrical relationship between the mitotic apparatus and the surface can also be changed by treatment with 0.06 M ethyl urethane, which reduces the size of the mitotic apparatus by about one third and blocks cleavage. When the mitotic apparatus of urethance-treated eggs is pushed closer to the surface, furrows develop, suggesting that in spherical cells the reduced mitotic apparatus is unable to establish furrows because its influence does not reach the surface. In order to determine where the critical geometrical deficiency lies, different surface regions were moved closer to parts of the mitotic apparatus. When treated cells are confined in short pieces of 80 μm i.d. glass capillary, the mitotic apparatus orients parrallel to the capillary axis, and the cell divides, even though the polar surfaces are displaced farther than normal from the asters. When similar cells are confined in 115 μm i.d. capillaries, they do not divide, and pushing the polar surfaces inward toward the asters has no effect. Insertion of urethane-treated eggs into 78 μm i.d. glass loops results in cleavage if the plane of artificial constriction lies in the equatorial region between the asters. There is no cleavage if the mitotic apparatus is located elsewhere.

Cells were doubly constricted by partially aspirating them into the reduced orifices (80 μm i.d.) of opposed micropipets. Simultaneous constriction of the sub-polar and sub-equatorial surfaces did not reverse the effect of urethane, although untreated controls cleaved. The results indicate that establishment of the division mechanism is not dependent upon a special geometrical relationship between the mitotic apparatus and the polar surface, and that deficiencies arising from reduction in the size of the mitotic apparatus can be remedied only by shortening the distance from it to the equatorial surface. This investigation was supported by NSF Grant PCM 7902624.

PRIMARY ROLE OF VOLUME EXPANSION IN THE STIMULATION OF RECTAL GLAND FUNCTION

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Previous studies have indicated that expansion of intravascular volume in the shark is the major physiologic stimulus of rectal gland chloride secretion (Amer. J. Physiol., 1984). These studies demonstrated that comparable stimulation of chloride secretion occurred despite the tonicity or chloride concentration of the volume load and regardless of the effect of the volume stimulus on plasma chloride concentration. To further define the role of intravascular volume expansion in this physiologic response, measurements of plasma volume were performed before, during,

and after infusion of either hypertonic saline or isotonic saline in the explanted rectal gland model. This model eliminates neural efferents to the rectal gland and permits evaluation of hemodynamic and humoral mediators of rectal gland function. The simultaneous measurement of chloride secretion confirms that expansion of plasma volume is necessary for stimulation of rectal gland function.

Male and female dogfish were prepared for study as previously described (Bull. MDIBL 21:16-19, 1981). Intravascular infusions consisted of either isotonic shark's Ringers solution (30 ml/kg given at 7 ml/min). In each animal, a 1 hour baseline collection was obtained following which the hypertonic saline was infused. Immediately prior to the infusion of hypertonic saline an equal volume of whole blood was removed from the fish. Two additional hourly collections were then obtained. Finally, isotonic saline was infused and a final hourly collection obtained. Plasma volume was determined at the end of each clearance period by the dye dilution technique using Evans Blue as the marker. One baseline sample and three additional samples taken 5 minutes apart after the infusion of a pre-weighed amount of Evans Blue were used to calculate plasma volume. Chloride secretory rate and rectal gland blood flow were calculated as previously described (ibid.). All venous blood collected was returned to the perfusing fish during the course of the experiment. Rectal gland perfusion pressure was monitored continuously and did not vary more than 5 cm H_2O in a given fish during the course of the experiment.

