Rat Sertoli cells in culture undergo metabolic co-operation with each other and with fibroblasts

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Summary. Metabolic co-operation between Sertoli cells from adult rats was detected by adding one group of cells, which were the recipients, to a second group of cells, which had been labelled for 3 h with [^3H]uridine and were the donors. Metabolic co-operation also was studied by co-culturing Sertoli cells, which were the donors, with human or Chinese hamster HGPRT⁻ fibroblasts (recipients) in the presence of [^3H]hypoxanthine. With both techniques the recipients in contact with donors had significantly more radioactive grains than did the recipients alone. In all cases the proportion of interactions that were positive for metabolic co-operation was greater than 80%.

Introduction

Cell-to-cell communication via gap junctions is one way that Sertoli cells could regulate spermatogenesis. Sertoli cells are in intimate contact with germ cells and are thought to aid the process of spermatogenesis (Russell, 1980). Gap junctions have been found between these two cell types in the rat and these structures are lost as the spermatids elongate (McGinley, Posalaky, Porvaznik & Russell, 1979). Gap junctions are also observed between Sertoli cells (Dym & Fawcett, 1970) and specifically in the tubulobulbar complexes that are formed between adjacent cells (Russell, 1979). These tubulobulbar complexes undergo cyclic formation and elimination during the spermatogenic cycle and this behaviour could influence the spermatogenic process (Russell, 1979). As rats mature, the number of gap junctions between Sertoli cells declines (Gilula, Fawcett & Aoki, 1976; Meyer, Posalaky & McGinley, 1977), and the gap junctions in adults are described as atypical (Gilula *et al.*, 1976).

Metabolic co-operation, which has also been termed contact-dependent metabolite transfer (Boliska & Bols 1982), is an in-vitro measure of cell-to-cell communication that is mediated by gap junctions (Hooper, 1982). The phenomenon was discovered by Subak-Sharpe, Burk & Pitts (1966) in co-cultures of cells that were positive (HGPRT⁺) or negative (HGPRT⁻) for hypoxanthine-guanine phosphoribosyl transferase. When the cultures were exposed to [^3H]hypoxanthine, the HGPRT⁻ cells became labelled only if they were in contact with HGPRT⁺ cells. Pitts & Simms (1977) later developed a method of measuring metabolic co-operation that did not require HGPRT⁻ cells. A population of cells, which were to be the donors, were labelled with [^3H]uridine, and the unincorporated [^3H]uridine was washed away before culture of these cells with an unlabelled population of cells (the recipients). The presence of grains over recipient cells in contact with donor cells and the absence of grains over recipient cells by themselves indicated the occurrence of metabolic co-operation. In both these assays [^3H]nucleotides were exchanged between cells (Cox, Krauss, Balis & Dancis, 1976; Pitts & Simms, 1977; Bols & Ringertz, 1979).

Although lymphocytes (Cox *et al.*, 1976) and macrophages (Kane & Bols, 1980) did not participate in metabolic co-operation, primary cultures and cultures that have been passaged only a

few times have shown selectivity or a lack of selectivity in this ability. Breast epithelial cells co-operated with one another, as did breast fibroblasts, but co-operation between the two different cell types did not occur (Fentiman, Taylor-Papadimitriou & Stoker, 1976; McKay & Taylor-Papadimitriou, 1981). With cells of a different origin Hunter & Pitts (1981) found a lack of selectivity: metabolic co-operation occurred between epidermal keratinocytes and dermal fibroblasts with either cell type acting as the donors, and these cells also acted as recipients with epidermal melanocytes. Another example of communication in a heterologous combination has been reported by Lawrence, Beers & Gilula (1978) who found that myocardial cells underwent metabolic co-operation with ovarian granulosa cells.

As a measure of cell-to-cell communication via gap junctions, the ability of Sertoli cells from adult rats to undergo metabolic co-operation in culture and to show selectivity in this property has been tested.

