Folliculo-stellate Cells: Paracrine Communicators in the Anterior Pituitary

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Abstract: Most research on the anterior pituitary (adenohypophysis) has concentrated on the endocrine cells characterized by their complement of cytoplasmic dense-cored vesicles containing the classic anterior pituitary hormones. However it has become increasingly clear over the last 20 years that cells first identified more than 50 years ago in the basis that they lack such dense-cored vesicles and now known generically as folliculo-stellate or follicular cells have important physiological functions and act as an adenohypophysis wide communication system. This brief review reveals the need for this communication system, what we know of the plethora of products secreted by Folliculo-Stellate cells, the many receptors to which they respond, and in particular, the role of these enigmatic cells in the physiology of the stress/immune axis, the gonadotroph cells and the pituitary vasculature. Finally we review the current evidence that cells in this category can act as stem cells in the adult pituitary.

Keywords: Annexin 1, ABC-transporter, folliculo-stellate.

THE NEED FOR COMMUNICATION IN THE ANTERIOR PITUITARY

The need for local communication in the nervous system has always been accepted, but has often been overlooked in the anterior pituitary gland [1]. Rather, since the discovery of control of the pituitary from the hypothalamus and from feedback by peripheral hormones, there has been the implicit assumption that these two are sufficient to control the multiple scattered cells that comprise at least five major types of classic endocrine cells. However, this assumption ignores the need for communication between the different hormonal systems in the gland. Carl Denef and his group have pioneered the study of communication between many of the cells types and this work has already been well reviewed [2]. This review will concentrate on recent findings on the role of the 'non-endocrine' F-S (F-S) cells in providing a communication system throughout the anterior pituitary, and in particular its roles in the hypothalamo-pituitary-adrenal (HPA) stress axis including links with the immune system, the hypothalamo-pituitary gonadal axis, and the pituitary vasculature.

F-S cells form a meshwork throughout the anterior pituitary, linked by gap junctions and are able to signal via calcium waves [1, 3]; they produce numerous bioactive molecules and express many different types of receptor, but the intracellular mechanism of action of the receptors and the precise functional role of the signalling molecules produced by F-S cells is still unclear in many cases.

IDENTIFICATION OF F-S CELLS: SUBTYPES

F-S cells were first clearly identified in early electron microscopic investigations of the anterior pituitary on the basis of morphological criteria [4]. They were named for their extensive star-like radiating processes and for the microvillus-lined follicles which are formed where the F-S cells come together. They form about 10% of the cell population of the adenohypophysis [5]. The introduction of immunocytochemistry added the presence of S-100 protein [6] and the absence of other classic anterior pituitary hormones as a second identification criterion which has been used extensively. Since then F-S cells and cell lines have been shown to produce many other markers (e.g. vimentin, annexin A1 (Anx A1), glutamine synthase) and bioactive molecules (see below). The finding that some F-S cells produce certain signalling molecules but apparently not others has led to an understanding that F-S cells are not a homogeneous group [7, 8]. However, apart from studies on laser-captured F-S cells which showed that these native F-S cells and the TtT/GF cell line (see below) expressed the mRNAs for glial fibrillary acidic protein (GFAP), S100 protein, transforming growth factor-β1 (TGFβ1), TGFβ receptor, interleukin-6, leptin, the leptin receptor, pituitary adenylate cyclase-activating polypeptide (PACAP), and PACAP receptors [9,10], there have been relatively few studies on gene expression by native F-S cells or on the stimulatory or inhibitory action of their numerous receptors on the synthesis of their biomarkers. This coupled with the difficulty of showing by immunocytochemistry that a cell does *not* produce a biomarker, means that the understanding of the heterogeneity of F-S cell subtypes is in its infancy.

F-S cells are linked by gap junctions which, like gap junctions elsewhere, are dynamic structures allowing variable degrees of communication. The extent of this communication has been investigated in a large series of experiments by Soji and Herbert [11, 12] and is increased by a number of the signals that act on F-S cells (see below). There is also some evidence for a few gap junctions between F-S and endocrine cells [13].

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Relatively little is know about the life history of F-S cells, including their origin. but their number appears to decrease during aging in both rats [14] and humans [15]. In contrast, the expression of glutamine synthase in rats increases markedly from the neonatal period to adulthood [16].

MORPHOLOGY OF F-S CELLS AND THE FOLLICLES

F-S cells are small cells which at first sight are of rather uninteresting appearance. The nucleus is ovoid with both hetero- and euchromatin, surrounded by relatively sparse cytoplasm containing mitochondria, some rough and smooth endoplasmic reticulum and generally few lysosomes. The feature that first identified the cells was their star-like shape and the lack of dense-cored secretory granules that characterise all the other 'endocrine' cells. The cytoplasmic processes that extend from the cell bodies are difficult to make out in immersion-fixed material in which the extracellular space appears minimal, but are much more obvious in perfusion-fixed tissues in which the extracellular space appears expanded (Fig. **1**). Such fixation also highlights the numerous robust connections between the F-S cells and the classic endocrine cells, and in which both gap and adherent junctions have been reported. Where F-S cells come together, they are linked at a number of points by obvious junctional complexes with both adherent and gap junctions (Fig. **2**) and, on the intervening space (but no other part of their surface), the membrane is expanded by numerous microvillus-like processes (Fig. **1**). This appearance strongly suggests some transport role, but what this might be is currently unknown. When grown in primary culture, bovine F-S cells form monolayers; shortly after reaching confluence the monolayers develop 'domes', a feature characteristic of cells that function as polarized transport epithelia. These monolayers cells express a high level of Na^+/K^+-ATPase and display an apical amiloride-sensitive Na^+ conductance, and basal Na/K-ATPase, allowing for transcellular Na^+ transport, which could contribute to ionic control in the pituitary [17]. The regulatory protein Anx A1 is localised around the follicles by pre-embedding immunocytochemistry [18]. At the cell surface Anx A1 is colocalized with the ATP-binding cassette transporter A1, which is involved in the export of Anx A1 (and could be involved with the export of other peptides such as S-100, VEGF, bFGF), at the ends of F-S cell processes, and Anx A1 immunoreactivity is concentrated at points of contact with endocrine cells [19] (Figs. **2**, **3**) . Such contacts are therefore well placed to influence the endocrine cells. Other transporters shown to be present in FS cells, but which have not been precisely localized subcellularly, include the thyroid hormone transporter MCT8 [20] the proton-peptide cotransporter PepT2 [21] and the cystine-glutamate transporter that is associated in other glial cells with glutamate release [22]. The dipeptides carnosine and homocarnosine are taken up by F-S cells [23]. The physiological function of this is unclear, but uptake of such peptides can be used to trace the F-S cell communication system [1, 3].