A total of 7 animals completed the entire protocol. Following the intravascular infusion of hypertonic saline, plasma chloride increased from 266±7 to 299±13, ($p < .05$) in the first hour and subsequently fell slightly to 283±8 in the second hour. As can be seen in Table 1, parallel changes in serum osmolality also occurred. These changes in the serum occurred in the absence of a change in plasma volume which in the baseline period was 63±7 ml/kg and one and two hours following the hypertonic infusions was 68±8 and 52±7 respectively. Despite these significant increases in

INFUSION PERIOD	CHLORIDE CONCENTRATION mEq/l	SERUM OSMOLALITY mOsm/kg	PLASMA VOLUME ml/kg
BASELINE	266 ±7	989 ±14	63 ±7
HYPERTONIC	299 * ±13	1035 * ±11	68 ±8
	283 * ±8	1025 * ±15	52 ±7
ISOTONIC	287 * ±7	993 ±7	88 ** ±2

All data are Mean ± SEM
* = $p<.05$; **=$p<.01$

Table 1

plasma chloride concentration and serum osmolality, rectal gland chloride secretion remained unchanged from baseline (Table 2.) A slight increase in the rectal gland blood flow was noted in the second hour following the hypertonic saline infusion but its magnitude was small.

Table 2

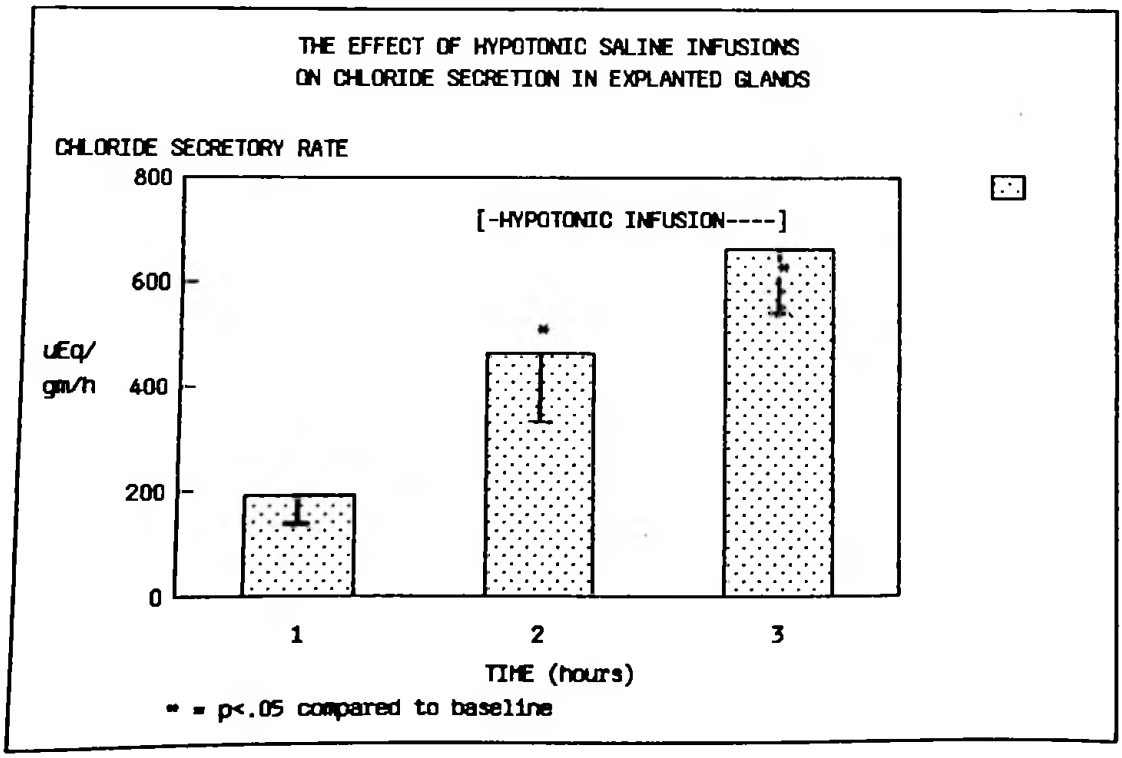
INFUSION PERIOD	DUCT FLOW ml/gm/h	CHLORIDE SECRETION ueq/gm/h	BLOOD FLOW ml/gm/h
BASELINE	.27 ±.09	82 ±21	11 ±1
HYPERTONIC	.30 ±.15	128 ±46	15 ±2
	.27 ±.07	106 ±32	16 ±2 *
ISOTONIC	.99 ±.22 *	361 ±118 *	31 ±6 *

All data are Mean ± SEM
* = p<.05

Following the infusion of an isotonic saline solution a significant fall in serum osmolality occurred such that the osmolality following the isotonic infusion was similar to the baseline serum osmolality. Parallel changes in plasma chloride were not seen although the explanation for this is not clear. However, a significant increase in plasma volume from 52±7 to 88±2 ml/kg, $p < .01$ was observed. This increase in plasma volume at a time that serum osmolality was falling resulted in a nearly threefold increase in chloride secretion and rectal gland blood flow (Table 2).

