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Gap junction modulation by extracellular signaling molecules: the thymus model

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Abstract

Gap junctions are intercellular channels which connect adjacent cells and allow direct exchange of molecules of low molecular weight between them. Such a communication has been described as fundamental in many systems due to its importance in coordination, proliferation and differentiation. Recently, it has been shown that gap junctional intercellular communication (GJIC) can be modulated by several extracellular soluble factors such as classical hormones, neurotransmitters, interleukins, growth factors and some paracrine substances. Herein, we discuss some aspects of the general modulation of GJIC by extracellular messenger molecules and more particularly the regulation of such communication in the thymus gland. Additionally, we discuss recent data concerning the study of different neuropeptides and hormones in the modulation of GJIC in thymic epithelial cells. We also suggest that the thymus may be viewed as a model to study the modulation of gap junction communication by different extracellular messengers involved in non-classical circuits, since this organ is under bidirectional neuroimmunoendocrine control.

Key words
• Thymus
• Thymic epithelial cells
• Connexin 43
• Hormones
• Interleukins

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Introduction

The establishment of cellular communication was a milestone for the origin and evolution of multicellular organisms. Such a communication can be mediated by 1) soluble factors, i.e., hormones, cytokines, growth factors and neurotransmitters, 2) membrane surface molecules, i.e., members of immunoglobulin gene superfamily, integrins, cadherins and other classes of recognition molecules, and 3) ion channels.

Ion channels have been recognized as a fundamental component involved in the mechanisms that most animals use to perceive the external world and the internal milieu (mechanoception, nociception, vision, taste, motion and hearing). Gap junction is a particular kind of ion channel that mediates direct transfer of molecules between adjacent cells by a diffusion process.

In vertebrates, the gap junction is an intercellular channel consisting of a dodecameric structure formed by hemichannels (or connexons) of six monomers in each adjacent cell (1). These monomers are proteins named connexins (Cx) that belong to a multigenic family, with at least 14 members already cloned in mammals (1). The most commonly used nomenclature defines a con-

nexin according to its predicted molecular weight deduced from cDNA sequences and species of origin (1).

These channels permit direct cell-cell exchange of small molecules such as ions, sugars and second messengers between adjacent cells (1,2). It was thought that these channels are permeable to molecules of up to 1 kDa in mammals. However, several studies have demonstrated that many connexins present selectivity for both charge and size. For instance, connexin 43 allows the passage of lucifer yellow while Cx45 does not (3). In addition, the functionality of heterologous gap junctions has been shown to depend on selective compatibility among different connexins (3,4). In agreement with this, Cx43 can form functional gap junctions with Cx37, 43, 45 and 46, but not with 26, 31, 32, 33, 40 or 50 (5).

Gap junction channels can be gated, i.e., "opened" or "closed", by several agents including Ca^{2+}, H^{+}, lipophilic substances, voltage gradients, and hormones (1), the latter generally acting via activation of protein kinases that phosphorylate the cytoplasmic regions of several connexins (6,7), or in some cases by phosphatases (6,7). The presence of connexins has been demonstrated in distinct lympho-hematopoietic organs where their function is not clear (reviewed in Ref. 8). In these organs cell-to-cell communication is a key event to tune performance.

In this review, we will focus on the modulation of gap junctions by extracellular messengers in a primary lymphoid organ, the thymus gland. In this organ, bone marrow-derived lymphoid precursors undergo a process of differentiation and maturation, culminating with the migration of mature T cells to the periphery. In this process more than 90% of thymocytes die intrathymically, whereas some are rescued from programmed cell death and are positively selected to ultimately generate the vast majority of the T cell repertoire (9).

Such a differentiation and maturation process is quite complex, involving a dynamic molecular cross-talk between T cells and the thymic microenvironment, a tridimensional network composed of distinct cell types including epithelial cells, macrophages and dendritic cells, as well as extracellular matrix (ECM) elements (10).

The thymic epithelium is the major component of the thymic microenvironment and determines thymocyte maturation through cell-cell contacts and secretion of a variety of polypeptides including thymic hormones and cytokines (11). Thymic epithelial cells (TEC) can bind to and interact with thymocytes by means of ECM ligands and respective receptors as well as by classical adhesion molecules (12) and, most importantly, by major histocompatibility complex gene products, which present endogenous peptides to the T cell receptors expressed on the cell membrane of differentiating thymocytes. Recently, we demonstrated the existence of a novel form of cell-to-cell communication in TEC which is mediated by gap junction channels (13).