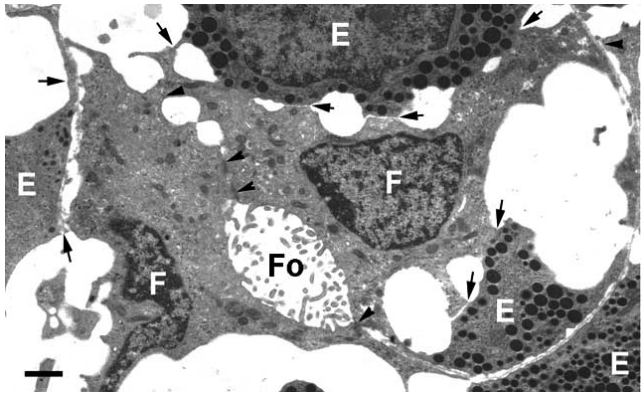

Fig. (1). Electron micrograph of mouse anterior pituitary to show the F-S (F) and classical endocrine (E) cells characterized, respectively, by the lack and the presence of dense-cored secretory vesicles, the expansion of the extracellular space that is seen in perfusion-fixed material, the microvilli decorating the membrane of the follicle (Fo) formed where F-S cells come together, and the numerous points of strong contact between the F-S cells (arrowheads) and between F-S and endocrine cells (arrows). Bar = 1μm. (Modified from Fig. **4** [19]).

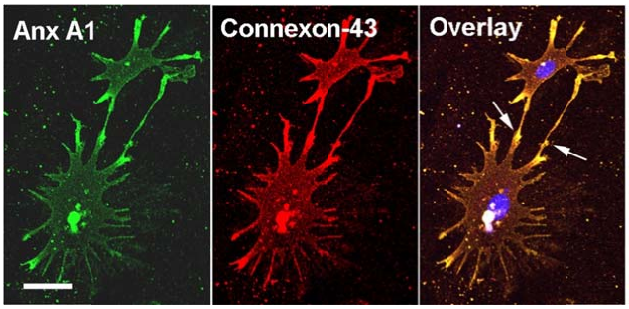

Fig. (2). Confocal fluorescence microscope images of F-S like TPit/F1 cells to show annexin A1 immunoreactivity, connexon-43 immunoreactivity, and the overlay image showing colocalization. The cells have been very lightly fixed to emphasize the proteins localized at the surface of the tips of the processes of the cells. Connexon immunoreactivity is particularly prominent where the cell processes have come together (arrows). Bar = 5μm.

Apart from the export of Anx A1 and other peptides, the role of transporters and particularly the follicles is unclear, though they could provide a novel intrapituitary means of circulation for paracrine signalling molecules and nutrient substrates required for hormone synthesis. F-S cells would be well placed to coordinate the pituitary response to changing physiological demands, e.g. during pregnancy, lactation, weaning and puberty, when the pituitary has the ability to expand and contract its activity and cell numbers several fold.

F-S CELL LINES

The isolation of F-S cells from intact pituitaries is not an easy task, so it is no surprise that the majority of recent experiments on F-S cells have used a number of cell lines. The F-S cell community is very indebted to Professor Kinji Inoue of Saitama University, Japan, who produced the two F-S cell lines that have been most widely used. TtT/GF cells

[24, 25] were derived from a mouse pituitary tumour and have been used most extensively; Tpit/F1 cells [26], derived from a temperature-sensitive large T antigen transgenic mouse; both have very similar morphology to native F-S cells and very similar properties. While the availability of these cell lines has been most useful, there is often the implicit assumption that these cell lines entirely represent the native F-S cells. However, TtT/GF cells lack nNOS [27] and we have recently found that, unlike native rat cultured F-S cells [28], TtT/GF cells do *not* respond to acetylcholine by a rise in intracellular calcium ($[Ca]_i$) although they can respond to acetylcholine by exporting Anx A1 [29]. The first human F-S cell line (PDFS) was derived spontaneously from a clinically non-functioning pituitary macroadenoma [30]. A further human hPit-1 cell line was derived which lacked classical pituitary hormones but expressed high levels of follistatin mRNA suggesting an origin from F-S cells [31]. PDFS cells show similar morphology to primary F-S cells, and express vimentin, S-100, follistatin, activin A and the activin intracellular signalling pathway. The PDFS cells also export Anx A1 [32] and express toll-like receptor (Tlr4), the receptor which induces lipopolysaccharide signals [33]. More recently a rat F-S cell line, FS/D1h cells, [34] has also been established. As with all cell lines [35], caution needs to be observed because there may well be species differences in F-S cell signals, receptors and intracellular mechanisms that are currently overlooked. More information about what is expressed by the native FS cells and how that expression is controlled, is urgently required.

