Short Communications

Electrophysiological properties of the follicle wall in the pig ovary

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Summary. The transmural potential difference and short-circuit current of the porcine Graafian follicle have been measured in an attempt to test whether antral fluid accumulates as a result of active transport of salt. The values obtained by mounting explants of follicle wall in Ussing chambers were close to zero and the specific electrical resistance was only 59 $\Omega \cdot cm^2$. The elemental composition of the follicular fluid was similar to that of ovarian venous plasma with the exception of follicular Na^+ which was slightly more abundant. Bicarbonate concentrations were slightly lower in follicular fluids. These findings were interpreted as evidence that the follicular wall is a leaky epithelium and, therefore, any charge resulting from net ion transport will be shunted along low resistance paracellular pathways.
Key words. Pig ovary; follicular fluid; active transport; trans-epithelial potential difference; short-circuit current; specific resistance.

Graafian follicles are characterized by an epithelium of granulosa cells which encloses a fluid-filled space, the antrum. The volume of the extracellular space in mature follicles is genetically determined and varies allometrically with body weight[1]. The rate of antrum expansion is slow at first but rises during the phase of preovulatory maturation[2]. The timing of this rise indicates that either the pituitary gonadotrophins or other hormones involved in preovulatory activation of the follicle are also responsible for stimulating the movement of fluid from blood to the antrum. However, the physiological mechanisms underlying water transport in the follicle are poorly understood.

The most widely held hypothesis for the accumulation of follicular fluid is based upon a presumptive hydrostatic pressure gradient across the follicle wall[3]. Experimental evidence is lacking, and the formation of small antra in cultured follicles would seem to indicate that other mechanisms are operating[4]. Furthermore, in many epithelia osmotic gradients created by active ion transport are responsible for net water movement[5,6]. The chemical composition and osmolality of follicular fluid and blood plasma are similar[7-9]; yet, this does not necessarily deny a role for active transport of salt because shallow osmotic gradients can cause net movement of water where the hydraulic conductivity of an epithelium is high[10]. Whilst the latter parameter has not been measured in follicles it is presumed to be high because tritiated water and small molecular weight markers rapidly enter the antrum[11,12].

Progress towards understanding ion transport in epithelia has depended heavily upon electrophysiological measurements, most notably of transmural potential difference (PD) and short-circuit current (SCC) under voltage clamp conditions[13]. Such methods have been particularly fruitful when studying electrically tight epithelia, such as frog skin and toad urinary bladder[5,14]. The electrical properties of the ovarian follicle wall have been seldom studied and measurements obtained using Ussing chambers have not previously been published. This paper reports the results of experiments on the preovulatory pig follicle, a structure which is large enough to be studied in this apparatus.

Materials and methods. Preovulatory follicles measuring 7–9 mm diameter were obtained from 5 Large White × Landrace pigs weighing approximately 95 kg. Oestrus was detected by teasing with a boar and ovaries were obtained surgically between 6–36 h of the normal 40-h preovulatory phase. Anaesthesia was induced with pentobarbitone sodium and maintained with a mixture of oxygen-halothane-nitrous oxide. The ovaries were exteriorized through a mid-ventral laparotomy.

Fluids were drawn into syringes from a number of follicles and blood was taken from the ovarian vein. They were centrifuged immediately and stored frozen at −20 °C. The ovaries were ligatured and severed at the hilum. The bulging walls of several large follicles were then dissected and immersed in Hepes-buffered Medium 199 (Flow Laboratories,

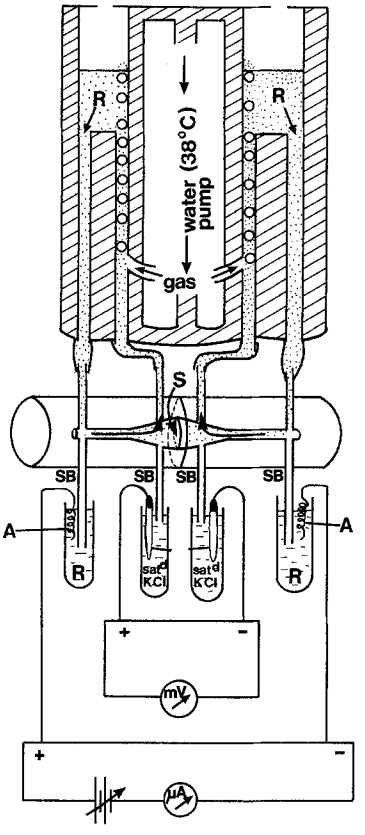

Figure 1. Ussing chamber and apparatus for voltage clamp experiments on the porcine follicle wall. The specimen (S) was spread flat and held in position with stainless steel pins in the perspex chamber. The chamber halves, which were thus separated by the follicle wall, contained identical mammalian Ringer's solution (R) at 38 °C which was circulated by rising gas bubbles (95% O_2, 5% CO_2). The lower half of the figure shows the electromotive force (battery) with the arrangements for measuring current and voltage. Calomel electrodes immersed in a saturated solution of KCl and silver/silver chloride electrodes (A) in Ringer's solution (R) were connected to the Ussing chamber by salt bridges (SB).

