ORGAN CULTURE OF ISOLATED SQUALUS ACANTHIAS RECTAL GLAND TUBULES

John D. Valentich, Department of Physiology and Cell Biology, University of Texas Medical School, Houston, Texas 77030

In 1975 Fine and Hays reported that shark rectal gland (SRG) tubules isolated by microdissection showed visual evidence of secretory activity when stimulated by theophylline (Bull. MDIBL 15:37, 1975). More recently, Greger et al. (NIPS 1:134, 1986) demonstrated that isolated perfused SRG tubules exhibit electrophysiologic properties consistent with previous work using isolated perfused glands, cell suspensions and plasma membrane vesicles.

We have found that collagenase-dissociated tubules from the SRG can be successfully maintained as monolayers in primary tissue culture (See this issue of the Bulletin). Best results were obtained when tubules were aliquoted onto the surface of type 1 collagen gels. Cell migration from tubules results in the formation of confluent monolayers. However, if dispersed tubules are suspended in ice cold collagen solution, which is then allowed to polymerize by incubating at room temperature, tubule segments become embedded in the collagen gel matrix. With regular feeding, these cultures can be maintained for at least 19 days with complete retention of tubular morphology (Fig. 1). The culture medium used in this study consisted of equal parts Eagle's MEM and Ham's F12 medium, ITS+ and 5% Nu-Serum (Collaborative Research). Urea, trimethylamine oxide and sodium chloride were added to adjust osmolarity to approximately 950 mOsm/L.

After a few days in culture, the cut ends of tubules become sealed and they exhibit narrow lumens. If 1mM dibutyryl cAMP/1mM theophylline or 1μM forskolin is added to these cultures, a significant increase in tubule diameter is seen (Fig. 2 and Table 1). In addition, the tubules developed a distinctly expanded lumen (Fig. 3). Tubules maintained in vitro for 19 days continued to exhibit a secretory response to forskolin. If stimulated tubules are returned to control culture media and fed every other day, they remain dilated for at least 10 days. These observations are consistent with the interpretation that cAMP/theophylline and forskolin stimulate chloride and fluid secretion in cultured SRG tubule segments. Since the ends of tubules are sealed, secreted fluid cannot escape, leading to dilation of their lumens. However, as shown in Fig. 2, all tubules do not respond to secretory agonists. It is possible that these tubules are insufficiently sealed or unable to sustain an internal hydrostatic pressure essential for dilatation. Cells comprising non-responding tubules were clearly viable, as judged by phase contrast microscopy.

Because of its simplicity and long-term stability, this culture procedure can be used for chronic studies of hormone interactions with SRG cells or as a screening system in environmental toxicology.

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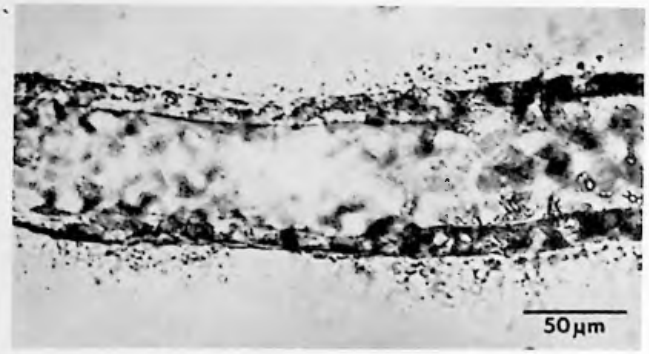

FIGURE 1

Longitudinal optical section through a SRG tubule maintained in culture for 19 days