PARACRINE SIGNALS PRODUCED BY F-S CELLS

F-S cells produce a large number of different signalling molecules; those not primarily associated with the immune system (see below) are summarised in Table **1**. Their actions are briefly summarised here. Anx A1 is a glucocorticoid-induced and exported molecule which mediates the early-delayed (30min-3h) inhibitory effects of glucocorticoid action on most pituitary endocrine cells (see below). Follistatin is produced both by F-S cells (Fig. **3**) and gonadotrophs. It acts on gonadotrophs to bind activin and thereby inhibit FSH secretion. Follistatin secretion in the pituitary is markedly increased by gonadotrophin releasing hormone (GnRH) at the time of the preovulatory surge [36] but the cellular source is unclear because there is no evidence that F-S cells express GnRH receptors. Activin, which likewise is also produced by gonadotrophs, has been reported only in the human PDFS cell line, but if expressed by F-S cells in primary tissue would act on gonadotrophs to stimulate FSH secretion and to decrease growth hormone (GH) and adrenocorticotrophic hormone (ACTH) secretion [30]. Basic fibroblast growth factor has been reported only in GH cells in the human pituitary [37] but is present in rat-derived F-S cells where it is responsible for determining the sensitivity of lactotrophs to the mitogenic effects of estradiol [38] and is stimulated by TGF-β3 [39]. Pituitary vascular endothelial growth factor (VEGF) was first described in bovine F-S cell-conditioned medium [40] and presumably controls pituitary vascularity in both physiological and pathological conditions such as tumour formation.

Leptin production appears to vary considerably among species. It is prominent in F-S cells in humans, but sparse in rat pituitary and in <1% of mouse pituitary cells though it is expressed by many cells in the murine TtT/GF cell line. Its local function seems to be to suppress proliferation of somatotrophs and F-S cells (which also express leptin receptors [41, 42]. Ciliary neurotrophic factor similarly suppressed proliferation, but also enhances gap-junction formation between F-S cells in castrated male rats [43].

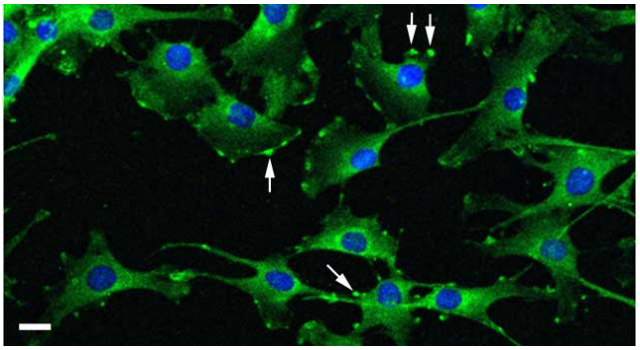

Fig. (3). Follistatin immunoreactivity revealed by confocal fluorescence microscopy of F-S like TtT/GF cells. The cells have been fixed a little more strongly than those in Fig. (**2**) so that the even distribution of immunoreactivity in the cytoplasm is apparent, together with the points of intense surface immunoreactivity at the tips of processes of the cells (arrows). Bar = 5μm.

There is relatively little information about the control of expression of some of these signalling molecules. Anx A1 synthesis is stimulated by glucocorticoids [18, 59] and also by oestradiol, causing Anx A1 expression appropriately through the oestrous cycle [60] in rats. Follistatin expression and export from F-S cells is stimulated by interleukin-1β and also by glucocorticoids and lipopolysaccharide [47].

Expression of bFGF, like that of Anx A1, is stimulated by estradiol [50]; it is also stimulated by transforming growth factor beta (TGFβ) by a mechanism that involves protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) [61]. Basic FGF can stimulate the proliferation of TtT/GF cells by an autofeedback mechanism that involves Src tyrosine kinase and protein kinase C [62].

VEGF expression is also stimulated by TGFβ [63] and by many other factors including: low oxygen tension via hypoxia-inducible factor 1 (HIF-1); IL-6; PACAP; (VIP); estradiol; but is inhibited by glucocorticoids [51]. All of these could act in the pituitary and we have demonstrated the stimulatory effects of PACAP and estradiol and the inhibitory effect of glucocorticoids on TtT/GF cells [55]. Gonadotrophins have been shown to induce VEGF secretion in the ovary, and thyroid stimulating hormone in thyroid carcinoma cell lines, but whether either of these act in the pituitary is unknown.

Nitric oxide production by F-S cells is stimulated by interferon γ [57], leptin [64] and adenosine triphosphate (ATP) [26] and has inhibitory effects on the secretion of prolactin (PRL) but not luteinizing hormone (LH) [65]. Interestingly, nNOS appears to be expressed mainly in F-S cells in male rats but in gonadotrophs in females;