Irvine), containing glucose (5.56 mM) which was chilled on ice and gassed with oxygen during transit to the laboratory (60 min after excision).

The elemental composition of the thawed fluids was determined using ion-sensitive microelectrodes (Na^+, K^+), colorimetry (Ca^{2+}, Mg^{2+}) and titration methods (Cl^-). The HCO_3^- composition of freshly obtained samples was measured using an automated blood gas analyser (Radiometer Ltd.).

Undamaged patches of follicle wall were mounted in a specially constructed Ussing chamber (Jim's Instruments Manufacturing Inc., Iowa City, USA). The internal cross-sectional area of the chamber was 0.16 cm^2. The apparatus was based on a conventional design [15, 16] (fig. 1) and loaded with mammalian Ringer solution (pH 7.4) containing glucose (5.56 mM) and sodium pyruvate (0.25 mM). Recordings were made at 38 °C after allowing the specimens to adjust to the experimental conditions for 30 min. Measurements of cellular respiration using an oxygen electrode demonstrated that the tissues were still alive at the end of experiments.

Results. The spontaneous PD of the follicle under open circuit conditions was < 1 mV and the short-circuit current when the voltage was clamped to zero was < 2 μA (table 1). In view of these low values, it was necessary to verify that the apparatus was functioning correctly. Frog skin was therefore mounted in the chambers and recordings were made at room temperature with the appropriate Ringer solution. High transepithelial PDs (> 70 mV) and electrical resistances (> 1000 Ω cm^2) were obtained as expected.

The resistance of the follicle wall was calculated from the slope of the current-voltage relationship obtained by passing current from an external source at predetermined voltages (fig. 2). In every follicle tested the relationship crossed the axes close to the origin and was linear for at least the range from − 35 to + 35 mV. Specific resistances, which averaged 59 Ω cm^2, were calculated on the basis of the area of exposed follicle surface.

The elemental composition of follicular fluid and plasma was identical with two exceptions (table 2). The concentrations of Na^+ were higher but those of HCO_3^- were lower in follicular fluid ($p < 0.01$).

Discussion. Numerous studies have shown that, apart from the high concentrations of locally produced hormones and the absence of suspended cells in healthy follicles, the composition of follicular fluid is similar to that of blood. This conclusion appears to hold for most if not all species, although some minor variations have been found. For example, some, but not all, studies of porcine follicular fluid have found greater concentrations of K^+ in the follicle than in bloos [17–20]. Such differences may, however, be attributed to using post-mortem material or sampling damaged or atretic follicles. In the present study of samples obtained under anaesthesia, K^+ concentrations were the same in the two fluids. On the other hand, a shallow but significant Na^+ gradient was found. This result agrees with findings in rabbits [21] and may indicate the existence of active ion transport by the Na pump. The significance of this gradient for the formation of follicular fluid is obscure at present because most studies have been unable to demonstrate any osmotic gradient across the follicle wall [9]. Whilst potential effects of anaesthetics on ion transport across membranes should normally be taken into account it is doubtful whether they apply in the present situation. Large changes in the flux of Na^+ would be required to explain the observed increase in ionic concentrations in follicular fluid, which amounts to 0.2–0.4 ml, and in the case of the isolated follicular tissue it is expected that anaesthetics would have been washed out or vaporized at an early stage.

Experimental evidence that active ion transport is occurring across an epithelium can be obtained electrophysiologically. In the only substantive study reported, McCaig [22] described a small, variable transmural PD in mouse follicles. Further, he demonstrated that this PD was metabolically coupled and changed with physiological state during the oestrous cycle. However, Mathews and Lipner [23] were unable to detect a PD in either rabbit or rat follicles. The present findings that the PD and short-circuit current across the pig follicle wall are

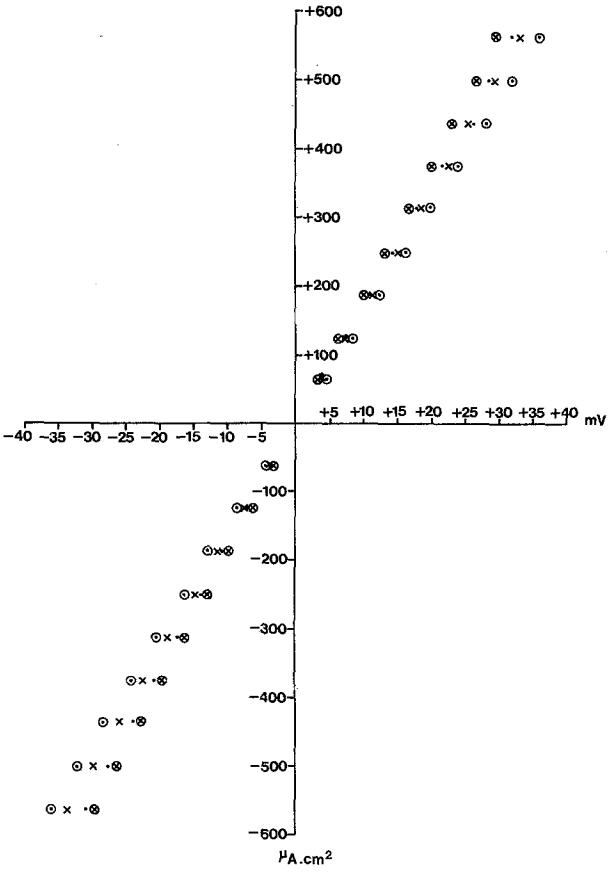

Figure 2. Current-voltage relationships obtained with four porcine follicles (different symbols). The relationships are linear and cross the axes close to the origin.