It is also noteworthy that the thymus is an important component of neuroimmunoendocrine circuits, synthesizing several classical hormones (for example, growth hormone, prolactin and glucocorticoids) which act pleiotropically upon the thymic epithelium (11). Furthermore, the intrathymic production of various neuropeptides, including the neurohypophyseal hormones, oxytocin and vasopressin (11), and vasoactive intestinal peptide (VIP) has been demonstrated (11). In this intriguing network, the data concerning influence of extracellular messenger molecules on gap junction mediated communication begin to be analyzed.

General aspects of gap junction modulation by extracellular signaling molecules

In vertebrates, gap junctions have been reported in virtually all cell types, with the

possibility of multiple connexin isoform expression (1). All members of the connexin family have the same putative structure: cytosolic amino and carboxyl termini, four transmembrane domains, and extracellular (two) and cytoplasmic (one) loops. In the primary sequence of these proteins there are several consensus sites for phosphorylation and dephosphorylation, which represent one of the most frequent mechanisms for hormonal modulation of gap junctions and other ion channels.

The first evidence of gap junction modulation by hormones can be credited to Hax et al. (14), who demonstrated the regulation of electrical coupling by cAMP in insect cell cultures, a finding that was confirmed by Loewenstein and co-workers (15). As expected, several extracellular signaling molecules that raised cAMP levels could modulate gap junction communication in different cell types.

Since then, regulation of these channels by hormones and other extracellular signaling molecules such as neurotransmitters, growth factors and cytokines has been demonstrated at the level of transcription, mRNA stability, translation, cytoplasmic traffic and gating (1,6,7). So far, the best studied mechanism operating during these regulatory processes is the phosphorylation/dephosphorylation (6) of connexins, transcription factors and other regulatory proteins. Except for Cx26, all connexins cloned so far can be regulated by phosphorylation/dephosphorylation. Table 1 illustrates some hormones and other extracellular messengers that modulate gap junctions in several cell types. Most of these effects are mediated by protein kinases A and C, mitogen-activated protein kinase and tyrosine kinase. These kinases can increase or decrease junctional communication depending on the cell type studied. It is not clear why this occurs but perhaps different mixes of connexin isoforms and/or selective activation of other intracellular messenger systems that act on gap junctions might explain the diversity of biological responses. Most of the effects studied thus far are on Cx43 but several other connexins can be modulated by such substances.

In endocrine glands, a rise in gap junction communication increases the cell response to a given stimulus, increasing hormonal secretion. In Beta cells in Langerhan's islets, the overexpression of Cx43 leads to an augmented secretion of insulin (16), whereas in adrenal cells an inhibitor of gap junction communication diminishes cortisol secretion (17).

Conversely, in exocrine glands, a rise of gap junction communication in general leads to a decrease in secretion as seen in salivary glands and in the exocrine pancreas (18).

Regulation of thymic gap junctions by extracellular messengers

We demonstrated that mouse and human TEC are coupled by gap junctions (13), a concept that had been previously postulated by Kendall in the 1980's (19). We also provided evidence for the possible existence of heterologous gap junctions between TEC and thymocytes as well as for the modulation of TEC hormonal secretion by gap junctions.

As shown in Table 1, the majority of the soluble messengers and the respective target cell/organs involved in gap junction modulation studies are those associated with classical endocrine, immunological and nervous circuits such as TSH:thyroid, epinephrine: heart, T_3,T_4:liver, oxytocin:uterus, FSH:testis, neurotransmitters:neuronal electric coupling, IL-1:liver, and so on. Looking for a more integrative view, the thymus gland could be an interesting model to study gap junction modulation by nonclassical circuits since it is under bidirectional neuroimmunoendocrine control.

Consistent with this idea, it has been demonstrated in rat primary TEC cultures that progesterone, estrogen, testosterone,

Table 1 - Gap junction modulation by neuroimmunoendocrine soluble products.