Table 1. Signalling Molecules Produced by F-S Cells and F-S Cell-Like Cell Lines

Signalling Molecule	Identified Source	Reference
Annexin A1 (Anx A1; Anx A1; lipocortin 1)	Rat pituitary	[44]
	TtT/GF cells	[44]
	Tpit/F1 cells	See Fig. 2
	PDFS cells	[30, 45]
Follistatin	Rat pituitary F-S cells	[46, 47]
Activin	Human PDFS cells	[30]
Basic fibroblast growth factor (bFGF)	Bovine F-S cells	[48]
	Rat pituitary F-S cell line	[49]
	Rat F-S cells	[27]
Vascular endothelial growth factor (VEGF)	Bovine F-S conditioned medium	[48, 51]
	Rat pituitary F-S cells	[52]
	Human pituitary F-S cells	[53]
	TtT/GF cells	[24, 54, 55]
Nitric oxide (NO) from neuronal NOS	Rat pituitary F-S cells	[56]
	TtT/GF cells	[57]
	Tpit/F1 cells	[26]
Novel neurotrophin-1/B-cell stimulating factor-3 (NNT-1BSF-3)	TtT/GF	[58]
Pituitary adenylate cyclase-activating peptide (PACAP)	Rat pituitary F-S cells	[42]
	TtT/GF cells	[42]
Transforming growth factor β1 (TGFβ1)	Rat pituitary F-S cells	[42]
	TtT/GF cells	[42]
Leptin	Rat pituitary F-S cells	[42]
	Human pituitary F-S cells	[40]
	TtT/GF cells	[42]

gonadectomy of males decreases nNOS in FS cells but increases it in gonadotrophs [66].

MECHANISMS OF EXTERNALISATION OF F-S SIGNALLING MOLECULES

A defining characteristic of F-S cells is their lack of dense-cored secretory vesicles. Whereas nitric oxide can simply diffuse from the cells, the proteins and peptides must either be secreted via the constitutive route, or be transported across the membrane. Anx A1, follistatin (Fig. **4**) and VEGF are all distributed diffusely in the cytoplasm of native and cultured F-S cells. The export of Anx A1 from F-S cell lines has been more extensively studied. Solito *et al.* [30] have shown in PDFS cells that dexamethasone, which causes rapid membrane association and export of Anx A1, induces rapid serine-phosphorylation of Anx A1 and involves a non-genomic action of the glucocorticoid receptor, protein kinase C, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase. Glucocorticoids cause the export of Anx A1 which is evident after 30 mins and maximal at 90 mins. The ATP-binding cassette (ABC) transporter family is known to export a number of proteins, including bFGF [67] and Anx A1 in inflamed gut mucosa [69]. Glyburide, an ABC transporter inhibitor, markedly inhibits glucocorticoid-stimulated (but not potassium-stimulated) export of Anx A1from TtT/GF cells and from pituitary tissue [69]. ABC transporter protein is co-localized with Anx A1 at the tips of processes of TtT/GF cells (Fig. **4**); both are increased by glucocorticoid exposure, and export of Anx A1 has been shown by immuno-electron microscopy of intact pituitary to occur where F-S cell processes abut adjacent endocrine cells [19] (Fig. **5**). Evidence for a role of ABC-A1 was strengthened by the finding that the ABC-A1 inhibitors geranyl-geranyl pyrophosphate and sulfobromophthalein both significantly inhibit Anx A1 export and that partial silencing of ABC-A1 expression by siRNA significantly decreased the amount of cell surface Anx A1. By contrast, ABC-A1-null mice appear to externalize Anx A1 normally, though compensation by other ABC transporters may have occurred. However, co-transfection of ABC-A1 and Anx A1 into *Xenopus* oocytes and of ABC-A1-GFP and Anx A1 into AtT20 corticotrophs both strongly suggest that ABC-A1 plays at least a major role in Anx A1 export [70].

The timescale of VEGF secretion (just detectable at 3h, well established at 24h) is reminiscent of constitutive secretion via small electron-lucent vesicles. The sulphonylurea glyburide (glibenclamide) is perhaps better known as an antidiabetic agent, acting by inhibiting ATP-sensitive potassium channels in pancreatic beta cells, thereby depolarising the cells, allowing voltage-sensitive calcium channels to open, increasing intracellular calcium and thereby stimulating insulin release. Our experiments [55] have shown that TtT/GF cells express K_{ATP} channels (Kir 6.1 and SUR2B). When VEGF secretion from the TtT/GF cells was tested, glyburide (100nM) on its own produced as great or greater stimulus of 24h VEGF secretion as did the well-known secretagogue PACAP (100nM), but also markedly facilitated the effect of PACAP when co-applied. By contrast diazoxide, which opens the K_{ATP} channels, inhibited both glyburide- and PACAP-induced secretion of VEGF. Enhancement of $[Ca]_i$ therefore appears to be important for stimulation of VEGF secretion. How this links with the known requirement of PKC for Anx A1 phosphorylation and export is currently unknown. Although follistatin secretion in response to interleukin 1β has been measured, the 48h stimulation period tested [47] provides no clue to the mechanism involved.

INPUTS TO F-S CELLS: RECEPTORS AND MECHANISMS OF ACTION

In order to act as the hubs of a signalling system, F-S cells must express a number of receptors. Those involved with interaction with the immune system are listed in Table **3**; other known receptors are listed in Table **2**. "Implied" indicates that the evidence for the receptor rests on a defined action, and not on actual identification of the receptor on the cells.

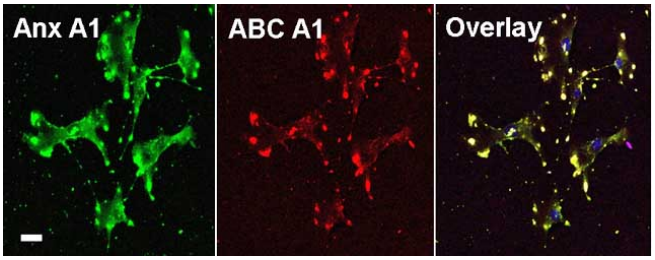

Fig. (4). Confocal fluorescence microscope images of F-S like TtT/GF cells to show annexin (Anx A1) immunoreactivity, (b) ABC-A1 transporter immunoreactivity, and (c) the overlay image showing colocalization. Annexin is evenly distributed through the cytoplasm and is co-localized with ABC-A1 at the tips of processes. Bar = 5μm.