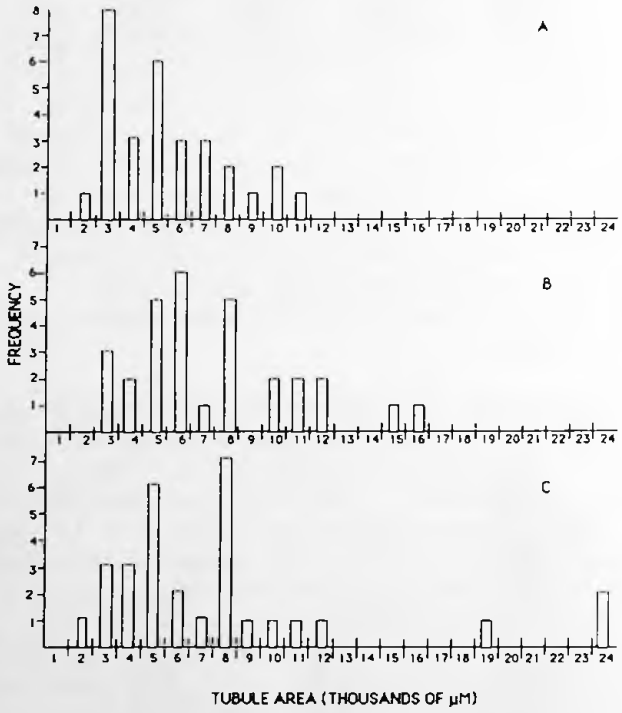

FIGURE 2

Distributions of SRG tubule cross sectional areas calculated from eyepiece micrometer measurements of tubule diameters. Thirty random tubules were measured in control (A), 1µM forskolin (B) or 1 mM dibutyryl cAMP/1 mM theophylline (C) treated cultures.

TABLE 1

	CONTROL	FORSKOLIN	cAMP/THEOPHYLLINE
MEAN TUBULE AREA (μm^2)	4953 (± 2377)	6957 (± 3350)	7406 (± 5493)
MEDIAN TUBULE AREA (μm^2)	4654	5632	5894
n	30	30	30
T-TEST (VS CONTROL)		P<0.01	P<0.05

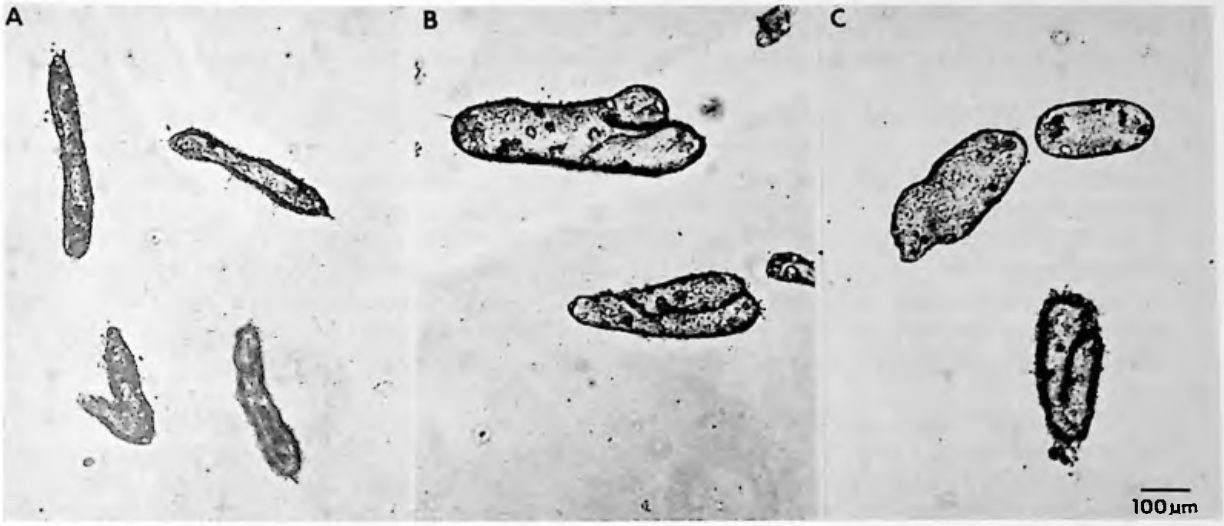

FIGURE 3

Effect of secretagogues on SRG tubules grown in type 1 collagen gels. (A) Control; (B) 1µM Forskolin; (C) 1mM Dibutyryl cAMP/1mM Theophylline.