ACh, Acetylcholine; BR, bone remodeling; CA, contractile activity; Cx, connexin; DI, differentiation; DTA, dye transfer assay; EF, electrophysiology; EM, electron microscopy; FFEM, freeze-fracture electron microscopy; FRAP, fluorescence recovery after photobleaching method; FSH, follicle-stimulating hormone; GnRHa, gonadotropin-releasing hormone analogue; hCG, human chorionic gonadotropin; HMC, human myoendothelial co-culture; HUVEC, human umbilical vein endothelial cells; ICC, immunocytochemistry; IHC, immunohistochemistry; IF, immunofluorescence; LH, luteinizing hormone; LH-RH, luteinizing hormone-releasing hormone; MAPK, mitogen-activated protein kinase; NB, Northern blotting; ND: not determined; PKA, protein kinase-A; PKC, protein kinase-C; PR, proliferation; PTH, parathyroid hormone; T_3, 3,3', 5-triiodo-L-thyronine; SLT, scrape-loading technique; T_4, L-thyronine; TGF-$ß_1$, tumor growth factor $ß_1$; TNFα, necrosis factor α; TSH, thyrotropin stimulating hormone; WB, Western blotting. *All these parameters depended on the cell type analyzed; **based on cell lines; ***the modulation of the junctional conductance (Gj) was also described (in some cases it involved exclusively Gj); ****based on goldfish Mauthner cells (M cells); #modulation of dye coupling and protein expression was represented by the following arrows: ↗ (positive modulation), ↙ (negative modulation) and ↙↗ (both effects were described, depending on experimental conditions, cell type or concentration tested, or even the connexin isoform analyzed).

Modulators	Analyzed cell/ organ	Techniques*	Connexin isoform*	Dye coupling#	Protein level#	Protein/kinase*	Associated function*	References
Cytokines								
Interleukin 1	Bone, HMC, HUVEC, liver**	DTA, FRAP, WB	Cx32, Cx43	↙	↙	ND	DI	36, 43, 55, 56
Interleukin 2	Liver	IHC	ND	ND	↙	ND	ND	33
Interleukin 6	Liver**	DTA, WB	Cx32	↙	↙	ND	ND	36
TGF-$ß_1$	Bone**, Schwann cells	EF, ICC, SLT	Cx43	↙↗***	↗	ND	ND	29, 54
TNFα	HMC, Liver**	DTA, WB	Cx32	↙	↙	ND	DI	36, 56
Hormones								
Estradiol	Bone, uterus	DTA, FFEM, IF, IHC, WB	Cx26, Cx32, Cx43	↙↗	↗	PKC	CA, BR, PR, DI	24, 28, 40, 44, 45
FSH	Testis	DTA, FRAP	ND	↗	ND	ND	ND	69
GnRHa	Ovary	WB	Cx43	ND	↙	PKC	ND	46
Glucagon	Liver	ICC, SLT	Cx26, Cx32	ND	↗	ND	ND	51
Glucocorticoid	Liver**	DTA, SLT, WB	Cx26, Cx32	↗	↗	ND	ND	51
hCG	Ovary, uterus	FRAP, IF, WB	Cx43	↗	↙↗	PKA	CA, DI	24, 25, 31
LH	Ovary, uterus	FRAP, WB	Cx43	↗	↙	PKC and probably PKA	CA	24, 46
Insulin	Xenopus oocytes	EF	Cx43	↙***	ND	Probably MAPK	ND	35
Melatonin	Liver	IHC, SLT, WB	Cx32	↗	↗	ND	Antiproliferative	26
Oxytocin	Ovary, uterus	IF, WB	Cx43	ND	↗	ND	ND	24, 31
Progesterone	Uterus	FFEM, IF,IHC, WB	Cx26, Cx32, Cx43	ND	↙↗	ND	CA, PR, DI	24, 28, 39, 45
Prolactin	Pancreas	DTA	ND	↗	ND	ND	ND	50
PTH	Bone	DTA, WB	Cx43	↗	↗	ND	ND	27, 70
T_3	Liver**	DTA, WB	Cx43	↗	↗	ND	ND	52
T_4	Liver**	DTA, WB	Cx43	↗	↗	ND	ND	52
Testosterone	Epididymis, pituitary gland	EM, IHC, WB	Cx43	↙↗	↙	ND	ND	23, 38
TSH	Thyroid	DTA	Cx32, Cx43	↗	↗	Probably PKA	DI	22, 41
Neurotransmitters								
ACh	Eye, hippocampus	DTA, EF	ND	↙***	ND	ND	ND	30, 42, 72
Dopamine	Hippocampus, retina	DTA, EF	ND	↙***	ND	ND	ND	34, 42, 53
Epinephrine	Heart	EF	ND	↗***	ND	ND	ND	71
Glutamate	Cerebellum, M cell****	DTA, EF	ND	↙***	ND	ND	ND	37, 47
Norepinephrine	Cortex, pineal gland	DTA, EF, WB	Cx26	↙↗***	ND	PKA	ND	32, 48, 49, 71
Serotonin	Cortex	DTA, EF	ND	↙	ND	PKC	ND	32

corticotropin, growth hormone, interleukin-1α, interleukin-1ß, γ-aminobutyric acid (GABA), neuropeptide Y, vasoactive intestinal peptide, substance P, and histamine reduce dye coupling whereas acetylcholine and the ß-adrenergic agonist isoproterenol have no effect (20,21; see Table 2).