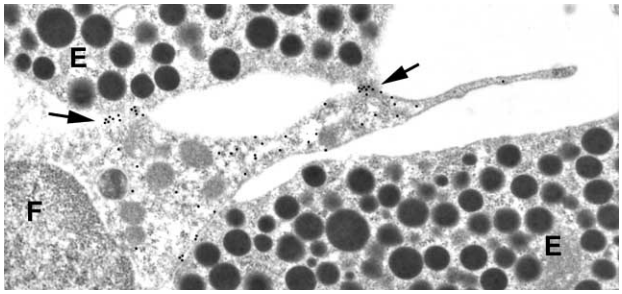

Fig. (5). Electron micrograph of mouse pituitary immunogold-labelled for annexin A1. Immunoreactivity is seen throughout the cytoplasm of the F-S cell (F) and concentrated at points on the surface of the F-S cells where they make contact with the membrane of surrounding endocrine cells (E). Bar = 1μm.

One immediately striking feature of Table **2** is that there is no direct evidence that F-S cells express receptors for any of the hypothalamic releasing/inhibiting peptides with the exception of PACAP, or for any of the classic anterior pituitary hormones with the possible exception of the thyrotrophin receptor in human pituitaries [80]. The hypothalamic cells expressing PACAP are found largely in the paraventricular (parvo- and magnocellular divisions), supraoptic and arcuate nuclei. Their axons project to the median eminence and the concentration of PACAP in portal blood is higher than that in the systemic circulation. Not only the F-S cells, but all the endocrine cells are influenced by PACAP primarily via the PAC1 receptor which induces rises in cytosolic calcium [81]. Thus PACAP can have effects both to stimulate release of ACTH, GH, and PRL (but not TSH), but also, via F-S cell Anx A1 to inhibit it; to stimulate FSH synthesis directly or to inhibit it via F-S cell follistatin; it also stimulates IL-6 secretion and VEGF secretion, thereby having indirect effects on differentiation of pituitary cells and pituitary vasculature ([82].

Estradiol, acting through nuclear ER stimulates F-S cell production of VEGF [83]. It also acts via a PKC-dependent MAP-K p44/42 pathway, interacting with TGF-beta 3 to increase bFGS and thereby cause proliferation of lactotrophs [38]. Estradiol can also block the stimulatory effect of glucocorticoids on 11β-hydroxysteroid dehydrogenase expression (Shun-Shin and Morris, unpublished observations) which could explain in part the desensitisation of the stress response in pregnant female rats [84]. Little is known of the action of androgens on F-S cells; they suppress follistatin production *in vivo*, but not in a F-S cell line [85].

F-S cells contain classic intracellular glucocorticoid receptors which migrate from the cytoplasm to the nucleus on binding. Glucocorticoids exert transcriptional actions to increase the synthesis of Anx A1 and follistatin and to decrease the synthesis of VEGF. They also induce the much faster non-genomic intracellular events that are blocked by cycloheximide but not actinomycin D [86] and which lead to the phosphorylation of serine-27 that is essential for externalisation of Anx A1 from F-S cells and cell lines within 30 minutes. These include activation of protein kinase C, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase and also required HMG-coenzyme A and myristoylation [87]. However, the exact route(s) by which these components are involved remains to be determined.

Leptin probably acts on the F-S cell long and short leptin receptors to activate STAT 3 and other pathways to inhibit F-S cell proliferation, but the mechanism has not been experimentally tested.

The adenosine receptors on F-S cells signal via Gq/PLC (A_1R, $A_{2B}R$) and Gs/adenyl cyclase ($A_{2B}R$), the stimulation of A_{2B} receptors causing proliferation of TtT/GF cells and increasing VEGF and IL-6 production [88, 89] and thrombomodulin expression and gap junction proteins [78]. The source of adenosine is unclear; it is derived from the diet, but may be generated locally because ecto-5'-nucleotidase is expressed by ~20% of pituitary cells, in particular lactotrophs and somatotrophs, and coculture of TtT/GF cells with somatomammotrophs increases the expression of connexon 43 [88].

The purinergic receptors P2Y and P2X are widely expressed in the pituitary. Adenosine triphosphate (ATP) acts on rat primary F-S cells via P2Y receptors, phospholipase C and IP3 to release calcium from intracellular stores. It causes a dose-dependent release of Anx A1 from TtT/GF cells. Acetylcholine acts on cultured primary rat F-S cells via muscarinic receptors to stimulate calcium transients by causing release from intracellular stores [28]. However, our TtT/GF cell cultures fail to generate calcium transients to even larger doses of acetylcholine, although they readily respond in this way to ATP (Morris, Williams, Christian, unpublished observations).

ATP is co-released with classical anterior pituitary hormones when the dense-cored vesicles are exocytosed. Given the proximity of F-S cell processes and the ability of ATP to stimulate Anx A1 release, this suggests an ultrashort negative feedback loop whereby an endocrine cell that has been particularly active will stimulate surrounding F-S cells to secrete Anx A1 and thereby damp down its secretory activity.

Choline is generally thought to act through nicotinic α7 receptors [90], but there is no evidence that these occur in pituitary F-S cells, or that choline causes an increase in intracellular calcium. Despite this, choline causes a dose-dependent increase in Anx A1 export from TtT/GF cells [29]. This implies the existence of another possible mechanism of choline action in these cells.