These experiments for the first time document that plasma volume changes resulting from various intravascular

FIGURE 1



infusions parallel the stimulation in rectal gland function. Infusions which significantly raise serum chloride and osmolality but which do not increase plasma volume are not effective in inducing stimulation of the rectal gland. Previous studies using hypertonic saline solutions (50 ml of 1 M NaCl) had demonstrated that such solutions were capable of stimulating rectal gland secretion (Amer. J. Physiol., 1984). Such solutions, however, probably induced a significant expansion of plasma volume at the same time as indicated by significant decreases in the blood hematocrit values. In the present experiments, plasma volume expansion was minimized over the two hours following the hypertonic infusion by the use of a small volume of fluid and the simultaneous removal of an equal volume of blood prior to the infusion. It should be noted that such a combination of maneuvers was not always successful as in three other experiments not reported here, plasma volume expansion did occur and an increase in rectal gland function was noted simultaneously.

Further support for the primary role of volume expansion can be inferred from the results of infusions of hypotonic (osmolality = 500 mosm/kg; Cl = 140 meq/l) shark Ringers solution. Such infusions in 3 separate experiments (Figure 1) resulted in a significant stimulation of rectal gland function. Although plasma volume and plasma chloride concentrations were not measured in these studies, such infusions would be expected to produce plasma volume expansion at a time that plasma osmolality actually fell. Thus these experiments add further support for the hypothesis that rectal gland function is regulated by mechanisms that are sensitive to and subserve the role of volume homeostasis.

RELATIONSHIP BETWEEN DORSAL AORTIC PRESSURE AND RECTAL GLAND SECRETION RATE IN S. acanthias: A PRELIMINARY REPORT

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In an elegant group of studies using either explanted perfused rectal glands (Solomon, et al., Bull. MDIBL, 21:16, 1981) or in situ glands perfused by blood from a volume loaded donor fish (Solomon, et al., Bull. MDIBL., 22:20, 1982), it was found that an increase in blood flow to the rectal gland always accompanied a volume induced increase in chloride secretion. Rectal gland perfusion pressure did not change in these studies. In a separate study of hemodynamic changes in dogfish in response to volume loading and hemorrhage (Kent, et al., Bull. MDIBL., 14:55, 1974) a comparable volume load, i.e., 150 ml of dogfish Ringers infused into the caudal artery during 15 minutes, caused only a slight increase in dorsal aortic pressure (20–22.5 mmHg). In that study, after 15 minutes the fish were hemorrhaged by 150 ml and dorsal aortic pressure dropped below control pressures. The present study was undertaken to re-examine the effect of volume loading on dorsal aortic pressure and to relate dorsal aortic pressure to rectal gland secretion rate.

Materials and Methods

Five female and one male dogfish were used. All were anesthetized with sodium pentobarbital (20 mg/kg), placed in a dorsal position in an aquarium with 15°C sea water equilibrated with 100% O_2 superfusing the gills at a rate of 1 L/min and prepared for pressure measurement with a 16 gauge needle placed percutaneously in the caudal artery. Rectal gland secretion was collected at 15 minute intervals in volume calibrated vials from a PE 90 catheter introduced into the rectal gland duct during evagination of the postvalvular intestine. Larger fish were given a 250 ml dogfish Ringers volume load via the caudal artery and the smaller fish a 100 ml infusion. The Ringers was infused over a 10 minute period. Epinephrine was injected into the caudal artery and blood was taken from that site during hemorrhage.