Published in final edited form as:

J Mol Neurosci. 2012 October ; 48(2): 413–419. doi:10.1007/s12031-012-9745-1.

Immune-Neuroendocrine Integration at the Adrenal Gland: Cytokine Control of the Adrenomedullary Transcriptome

Stephen J. Bunn¹, Djida Ait-Ali², and Lee E. Eiden²

¹Centre for Neuroendocrinology Department of Anatomy School of Medical Sciences, University of Otago P.O. Box 913, Dunedin, New Zealand ²National Institutes of Health/National Institute of Mental Health Section on Molecular Neuroscience Laboratory of Cellular and Molecular Regulation Building 49, Room 5A-38 9000 Rockville Pike Bethesda MD 20892

Abstract

The bovine chromaffin cell represents an ideal model for the study of cell signaling to gene expression by first messengers. An abundance of GPCR, ionotropic, and growth factor receptors are expressed on these cells, and they can be obtained and studied as an abundant highly enriched cell population: importantly, this is true of no other post-mitotic neuroendocrine or neuronal cell type. Chromaffin cells have now been shown to bear receptors for cytokines whose expression in the circulation is highly elevated in inflammation, including TNF, IFN, IL-1 and IL-6. The use of bovine-specific microarrays, and various biochemical measurements in this highly homogenous cell preparation, reveals unique cohorts of distinct genes regulated by cytokines in chromaffin cells, via signaling pathways that are in some cases uniquely neuroendocrine. The transcriptomic signatures of cytokine signaling in chromaffin cells suggest that the adrenal medulla may integrate neuronal, hormonal, and immune signaling during inflammation through induction of paracrine factors that signal to both adrenal cortex and sensory afferents of the adrenal gland, and autocrine factors, which determine the duration and type of paracrine secretory signaling that occurs in either acute or chronic inflammatory conditions.

Keywords

adrenal medulla; chromaffin cell; cytokine; IFN; IL-1; IL-6; inflammation; PACAP; stress; TNF

Introduction

Cytokines were first identified as small glycoproteins of apparent molecular weight 21–28 kD produced by lymphocytes (lymphokines) or macrophages (monokines) to facilitate innate immune response to bacterial and viral infection via autocrine, paracrine and hormonal signaling. Most cytokines act following secretion from the cell of origin, while some, such as tumor necrosis factor- α (TNF- α), may also act on adjacent cells while remaining anchored to the plasma membrane of the producing cell. A major role of the cytokines considered here (TNF- α ; interferon- α , IFN- α ; interleukin-1 α/β , IL-1 α/β ; interleukin-6, IL-6) is the generation of the acute phase of the inflammatory

Correspondence for communication during review and publication to: Lee E. Eiden Building 49, Room 5A-38 9000 Rockville Pike Bethesda MD 20892 Tel: (301) 496-4110 Fax: (301) 402-1748 eidenl@mail.nih.gov Correspondence for communication after publication to: Lee E. Eiden Building 49, Room 5A-38 9000 Rockville Pike Bethesda MD 20892 Tel: (301) 496-4110 Fax: (301) 402-1748 eidenl@mail.nih.gov and Stephen J. Bunn Centre for Neuroendocrinology Department of Anatomy School of Medical Sciences, University of Otago P.O. Box 913, Dunedin, New Zealand Tel: (64)(3) 479-7366 Fax: (64)(3) 479-7366 stephen.bunn@anatomy.otago.ac.nz.

reaction, which includes C-reactive protein and alpha-2-microglobulin secretion from the liver, induction of sickness behavior within the brain, lymphocyte, macrophage, neutrophil, and fibroblast activation in various organs, and muscle proteolysis. These protean cellular effects are further fine-tuned in each organ system by tissue-specific regulation of cytokine receptor expression, and generation of additional cytokines, soluble cytokine receptors that damp cellular function, and glucocorticoids in the adrenal cortex that potently down-regulate cytokine biosynthesis in virtually all cells that produce them.