Yet, in contrast to some of these data, we found that VIP and vasopressin increase dye coupling in one mouse TEC line and primary cultures of thymic nurse cells. Supporting this idea, 8Br-cAMP, a permeable analog of cAMP (secondary messenger of VIP and vasopressin), can also increase the degree of inter-TEC dye coupling (Alves LA, Figueira G, Savino W and Campos-de-Carvalho AC, unpublished results). Additionally, we found an increase in dye coupling in a rat cell line (clone IT45-R1, provided by Dr. Tsumeroshi Itoh, Tohoku University, Sendai, Japan) when cells were treated with dexamethasone (Figure 1). Much work is still necessary in order to obtain a clear view of the possible role of gap junctions in thymus tissue. Furthermore, it is essential to understand the multiple levels of regulation of intercellular communication by way of gap junctions in the thymus gland. Presently, the mechanisms by which dye coupling increases or decreases in thymic epithelial cells when these cells are treated with extracellular messenger molecules are not known. Table 2 summarizes the effects of several extracellular molecules on inter-TEC dye coupling, as well as the expression of the respective receptors. One can see that receptors for some putative modulators have not yet been formally characterized in TEC, and that several hormones for which receptors are expressed have not been studied for their potential effects on

Table 2 - Neuroendocrine modulators of inter-TEC gap junctions.

ACh, Acetylcholine; ACTH, adrenocorticotropin hormone; AR, auto-radiography; CGRP, calcitonin gene-related peptide; EF, electrophysiology; GABA, γ-aminobutyric acid; GH, growth hormone; 5-HT, 5-hydroxytryptamine; IHC, immunohistochemistry; IL1-α, interleukin-1α; IL1-ß, interleukin-1ß; LM, light microscopy; NB, Northern blotting; NE, no effect; NPY, neuropeptide Y; PA, pharmacological analysis; RT-PCR, reverse transcription-polymerase chain reaction; RTM, reticuloepithelial cells of thymus medulla; SPA, Scatchard plot analysis; SP, substance P; TEC, thymic epithelial cells; VIP, vasoactive intestinal peptide; WB, Western blotting. *Summarized from Refs. 20 and 21; **the receptor expression is represented by the following signals: + (positive expression), - (not found) or ND (not determined); ***related to receptor characterization studies; #modulation of dye coupling is represented by the arrows: ↘ (negative modulation).

Modulators*	Dye coupling*#	Cell type***	Receptor**	Approach	References***
ACh	NE	Thymic extract	+	NB, RT-PCR	59
ACTH	↘		ND		
CGRP	↘	TEC	+	RT-PCR, IHC	62
GABA	↘		-	EF	68
GH (rat)	↘	Type-1 TEC	+	NB, WB, RT-PCR, IHC	61
Histamine	↘		ND		
5-HT	NE		ND		
IL1-α	↘		ND		
IL1-ß	↘		ND		
Isoproterenol	NE	Thymic extract, TEC	+	PA, NB, RT-PCR	66, 67
NPY	↘		-	LM, IHC	63
Oestrogen	↘	RTM	+	IHC	58
Progesterone	↘	RTM	+	SPA, IHC	57, 58
SP	↘		-	AR	60
Testosterone	↘	Thymic extract	+	SPA	64, 65
Thymulin	NE		ND		
VIP	↘	Cortical/medullar TEC	+	RT-PCR, IHC, AR	60, 62

TEC gap junctions. For instance, in human TEC the GABA receptor was not found. Yet, when this neurotransmitter is applied to rat TEC, a decrease in dye coupling is observed, indicating a possible species-specific modulation or even an epiphenomenon.

