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Growth factor activity in luminal fluids from the male reproductive tract of the ram, rat, tammar wallaby (*Macropus eugenii*) and Japanese quail (*Coturnix coturnix japonica*)

R. C. Jones, A. L. Walsh, B. P. Setchell* and J. Clulow

*Department of Biological Sciences, University of Newcastle, New South Wales 2308, Australia; and *Department of Animal Sciences, Waite Agricultural Research Institute, University of Adelaide, South Australia 5064, Australia*

Summary. Growth factor activity in luminal fluids from the male reproductive tract was assayed by measuring the stimulation of [^{3}H]thymidine incorporation into BALB/c 3T3 fibroblasts. The potency of fluids from the rete testis of the rat, ram, tammar wallaby and Japanese quail was much the same. However, about 90% of the activity in fluid from the rete testis of the rat and tammar was lost during its passage through the epididymis.

Keywords: seminiferous growth factor; rete testis fluid; epididymis

Introduction

A potent mitogen that induces DNA synthesis and cell division in cultures of confluent mouse 3T3 fibroblasts was identified in Sertoli cells of the prepubertal and adult mouse and the seminiferous epithelium of the rat, guinea-pig and calf (Feig *et al.*, 1980, 1983), and in fluid collected from the rete testis of the ram (Brown *et al.*, 1981, 1982). The seminiferous growth factor has been identified as a peptide which is sensitive to proteases, heating at 100°C for 2 min, but not sensitive to dithiothreitol. The molecular weight was estimated to be 15 700 when assessed by denatured polyacrylamide gel electrophoresis (SDS-PAGE: Feig *et al.*, 1983), but after some purification it formed 7 bands in SDS-PAGE, corresponding to molecular weights of 14 000 to 20 000 (Feig *et al.*, 1983). The growth factor in rete testis fluid was inactivated by proteases and β-merceptoethanol, but not by heating to 100°C for 20 min. Its molecular weight was 45 000 as assessed by gel filtration through Ultragel AcA 34 (Brown *et al.*, 1982).

Feig *et al.* (1980, 1983) suggested that the growth factor could play a role in regulating spermatogenesis whilst Brown *et al.* (1981, 1982) added that, as it is present in rete testis fluid, it could play a role in regulating the activity of the initial segment of the epididymis. This initial segment is a structurally distinct region of the ductus epididymidis which is unique to mammals, and is dependent for its normal development and function on a luminal connection with the testis (see Jones *et al.*, 1987, 1988).

The studies described in this report examined the occurrence of the seminiferous growth factor in mammals by determining its activity in fluid from the rete testis of an Australian marsupial, the tammar wallaby (*Macropus eugenii*), and comparing its potency with rete testis fluid from the ram and rat. The fate of the growth factor was determined by comparing its activity in fluid from the rete testis and cauda epididymidis of the tammar and rat. Further, to obtain circumstantial evidence about the potential role of the growth factor in regulating the function of the initial segment of the epididymis, rete testis fluid from the Japanese quail (*Coturnix coturnix japonica*) was

examined for activity as there is no initial segment in the ductus epididymidis of the bird (see 'Discussion').

Materials and Methods

Samples of luminal fluids were collected from adult animals. Rete testis fluid was collected from Merino rams after cannulation of the rete testis of animals under pentobarbitone-halothane anaesthesia (Suominen & Setchell, 1972). Samples of rete testis fluid from the rat, tammar and Japanese quail and fluid from the cauda epididymidis of the rat and tammar were collected using micropuncture procedures (Jones, 1987).

BALB/c mouse embryonic fibroblasts (3T3, Commonwealth Serum Laboratories, Melbourne, Victoria, Australia) were maintained in tissue-culture flasks (Lux, Miles Scientific, Naperville, IL, USA) containing Dulbecco's modified Eagle's medium (DMEM: Gibco Laboratories, Grand Island, NY, USA) supplemented with 5% (v/v) fetal calf serum (Flow Laboratories, North Ryde, NSW, Australia), 60 μg penicillin/ml and 50 μg streptomycin sulphate/ml. The cells were passaged at intervals of about 7 days so that they did not reach confluence.