The tight control of the 'cytokine storm' produced by inflammatory stimuli via glucocorticoid secretion from the adrenal cortex has long been recognized, for example through the classical finding that after adrenalectomy, doses of lipopolysaccharide (LPS) or the cytokines elevated by bacterial infection or LPS (IL-1 and TNF- α) that are innocuous in intact mice become lethal, with lethality reversed by dexamethasone administration (Bertini et al. 1988). Appreciation of the adrenal medulla as a link in facilitation and modulation of the cytokine response developed somewhat less rapidly, in part because the secretory products of the adrenal medulla have less dramatic anti-inflammatory effects than glucocorticoids. Although it is now abundantly clear that psychogenic and cold stress can be profoundly immunosuppressive, the connection between stress and immune function has previously been localized to the effects of altered sympathetic tone on the innate immune system (Elenkov et al. 2000) rather than at the level of cytokine modulation of the adrenomedullary hormonal system (Goldstein 2010).

Cytokine interactions with adrenomedullary cells leading to altered catecholamine secretion and biosynthesis has been recently reviewed (Douglas et al. 2010). The purpose of this short review is to bring up-to-date emerging evidence that cytokine signaling potently and profoundly alters gene expression in the adrenal medulla, changing its secretory properties both qualitatively and quantitatively. Special features of cytokine signaling to chromaffin cells include the induction of multiple intercellular signaling molecules such as neuropeptides, other cytokines, and catecholamines (through tyrosine hydroxylase induction) which creates a dense paracrine/autocrine interplay allowing cytokines to 'program' chromaffin cells for both short- and long-term altered responsiveness, and integration through cross-talk and synergistic effector function. Adrenomedullary response to cytokine signaling via a cascading induction of secreted molecules suggests a mechanism whereby 'immune memory' is programmed into this stress-transducing organ, allowing the gland to respond differently to stress in the context of inflammation, and vice versa.

Cytokine receptors and their signaling pathways in chromaffin cells

A key to understanding how cytokines regulate adrenomedullary function begins with the observation that receptors for TNF- α , IFN- α , IL-1 α/β and IL-6 are all present on chromaffin cells (vide infra). Receptor localization and function is deduced mainly in three ways: presence of receptors or mRNA encoding them in chromaffin cells; rapid activation of second and third messengers in highly purified chromaffin cell cultures upon exposure to cytokines; and correlation of blockade of third messenger activation and altered chromaffin cell function (secretion, tyrosine hydroxylase induction, etc.) upon exposure to specific cytokines. Each of these approaches has merits and limitations, and these are commented upon as appropriate. We also note here that cytokines activating identical pathways, such as Signal Transducer and Activator of Transcription (STAT), Extracellular Signal-Regulated Kinases 1 and 2 (ERK1/2) and Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NF- κ B) often do so with very different time-courses, and likely quite different cellular outcomes, reminiscent of the dramatically different temporal patterns of activation of ERK signaling in endocrine cells by the trophic factors EGF and NGF, with acute elevation (EGF)

leading to cellular proliferation, and prolonged elevation (NGF) promoting growth arrest and differentiation (Marshall 1995).

In the case of TNF- α , receptor-mediated first messenger signaling to chromaffin cells has been documented in all three domains: documentation of specific receptor expression in chromaffin cells; characterization of intracellular signaling component activation (phosphorylation), and pharmacological correlation of chromaffin cell read-outs (secretion, gene expression, etc.) with signaling pathway activation using specific inhibitors of known signaling pathways. TNF- α signaling occurs through one of two receptor types, TNFR1 and TNFR2. While both TNFR1 and TNFR2 activate NF- κ B in response to occupancy by TNF- α , there are differences in both signaling profiles, and cellular responses, upon activation of either receptor (Baud and Karin 2001; MacEwan 2002; Thommesen and Laegreid 2005). In the central nervous system, for example, TNFR1 activation mediates programmed cell death, and TNFR2 provides neuronal protection from apoptosis, in the context of brain injury or neurodegenerative disease (Marchetti et al. 2004; Tweedie et al. 2007). Bovine chromaffin cells exclusively express TNFR2 (Ait-Ali et al. 2008). Exposure of these cells to TNF- α results in activation of the MAP kinases ERK1/2 and p38 and transcription factor NF- κ B, as deduced from a combination of measurement of protein activation by phosphorylation (ERK1/2), induction of genes characteristic of the signaling pathway's activation (I κ B, in the case of NF- κ B), and blockade of gene induction by specific inhibitors of the pathway (U0126 for ERK1/2; SB 203580 for p38; PDTC for NF- κ B) (Ait-Ali et al. 2008).