Materials and Methods

The technique of prelabelled donor cells that was developed by Pitts & Simms (1977) was used to measure metabolic co-operation between Sertoli cells. Cultures of Sertoli cells were prepared from adult Wistar rats by the method of De Martino *et al.* (1977) and maintained at 37°C in 6-cm Petri dishes in an atmosphere of 5% CO₂ and 95% air. The cells were maintained for up to 7 days with Eagle's modified minimal essential medium (MEM) supplemented with 10% newborn-calf serum. The cells that were to be the donors were removed from the growth surface with trypsin and plated onto haemocytometer coverslips, and the culture of cells that were to be the recipients received 20 µl of a latex bead suspension (Polysciences Inc., Warrington, PA, U.S.A.). On the next day the coverslip cultures were labelled for 3 h with [³H]uridine (Amersham Radiochemical Center, Arlington Heights, IL, U.S.A.) at 2.5 µCi/ml and then rinsed 4 times with medium. At this point the recipients were rinsed free of beads that had not been phagocytosed, trypsinized from the growth surface, and added to the coverslip culture of donors. After 3 h these cultures were fixed and processed for autoradiography as described previously (Bols & Ringertz, 1979). The autoradiograms were stained with the fluorochrome H33258 under conditions that stain nuclei (Hilwig & Gropp, 1975).

The specificity of the interactions that Sertoli cells can develop was tested by co-culturing the HGPRT⁺ Sertoli cells with human (GM0377) or Chinese hamster (GM0459 or CHW-1102) HGPRT⁻ fibroblasts, which were purchased from the Human Genetic Cell Repository, Camden, NJ, U.S.A. The HGPRT⁻ cells were grown overnight with latex beads before the excess beads were washed away; the cells were then trypsinized and added to coverslip cultures of Sertoli cells. The cells were allowed to make contact overnight and on the next day [G-³H]hypoxanthine (sp. act. 1.2 Ci/mmol) (Amersham) was added to give 5 µCi/ml. The cultures were fixed after 4 h.

Autoradiograms were examined with a Nikon fluorescence microscope that was equipped with phase optics. Each microscope field was examined under phase contrast to determine the cell contacts and the presence of beads, and under u.v. illumination to detect nuclei. Recipient cells were identified in all experiments by the presence of 3 or more beads in the cytoplasm; the donor cells were identified by the absence of beads. In heterologous cell combinations morphological differences could also be used to distinguish recipient from donor cells: the epithelial-like morphology of the Sertoli cells contrasted with the fibroblast-like morphology of the HGPRT⁻ cells; in addition the CHW-1102 cells were smaller than the donor cells. Recipient cells in direct contact with donors were termed primary recipients those that were not in contact with donor cells, background cells (Pederson, Sheridan & Johnson, 1980). Indirect interactions, such as a recipient in contact with a donor via contact with a primary recipient were not scored. The number of grains over the nuclei of primary recipients was compared to the number over the nuclei of background cells by the Mann-Whitney U test (Zar, 1974; p. 109). Interactions in which the primary recipient

Figs 1—3. Metabolic co-operation between Sertoli cells is demonstrated in this three-stage analysis of an autoradiogram. In **Fig. 1** the cell outlines are in focus, and one primary recipient (P), two background cells (B), and two donors (D) are seen. The arrows indicate latex beads. In **Fig. 2** the grains are in focus, and grains can be seen over the nucleus (arrow) of the primary recipient but not over the nuclei of background cells. In **Fig. 3** the location of the nuclei is revealed by fluorescence microscopy. 200 $\times$.

cell had more grains than the value of either the 95 percentile (Pederson *et al.*, 1980) or twice the 90th percentile (Gaunt & Subak-Sharpe, 1979) in the background-cell distribution were deemed positive for metabolic co-operation. These represent a liberal and a conservative estimate respectively.

Results

Metabolic co-operation was examined between Sertoli cells (Pl. 1, Figs 1–3) and between Sertoli cells and fibroblasts. A comparison of the distribution of grains over Sertoli cells in contact and not in contact with donors cells is illustrated in Text-fig. 1. In all combinations the distribution of grains over recipient cells in contact with donors was significantly greater than the distribution over recipient cells not in contact ($P < 0.01$), i.e. in all co-cultures metabolic co-operation took place.