Table 2. Receptors Expressed by F-S Cells but not Primarily Associated with Immune or Inflammatory Mechanisms System

Receptor/known response	Tissue	Reference
PACAP (PACI > PACII)	Rat pituitary F-S cells	[71, 72]
Estradiol (ERα, ERβ)	TtT/GF cells	[60]
Androgen	TtT/GF cells	Morris & Christian, unpublished
Glucocorticoid (classic & non-genomic actions)	Rat pituitary	[73]
	TtT/GF cells	[19]
Leptin (common, ObRa and long, ObRb forms)	Mouse pituitary	[42]
	Rat pituitary	[42]
	TtT/GF cells	[42]
	Human pituitary	[41]
Vascular endothelial growth factor (VEGF; Flk-1)	Rat pituitary	[74]
Ciliary trophic nerve factor (CNTF)	TtT/GF cells	[75]
Transforming growth factor β (TGFβ) implied	Rat F-S cell line	[42, 76]
	TtT/GF cells	[42]
Platelet-derived growth factor (PDGF)	Rat pituitary	[77]
	TtT/GF cells	
Adenosine A_1R, $A_{2B}R$	TtT/GF. Tpit/F1 cells	[78]
Adenosine trisphosphate (ATP; P2Y, P2X)	Rat pituitary F-S cells	[79]
	TtT/GF cells	Williams, Morris, Christian, unpublished
Acetylcholine, muscarinic (implied)	Rat pituitary F-S cells	[28]
Choline (implied)	TtT/GF cells	[29]
Bradykinin (implied)	Rat pituitary F-S cells	[72]
Angiotensin-II (AT-1; implied)	Rat pituitary F-S cells	[72]
Endothelin-1 (implied)	Rat pituitary F-S cells	[72]

TGFβ from lactotrophs increases gap-junction communication among F-S cells and stimulates the release of bFGF which acts back on lactotrophs. In this way the stimulation of lactotrophs by estradiol can lead to lactotroph tumours via F-S cell bFGF [76].

F-S cells express a PDGF receptor which acts via PI3 kinase and Akt to increase F-S cell proliferation and VEGF secretion [77].

COMMUNICATION WITH THE HYPOTHALAMO-PITUITARY ADRENAL STRESS AXIS

One of the prime ways in which F-S cells communicate with the endocrine cells in the anterior pituitary is via the action of glucocorticoids feeding back negatively in the stress response. There is at present no evidence that either corticotrophin-releasing hormone or vasopressin (both stimulants of ACTH release) act on F-S cells. It is not often appreciated that glucocorticoid negative feedback occurs in three different but overlapping time frames and by three different mechanisms, although much attention is paid to pulses and circadian rhythms of ACTH secretion. There is a rapid feedback which occurs within 30 minutes. This inhibits release of ACTH by a mechanism which involves a non-genomic change in membrane potential and inhibition of CRH-induced cAMP production [91]. After several hours, the well understood GR-induced inhibition of POMC transcription and processing to ACTH occurs. Between these two (30 minutes to 3 hours) is the 'early-delayed' feedback period and it is here that F-S cell Anx A1 is essential. Exposure of F-S cells to glucocorticoids not only increases the synthesis of Anx A1 but also causes its externalisation from the cells (see above). This occurs at particular foci in the cell membrane in both native F-S and TtT/GF cells where the Anx A1 is co-localized with the ABC-A1 transporter (Fig. 4) and also with cytoskeletal elements, S-100 and connexin 43 (Fig. **2**).

Stress levels of glucocorticoids inhibit the secretion of all pituitary endocrine cells, not only the corticotrophs. Most of the endocrine cells contain glucocorticoid receptors and so are subject to transcriptional inhibition by glucocorticoids, but lactotrophs apparently do not [73] and all are subject to the early delayed glucocorticoid-induced inhibition that requires Anx A1 as demonstrated by the blocking effects of both antibodies and antisense [92, 93]. F-S cells make functional contacts with all the classical endocrine cell types in proportion to their number and the Anx A1 that they export can therefore exert the early-delayed feedback on secretion of all adenohypophyseal hormones. Immunoelectron microscopy (Fig. **5**) shows Anx A1 immunoreactivity concentrated at some of these points of contact between F-S and endocrine cells [19] so that F-S cells appear to be able to deliver Anx A1 very precisely to endocrine cells through what could be considered a sort of 'endocrine synapse'. The extent to which this is controlled is completely unknown but will be interesting to investigate. Certainly, co-culture experiments involving TtT/GF cells and AtT20 corticotrophs show that glucocorticoid inhibition of CRH-induced ACTH secretion is proportional to the number of TtT/GF cells present [94].

Glucocorticoids can also influence the HPA axis through interleukin-6 (IL-6; see section on Communication with immune system) produced by F-S cells. IL-6 is a strong stimulant of the HPA axis and its expression is stimulated by TGFβ, PACAP and glucocorticoids [95, 96]. At present it is

not known how the apparently conflicting effects of glucocorticoid-induced secretion of Anx A1 and IL-6 are physiologically controlled. Perinatal glucocorticoid treatment of rats increases IL-6 production, but decreases Anx A1 production and the size of F-S cells [97]. This suggests that F-S cells are implicated in the known influence of perinatal stressful events in the heightened sensitivity of the HPA stress axis in adults.

One other way in which F-S cells can influence the stress axis is during development via an effect on the number of corticotrophs. Lack of Anx A1 in Anx A1-null mice is associated with a 4-fold increase in the number of corticotrophs in the male but not the female null mice [98] and the same is seen in Anx-receptor null animals. However, the individual corticotrophs in the male null mice were smaller and had fewer dense-cored secretory vesicles. In null males pituitary ACTH and IL-6 were increased, but plasma corticosterone and ACTH were unchanged.