IFN- α responsiveness of chromaffin cells was demonstrated in studies revealing that exposure to this cytokine suppressed both catecholamine release and its reuptake (Tachikawa et al. 1997; Toyohira et al. 1998). It was suggested that these actions resulted from a suppression of nicotinic acetylcholine receptor and norepinephrine transporter expression, respectively. More recent studies have identified IFN- α -activated signaling pathways in chromaffin cells, with a rapid but transient increase in ERK1/2 activity followed by a slower but more sustained rise in phosphorylated STAT1 and STAT2 levels (Douglas and Bunn 2009). Activated ERK1/2 may target a number of substrates including tyrosine hydroxylase, while the STAT proteins directly regulate gene transcription. Thus, while there is no definitive evidence regarding the molecular nature of the IFN- α receptor expressed on chromaffin cells, there is good evidence that they are responsive to this cytokine (Douglas and Bunn 2009).

Immunohistochemical and in situ-hybridization studies have demonstrated the presence of IL-6 receptors (IL6R) within the adrenal medulla of both rodent and human, with only some chromaffin cells expressing IL6R in human, and most expressing IL-6R in rat (Gadient et al. 1995; Path et al. 1997). The obligate coreceptor for IL-6 signaling, gp130, is ubiquitously expressed. Thus, as expected, isolated chromaffin cells are IL-6 responsive, with our own preliminary studies demonstrating a rapid but transient ERK1/2 activation followed by STAT3 phosphorylation upon acute exposure to this cytokine (Bunn et al. 2009).

IL-1 receptor activation of chromaffin cells has been reported to have both acute and prolonged effects. An acute inhibition of acetylcholine-induced catecholamine release occurs via an ERK1/2-mediated suppression of Ca²⁺ influx (Morita et al. 2004). Continued exposure to IL-1 leads to increased basal catecholamine release (Gwosdow 1995; Yanagihara et al. 1994). There is also a neuropeptide Y-mediated increased release of catecholamine in both mouse and human chromaffin cells after exposure to IL-1, which is associated with both enhanced tyrosine hydroxylase expression and increased tyrosine hydroxylase serine-40 phosphorylation (Rosmaninho-Salgado et al. 2007; Rosmaninho-Salgado et al. 2009). Prolonged exposure to IL-1 also results in enhanced neuropeptide

secretion upon chromaffin cell depolarization, suggesting that this cytokine, like TNF- α , may facilitate the effects of other secretagogues on chromaffin cells (Ait-Ali et al. 2004).

Although not all of the major features of signaling in chromaffin cells have been worked out, in general cytokine effects are consistent with the scheme shown in Figure 1. Perhaps the most physiologically relevant questions yet to be resolved are how the signaling pathways depicted in Figure 1 are executed *temporally* in chromaffin cells, compared to non-neuroendocrine cytokine target cells; how this affects the transcription of genes encoding autocrine, paracrine and hormonal factors secreted by the adrenal medulla; and how cytokines and neurotransmitters might interact, synergistically or antagonistically, to integrate inflammatory and neural signaling in vivo (see below).