The assay system is based on the method described by Feig *et al.* (1980, 1983) who counted 3T3 cells to demonstrate a correlation between uptake of [^{3}H]thymidine and mitosis of the 3T3 cells. For the assays, the cells were washed twice with phosphate-buffered saline, harvested by treatment with trypsin, and resuspended at a concentration of 5×10^4 cells/ml in DMEM containing 5% (v/v) fetal calf serum. Samples (200 μl) of the suspension were transferred to individual culture wells (Tissue Culture multi-well plate, Limbro, Flow Laboratories, Inc., McLean, VA, USA) and incubated at 37°C in a humid atmosphere of 5% CO_2 in air until the cells were confluent (7–10 days). The wells were then washed 3 times with phosphate-buffered saline, and fresh DMEM containing [^{3}H]thymidine (1 μCi/well: sp. act. 21 Ci/mmol: Amersham International, Amersham, UK) and aliquants of the test reproductive fluids were added in a total volume of 250 μl. The cultures were incubated again as described above for a further 48 h. Subsequently, the cells were washed 3 times with 5% trichloracetic acid and dissolved in 0·3 M-sodium hydroxide (300 μl). Scintillation fluid (4 ml; Liquiscint, National Diagnostics, Somerville, NJ, USA) was added and the samples counted for tritium. The incorporation of [^{3}H]thymidine into the 3T3 cells is expressed as counts per minute (c.p.m.) per well.

The percentage of solute (growth factor activity) which was reabsorbed from the seminal fluid between the rete testis and cauda epididymidis was calculated by the formula:

$$\% \text{ solute reabsorbed} = \frac{(C_1 - C_2 \cdot S_1/S_2)}{C_1} 100$$

where C and S are solute concentrations and spermatocrits respectively and the subscripts refer to the proximal and distal sites respectively.

Results

Rete testis fluid from the ram, rat, tammar and quail all contained something which stimulated the incorporation of [^{3}H]thymidine by the cultures of 3T3 cells (Fig. 1). The slope of the dose–response lines was much the same for fetal calf serum and all the fluids and there was no significant difference in the potency of rete testis fluid from the 4 species. However, fetal calf serum was about 8 times as potent as the samples of rete testis fluid.

Table 1 compares the effect of fluids from the rete testis and cauda epididymidis of the rat and tammar on the incorporation of [^{3}H]thymidine by the 3T3 cells. Based on the change in spermatocrits which occur between the ductuli efferentes and cauda epididymidis (Table 1) it was calculated that most of the growth factor activity was removed from the seminal fluid as it passed through the epididymis (96% for the rat and 87% for the tammar).

Discussion

As the slopes and mean responses of the dose–response lines for the determination of mitogenic activity of fluid from the rete testis of the rat, tammar and quail were much the same as for the ram, it is concluded that we are dealing with the same growth factor reported by Brown *et al.* (1981, 1982). Its presence in rete testis fluid from 3 mammals, representing 2 subclasses of Mammalia, and one bird, indicates that its occurrence may be widespread amongst endotherms. Its potency in rete