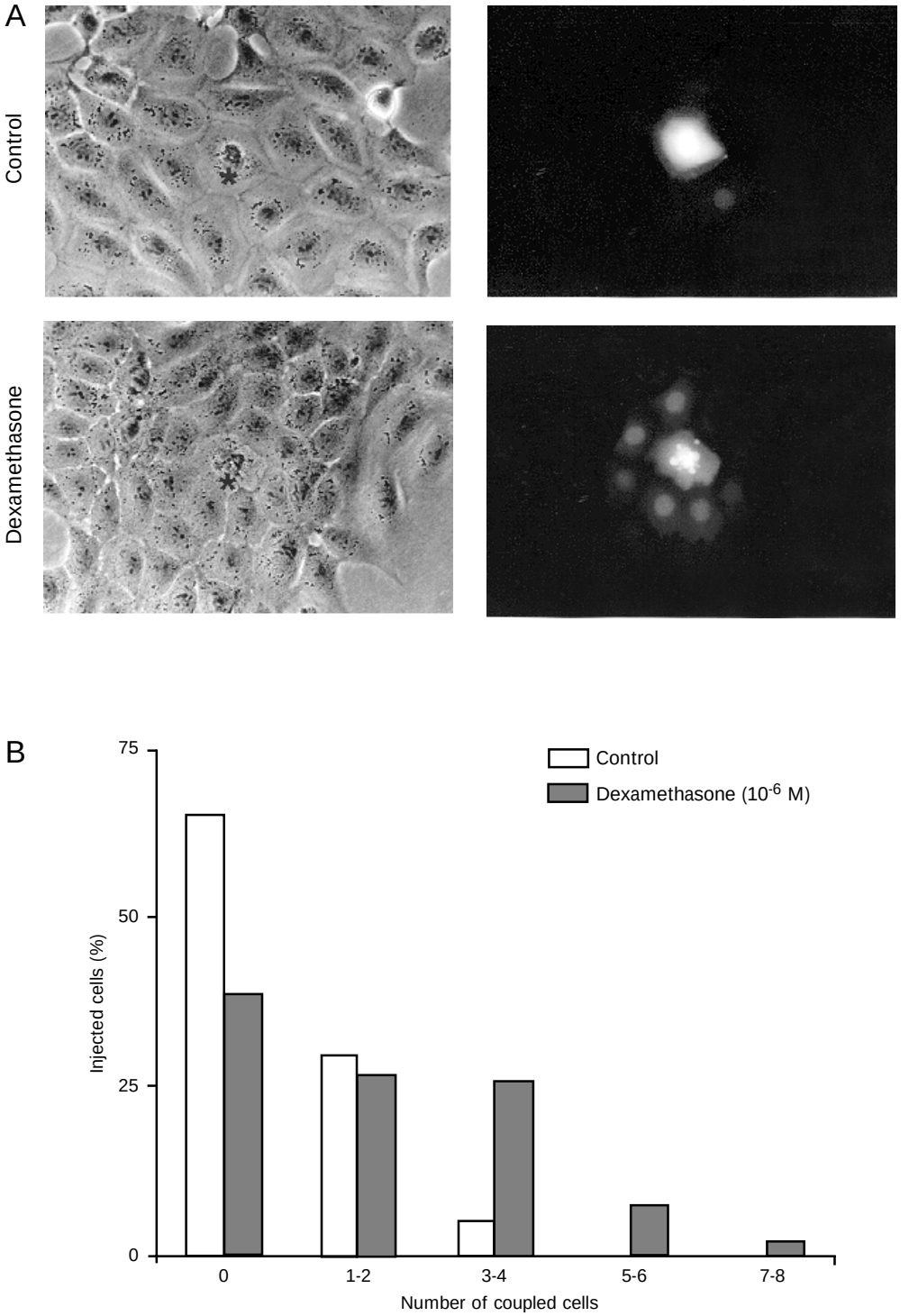

Figure 1 - Increase in gap junctional communication induced by dexamethasone in a rat epithelial cell line. TEC were treated with 10^{-6} M dexamethasone for 48 h, and intercellular communication was evaluated by dye transfer assay using the fluorochrome lucifer yellow (LY). *A*, Microscopy fields (phase contrast and fluorescence, respectively, in the left and right panels) depicting the injected cell (*) and those that were coupled when LY was injected (magnification 320X). *B*, Histograms showing the pattern of coupling degree of control and dexamethasone-treated cells. The analysis comprises 100 microinjections per group.

Conclusion and perspectives

Taken together, the data discussed herein indicate that inter-TEC gap junctions in the thymus gland can be modulated by several extracellular messenger molecules, which may influence the putative functions of gap junctions in the thymic epithelium. It remains to be defined at what level this modulation occurs and to what extent it influences thymus physiology.

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