Table 1. Electrophysiological characteristics of the porcine follicular wall

Transmural potential difference (mv)	Short-circuit current (μA)	Specific resistance (Ω · cm^2)
+ 0.2 ± 0.1	< 2	58.9 ± 2.1

4 animals with 2 or 3 follicles from each means ± SEM given.

Table 2. Concentrations of major electrolytes and bicarbonate ions in porcine follicular fluid and ovarian venous plasma (mmol · l^{-1})

Fluid	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-
Follicular fluid	141.1 ± 0.3*	3.80 ± 0.25	2.30 ± 0.03	0.75 ± 0.02	95.3 ± 1.7	26.0 ± 1.0*
Plasma	137.5 ± 1.1	3.77 ± 0.41	2.27 ± 0.05	0.77 ± 0.04	95.7 ± 3.4	29.9 ± 1.1

5 animals with 4 or more follicles from each. * significantly different compared with plasma ($p < 0.01$ by Student's t-test).

small are consistent with both of the earlier studies. It is not yet clear whether these are biologically negligible.

Such findings could be interpreted as denials of the active transport hypothesis for follicular fluid formation, at least during the more rapid phase of preovulatory expansion. They also show that the specific electrical resistance of the follicle wall (including the granulosa and ovarian surface epithelia) is low and, therefore, electro-chemical gradients are unlikely to be stable. It can be concluded then that the results are not in conflict with the hypothesis which will require other methods for further testing. The near or complete absence of a measurable PD and short-circuit current across the follicle wall is not particularly surprising since the same properties are encountered in other 'leaky' epithelia, e.g. gallbladder, choroid plexus, renal proximal tubule, small intestine [5]. In these cases, the cell membrane resistance typically exceeds the transepithelial resistance by a large margin [24] and the linear current-voltage relationship indicates that current is being conducted paracellularly, a conclusion which agrees with the absence of morphologically recognisable 'tight' junctions between granulosa cells [25]. Consequently, it is probable that the major pathway for entry of water into the antrum is paracellular, although the forces involved, whether active transport and/or transudation, require further clarification.

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Ventricular fibrillation threshold during acute ischemia in hypertrophied rat hearts

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Summary. Ventricular fibrillation threshold was significantly lower in hypertrophied hearts than in normal hearts. Ischemia produced by coronary occlusion reduced fibrillation threshold in both normal and hypertrophied hearts, but the maximum reduction in fibrillation threshold was observed earlier in hypertrophied hearts.
Key words. Ventricular fibrillation threshold; cardiac hypertrophy; pressure overload; ischemia.

Left ventricular hypertrophy is associated with an increased risk of sudden cardiac death in coronary artery disease [1, 2]. The mechanisms responsible for this high incidence of sudden death are still obscure. One possible explanation is an increased vulnerability to ventricular tachyarrhythmias, especially ventricular fibrillation (VF), during ischemia in hypertrophied hearts [3]. Ventricular fibrillation threshold (VFT) has long been used to assess vulnerability to arrhythmias in experimental animals [4]. Using this technique, we compared VFT changes during ischemia between normal and hypertrophied rat hearts.

Methods. Left ventricular hypertrophy was created by partial occlusion of the abdominal aorta to produce chronic pressure overload. Male Sprague-Dawley rats (370–485 g) were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). A polyethylene cannula and a small animal respirator were used to provide mechanical ventilation. Under aseptic conditions, a 4-cm midline incision was made terminating at the xiphoid process. The abdominal aorta (between the diaphragm and celiac artery) was exposed and looped with 3–0 silk suture. The suture was tightened around a 25-gauge needle, and then the needle was withdrawn. The rats were placed in an incubator until they regained consciousness, and kept in colonies until the day of study. At terminal study, 6–8 weeks after surgery, the animals were weighed, anesthetized, and treated with heparin sodium (300 μg/kg, i.p.). Age-matched normal rats were treated in the same way to provide control data. Systemic blood pressure was recorded through a polyethylene catheter inserted in the carotid artery. After thoracotomy, the hearts were quickly excised and placed in preoxygenated Tyrode's solution. Each heart was mounted by the Langendorf method on a perfusion apparatus by cannulation of the aorta, and perfused retrogradely with Tyrode's solution from a reservoir at a constant pressure of 95 cm H_2O in hypertrophied hearts, and of 65 cm H_2O in normal hearts. These perfusion pressures were deter-