F-S cells therefore play a variety of different roles in the stress axis. They determine the number of corticotrophs, mediate an important component of glucocorticoid negative feedback on ACTH during stress, but also play a role in the stress-induced inhibition of all the other pituitary hormones.

COMMUNICATION WITH THE HYPOTHALAMO-PITUITARY GONADAL AXIS

F-S cell communication with the reproductive axis occurs largely via the effects of follistatin on gonadotrophs, though the action of Anx A1 to inhibit gonadotrophs [99] and lactotrophs and of bFGF to mediate oestrogen-induced lactotroph proliferation will have indirect effects on the gonadotrophs [2]. Follistatin in F-S cells is increased by glucocorticoids, IL-1β and by this mechanism stresses of many types can inhibit FSH production and also sensitivity to gonadotrophin-releasing hormone (GnRH) by inhibition of GnRH receptor expression [100]. PACAP also stimulates follistatin expression in FS cells [101] but this is difficult to interpret until the controls on PACAP secretion are better understood. A human F-S cell line is also said to produce activin, but the controls on this are not known [30]. A further complication in assessing the role of F-S cell signals is that the gonadotroph cells themselves produce both activin and follistatin [102, 103].

COMMUNICATION WITH THE IMMUNE SYSTEM

F-S cells display many characteristics of immune cells and there is good evidence that they mediate and modulate the neuroendocrine response to immune stress and inflammation [104]. F-S cells respond to bacterial endotoxins by producing cytokines that in turn influence hormonal output from the anterior lobe 'classical' secretory cells [2]. The expression of many immune cell markers and the release of cytokines by F-S cells (summarised in Table **3**) together with ultrastructural similarities led to speculation that FS cells might represent either pituitary specific macrophage or dendritic-like cells [105]. Dendritic cells and macrophages are two groups of non-lymphoid mononuclear cells involved in immune responses through the production of a variety of cytokines, presentation of antigens to lymphocytes and phagocytosis/degradation of unwanted material [106]. Whereas FS cells undoubtedly show some characteristics of macrophages, a number of reports have been unable to detect cells in rat or human pituitary that colocalise the F-S marker S100 with well known macrophage markers such as OX42, ED1 or CD45 [107-109]. However, Allaerts *et al.* [7, 107] demonstrated co-immunolabeling for S100 and the dendritic specific marker OX6 in a subpopulation (10-20%) of F-S cells in the rat pituitary. These findings raised the possibility of a myeloid origin of a proportion of F-S cells (reviewed in [110]). It is possible that only this subset of $S100^{+ve}$ $OX6^{+ve}$ F-S cells supports the intrapituitary response to immune stimuli. Therefore, because many studies investigating immune properties of F-S cells have relied on TtT/GF cells it may be that the TtT/GF cell line was established from this particular subpopulation of FS cells. However to our knowledge the expression of OX6 or other dendritic markers has not been investigated in TtT/GF cells. Ultrastructural studies in rat and guinea fowl have suggested a phagocytic role for F-S cells [111-113]. We are not aware of functional studies to support this and have been unable to demonstrate phagocytic activity by native F-S cells or TtT/GF cells, though the very slow turnover of endocrine cells may make this a rather rare event in the adenohypophysis. However, it is reported that Anx A1 is a pro-engulfment ligand which, colocalized with phosphatidylserine in the outer membrane leaflet, is required for the efficient clearance of apoptotic cells [114]. FS cells also respond to a number of cytokines (Tables **3**, **4**).

COMMUNICATION WITH PITUITARY VASCULATURE

In any tissue, increased cellular activity is usually accompanied by increased blood flow. The blood supply to the anterior pituitary is unusual in that it comes almost exclusively from hypothalamo-pituitary portal veins originating from the capillary plexus in the median eminence and descending the pituitary stalk, so one might expect the oxygen tension in the tissue to be low. Also, flow is likely to be slow both because the input is from the portal veins and also because of the very large vascular sinusoids between the clusters of cells. However, Lafont *et al.* [130] report a pituitary oxygen tension of ~34mmHg in anaesthetized rats breathing air.

The principal way in which F-S cells communicate with the vasculature is via the secretion of VEGF [40, 51, 126] which stimulates the endothelium. F-S cells can also produce nitric oxide [26, 57], and this is stimulated by interferon-γ [57, 129] and by ATP [26], though whether this can cause vasodilation in the pituitary is unclear. However, F-S cells also produce LIF, which inhibits the proliferation of endothelial cells in the aorta at least [119]. Whether it has a similar effect on pituitary vasculature, how its secretion is regulated and coordinated with that of VEGF and nitric oxide remains unknown.

Unlike the very close anatomical relationships between F-S cell processes and the endocrine cells, F-S cells are separated from the vascular endothelial cells by a substantial perivascular space. VEGF secretion from F-S cells is presumably increased by hypoxia, though this does not appear to have been tested. It is known to be increased by PACAP [126], IL-6 [128], and TGFbeta [63] but the functional important of this is unclear. VEGF secretion is