Text-fig. 1. Number of grains over the nuclei of 100 Sertoli cells that have been co-cultured with prelabelled Sertoli cells (donors) and are (a) in contact with the donor cells or (b) not in contact. The 95th percentile is shown by the thin arrow, $2 \times$ the 90th percentile, by the thick arrow.

Table 1. Ability of rat Sertoli cells to act as donors in metabolic co-operation

Recipient cells	Metabolic co-operation technique	Exp.	Mean $\pm$ s.e.m. no. of grains†		Ratio of means	Proportion of cells co-operating‡	
			Primary recipient cells	Background cells		95th percentile	$2 \times$ 90th percentile
Sertoli cell	Prelabelled donor	1	57.3 ± 2.0	10.3 ± 0.6	5.6	100	88
		2	94.0 ± 1.7	5.7 ± 0.4	16.5	98	98
HGPRT ⁻ Chinese hamster fibroblasts	Co-culturing with [³ H]hypoxanthine	1	31.2 ± 1.7	3.0 ± 0.3	10.4	90	86
		2	61.8 ± 4.0	1.9 ± 0.2	32.5	88	81
HGPRT ⁻ human fibroblasts	Co-culturing with [³ H]hypoxanthine	1	62.7 ± 2.5	1.9 ± 0.2	33.0	100	100
		2	93.2 ± 1.8	15.2 ± 1.3	6.1	97	90

* Each experiment represents a separate preparation of Sertoli cells.

† Per 100 cells.

‡ i.e. liberal and conservative estimates.

With Sertoli cells or human fibroblasts as the recipient cells, close to 100% of the interactions were positive and about 90% of the interactions with CHW-1102 cells were positive. These results are summarized in Table 1.

Discussion

The demonstration of metabolic co-operation between Sertoli cells *in vitro* suggests that these cells are capable of cell-to-cell communication *in vivo*. However, the possibility remains that communication-competent junctions develop only when the cells are put into culture. In the case of Sertoli cells in adult rats, the gap junctions have been described as unusual and infrequent (Gilula *et al.*, 1976; McGinley *et al.*, 1979). Whether this junctional phenotype is maintained *in vivo* is not known. However, the variety of junctions between Sertoli cells that occur *in vivo* are seen when testis fragments are maintained in organ culture (Bigliardi & Talluri, 1976; Posalaky, Meyer & McGinley, 1981) and the unique tight junctions between these cells are present in cell culture (Tung, Dorrington & Fritz, 1975; Janecki, Lukaszyk & Jakubowiak, 1981).

Despite their unusual gap-junction morphology *in vivo*, Sertoli cells *in vitro* did not show selectivity in cell-to-cell communication. This is supported by the work of Hutson (1983) who found that rat Sertoli cells exhibited metabolic co-operation with peritubular cells. A lack of specificity in metabolic co-operation has been observed for other primary cultures (Lawrence *et al.*, 1978; Hunter & Pitts, 1981; Larson & Sheridan, 1982). This could mean that the restriction on cell-to-cell communication *in vivo* is simple physical separation and/or a more subtle mechanism that is lost *in vitro*. Primary cultures that do show selectivity (McKay & Taylor-Papadimitriou, 1981) might retain such a mechanism.

The measurement of cell-to-cell communication between Sertoli and germ cells is unlikely to be an appropriate assay for metabolic co-operation. The technique of metabolic co-operation requires the bringing together of two separate populations of cells and having them attach to a substrate suitable for autoradiography. Germ cells do not attach to growth surfaces (Davis & Schuetz, 1975) and although they might be made to adhere to Sertoli cells that are attached (Grootegeod, Jutte, Rommerts, van der Molen & Ohno, 1982), this configuration of cells would be difficult to analyse in autoradiograms. A better approach would be to prepare testicular cultures in a manner that maintains the physical link between the two cell types (Palombi *et al.*, 1979). In these cultures cell-to-cell communication could perhaps be measured by ionic coupling or dye transfer (Loewenstein, 1981).

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