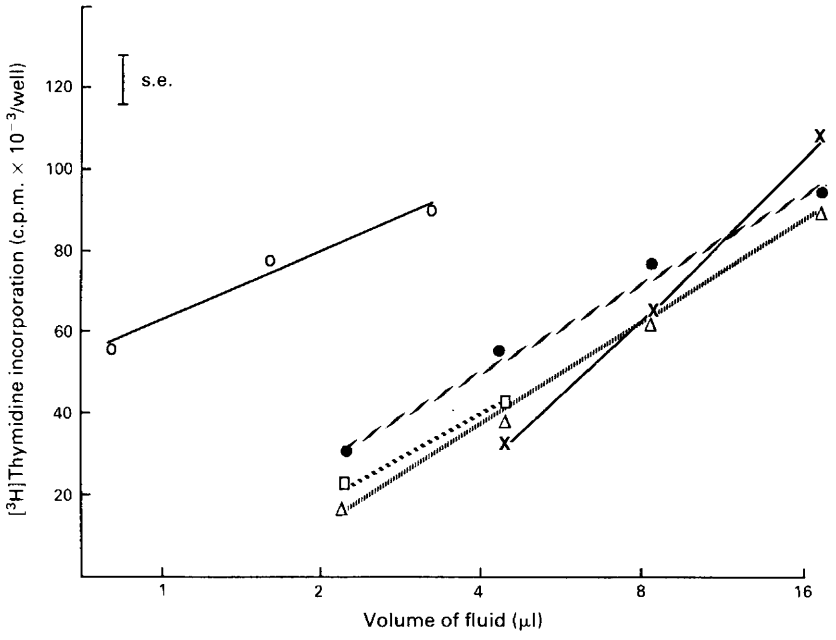

Fig. 1. The incorporation of [^{3}H]thymidine into cultures of 3T3 cells in the presence of fetal calf serum (○——○) or samples of rete testis fluid from the rat (●– – –●), ram (×——×), tammar (△···△), and Japanese quail (□– – –□). Values are means from 5 animals, of c.p.m. per well minus control values for cells with no added biological fluid. The magnitude of the pooled s.e. of the means is shown.

Table 1. The effect of fluid from the rete testis (RTF) and cauda epididymidis (CEF) from the rat and tammar on the incorporation of [^{3}H]thymidine (c.p.m. × 10^{-3}/well) into 3T3 cells

Sample	Volume of fluid			Spermatocrit* %
	0 μl	2 μl	4 μl	
Rat				
RTF	12 ± 3·2	36 ± 6·8	63 ± 14·4	3·3 ± 0·3
CEF	16 ± 4·5	—	50 ± 8·0	55·5 ± 1·7
Tammar				
RTF	12 ± 3·2	29 ± 6·2	48 ± 6·1	1·5 ± 0·2
CEF	7 ± 0·5	130 ± 7·2	147 ± 14·0	61·3 ± 2·6

Values given are means ± s.e.m. for samples from 5 animals.
*Spermatocrits from Hinton (1979) and Jones (1987) show the magnitude of fluid reabsorption in the epididymis.

testis fluid was only one eighth that in fetal calf serum when expressed per unit volume of fluid. However, as the protein content of fetal calf serum (50 μg/μl) is much greater than that of rete testis fluid (1 μg/μl; Setchell, 1978; Jones, 1987; Clulow, 1988), the growth factor activity of rete testis fluid is 6-times more potent than fetal calf serum when the potency is compared on the basis of protein in the fluid. This is about half the relative potency determined for ram rete testis fluid by Brown *et al.* (1982) who used sources of fetal calf serum and mouse fibroblasts different from those used in this study.

The finding that the potency of the seminiferous growth factor activity is much the same in samples of rete testis fluid from the quail and the three mammals which were examined in this study suggests that regulation of the activity of the initial segment of the epididymis cannot be its main function, as this structure had not been found in any of the birds examined to date (e.g. Aire, 1979; Tingari, 1972).

It is not possible to resolve the significance of the reabsorption of about 90% of the growth factor activity present in rete testis fluid by the excurrent ducts of the testis. Work on the tammar (Jones, 1987) indicates that about 50% of the protein leaving the testis is reabsorbed by the ductuli efferentes and more is reabsorbed in the corpus epididymidis. A similar pattern also occurs in the elephant (Jones, 1980) and echidna (Djakiew & Jones, 1983) and the process seems to involve the specific and obligatory uptake of proteins by the epithelium lining the ducts (Djakiew & Jones 1983; Hermo & Morales, 1984; Djakiew *et al.*, 1985; Jones, 1987). The only known quantitative data for the uptake of a specific protein by the excurrent duct epithelium is for androgen-binding protein in the rat (Turner *et al.*, 1984): estimates from these data indicate that 52% is removed from the luminal fluid between the rete testis and middle–distal caput epididymidis and 95% is removed between the rete testis and cauda epididymidis.

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