Table 3. Immune, Pro- and Anti-Inflammatory Markers Expressed by FS Cells

Immune Family Marker	Tissue Studied & Technique Used	Reference
Interleukin-6 (IL-6)	Rat & mouse pituitary cells; indirect evidence that F-S cells derived (hybridoma growth factor assay	[115, 116]
	TtT/GF cells (ELISA)	[117]
	Tpit/F1 cells (ELISA)	[26]
Macrophage migration inhibitory factor (MIF)	TtT/GF cells (ELISA) Adult male rat (immunofluorescence)	[118]
Leukaemia inhibitory factor (LIF)	Bovine pituitary follicular cells	[119]
Inducible nitric oxide synthase (iNOS)	20% of F-S cells in female rat after IFNγ induction	[57]
	Male rat (immunoreactivity)	[56]
Dendritic cell markers OX6, OX62	Rat pituitary (immunoreactivity in 10-20% of S100-positive cells	[107]. not confirmed by [109]
CD14 (binds LPS complex)	TtT/GF cells	[117]
	Mouse pituitary (RT-PCR)	[33]
Toll-like receptor 4 (TLR4; induces LPS signals)	PDFS cells	[33]
Complement C3a receptor	Rat pituitary; colocalization with S100β by immunofluorescence	[120]
Nucleotide-binding oligomerisation domain intracellular receptor (NOD2; innate immune response after bacterial infection	Human pituitary and TtT/GF cells	[121]
Endothelial protein C receptor, thrombomodulin (anti-inflammatory agents)	TtT/GF and Tpit/F1 cells; RT-PCR and western blot	[78]
Adenosine A1, A2b receptor	TtT/GF and Tpit/F1 cells; RT-PCR and immunofluorescence	[122, 123]
Annexin A1 (Anx A1); mediator of glucocorticoid anti-inflammatory and negative feedback actions	Rat pituitary; TtT/GF cells; western blot, immunofluorescence, immunogold	[44]
	Human pituitary adenomas	[124]

also increased by estradiol which would be consonant with the increased secretory activity at the time of the preovulatory gonadotropin surge, and the lactotroph proliferation during pregnancy. The inhibition of VEGF secretion by glucocorticoids would also be consonant with the inhibition of much pituitary endocrine activity during stress. The observation that the sulphonylurea gliburide markedly enhances VEGF secretion [55] should perhaps sound a note of caution when treatment with sulphonylureas is considered in patients with a known pituitary tumour, as the activity of such tumours is correlated with their vascularity [131] and F-S cells are often observed clustered around pituitary adenomas.

STEM CELLS IN THE ANTERIOR PITUITARY

The identity of stem cells in the pituitary and the possible involvement of F-S cells or a subset of F-S cells has long been an object of discussion and dispute [132-134]. The turnover of cells in the anterior pituitary is very slow (~0.05%; [135]). And there have been repeated suggestions that F-S cells, or a subset of those cells (see above) might be involved. Inoue *et al.* [27, 136] suggest that F-S cells might have stem cells properties because of the ability of the Tpit/F1 cell line to transform into other tissues [137] and this suggestion was supported by Horvath and Kovacs [138] on the basis of structural observations on normal and adenomatous pituitaries. More recently specific markers have been used in the search for pituitary stem cells. Nestin immunoreactivity [139] has identified a population of non-endocrine cells that are also not typical F-S cells; the stem cell antigen Sca1 likewise identifies a small proportion ('side population') of cells some of which express the F-S cell marker S-100 [132]. SOX-2 has been used to identify a population of adult pituitary cells that form spheres which can differentiate into all pituitary cells types including F-S cells. A small proportion of these cells co-express S-100 and SOX2 [140]. A molecular approach, using nestin coupled to green fluorescent protein, has identified a population of cells surrounding the pituitary cleft (where, incidentally, Anx A1 is also expressed [44]) which also generate subsets of all the pituitary cell types and apparently provide the majority of cells in the adult organ [141].

Table 4. Summary of FS Cell Responses to Immune and Inflammatory Mediators

F-S cells respond to	Response	Model system	References
Interleukin-1 (IL-1)	Gap-junction uncoupling	TtT/GF cells	[125]
	Release of IL-6 and follistatin	FS/D1h cells	[47]
Interleukin-6 (IL-6)	Stimulation of VEGF release	TtT/GF cells, rat pituitary monolayers	[126]
Tumour necrosis factor α (TNFα)	Gap junction coupling and uncoupling	TtT/GF cells	[125, 127]
Lipopolysaccharide (LPS)	IL-6 production	TtT/GF cells	[117]
		Mouse pituitary	[128]
	Macrophage inhibitory factor (MIF) production	TtT/GF cells	[118]
Interferon γ (IFNγ)	iNOS induction	Female rat pituitary	[129]
MIF	Reverse inhibitory actions of glucocorticoid on IL-6 release	TtT/GF cells	[118]

CONCLUSIONS

The research analysed for this review makes it clear that the group of cells classed as F-S cells, so long the rather ignored 'poor relation' in the anterior pituitary in comparison with the classical endocrine cells, nevertheless have many potentially important functional properties. Furthermore, it seems very likely that, at any one time, there are many subtypes, each with different properties in terms of the signals produced and receptors expressed. The extent to which these subtypes are fixed or plastic is completely unknown, but given the range of signals to which the cells respond and the range of responses it would seem very likely that these are functionally very plastic cells, responding with exquisite precision to the varying physiological conditions. What is clear is that F-S cells form a communication system within the anterior pituitary, interacting with each other and with the surrounding endocrine cells and the vasculature, and using the release of intracellular calcium as an intracellular messenger. Yet even here, although there is now evidence that various signals can alter the expression of gap junction proteins and the intracellular signalling pathways within the cells, we have only a very hazy and limited understanding of how the communication systems within the cells are controlled. Finally, it seems increasingly clear that cells with at least some of the characteristics that have been used to class them as 'F-S' have a stem-cell role in the pituitary. Undoubtedly these 'Cinderella' cells have a lot of secrets that we have yet to uncover.

ABBREVIATION

Anx A1 = Annexin 1

ABC-A1 = ABC transporter

F-S = Folliculo-stellate

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