Cytokine gene targets in chromaffin cells

One of the earliest hints that adrenomedullary function might be cytokine-regulated was detection of IL-1 α/β immunoreactivity in chromaffin cells themselves (Schultzberg et al. 1989). The ability to examine purified chromaffin cells in culture led to the further observation that these cells were also responsive to interleukins, with both TNF- α and IL-1 α mediating pronounced up-regulation of neuropeptide expression, especially that of vasointestinal peptide (VIP) in cultured bovine chromaffin cells (Eskay and Eiden 1992). A hallmark of this regulation was its delayed effect: both cytokines increased peptide expression only after several hours in culture. Later demonstration of VIP and galanin (GAL) mRNA up-regulation by each of these cytokines in cultured chromaffin cells indicated that this delay (18-24 h) was due not to the time required for neuropeptide processing from the prohormone precursor, but to a lag phase in induction of mRNA synthesis (Ait-Ali et al. 2004). These observations provided an indication of complex temporal regulation, and possibly a cascade of autocrine/paracrine effects leading to gene induction in the adrenal medulla. Induction of GAL and VIP provide a possible physiological role for cytokine action on the adrenal medulla beyond modulation of catecholamine biosynthesis and release (see above). Thus, both VIP and GAL are reported to affect steroidogenesis in adrenocortical cells (Andreis et al. 2007; Bodnar et al. 1997; Tortorella et al. 2007; Toth and Hinson 1995), providing a potential mechanism for paracrine regulation of the adrenal cortex through induction of these factors by cytokine stimulation of chromaffin cells.

A more comprehensive transcriptomics approach, which has been facilitated by commercial availability of bovine oligonucleotide chips for species-specific hybridization of cDNA and cRNA from bovine chromaffin cells (Ait-Ali, D., Bunn, S., Eiden, L.E., Mustafa, T., Samal, B., in preparation), has led to the emerging view that cohorts of genes regulated by cytokines in chromaffin cells, or at least by TNF- α , are indeed biased towards enhanced expression of secreted proteins/prohormones, consistent with a highly integrative function for the adrenomedullary cytokine response via propagation of signaling to the adrenal cortex (paracrine), distant organs ('hormonal'), and the medulla itself (autocrine) (Ait-Ali et al. 2010a). It remains to be seen first, if other cytokines similarly enhance the type and number of informational molecules/first messengers secreted from chromaffin cells and second, whether the effects of TNF- α itself on secretory protein gene expression may be mediated by the intermediate induction of other cytokines from the chromaffin cell (i.e. an autocrine cascade mechanism, see below).

Cytokine signaling cascades in chromaffin cells

TNF- α effects on the chromaffin cell transcriptome occur in two waves, early (6 h, NF- κ B-dependent) and late (ERK-dependent, including induction of neuropeptides such as GAL and VIP) (Ait-Ali et al. 2010b; Ait-Ali et al. 2008). This suggests the possibility that TNF- α

affects neuropeptide biosynthesis via an early induction of other cytokines, such as IL-6, which then, in paracrine fashion, induces neuropeptides such as GAL and VIP. To deduce this regulatory web fully will require complete transcriptomic analysis for each of the cytokines in bovine chromaffin cells, an effort currently underway (Ait-Ali, D., Bunn, S., Eiden, L.E., Mustafa, T., Samal, B., in preparation). We know already that the major stress transmitter at the splanchnic-adrenal synapse, pituitary adenylate cyclase-activating peptide (PACAP), regulates some of the same pathways, and is itself regulated by TNF- α (Ait-Ali, D., Bunn, S., Eiden, L.E., Mustafa, T., Samal, B., in preparation). A cascade that is initiated by either cytokines or stress-related PACAP release, and propagated by additional cytokines, or later changes in splanchnic firing, would control the induction of various bioactive factors during periods of prolonged inflammation, stress, or both. This could provide for stress priming of cytokine modulation of adrenomedullary output, and cytokine facilitation of stress-mediated adrenal cell plasticity, and 'immunological memory' for integration of stress and inflammatory signaling.

Cytokine effects in vivo

Much of the information about cytokine signaling properties discussed so far has been obtained in bovine chromaffin cells, which highlights that the cytokine effects are truly direct and not mediated through the action of fibroblasts, endothelial cells or other non-parenchymal cells. That this signaling actually occurs in vivo and has physiological and pathophysiological consequences has been shown by experiments in which LPS treatment causes the induction of neuropeptides including both VIP and GAL, with a delay similar to that seen in cultured chromaffin cells treated with TNF- α . Interestingly, this induction is attenuated in PACAP-deficient mice, reminiscent of the synergistic stimulation of VIP and GAL by PACAP and TNF- α in cultured chromaffin cells, and supporting the idea that adrenomedullary cytokine actions both condition, and are conditioned by, stress signaling via the splanchnic nerve (Ait-Ali et al. 2010b). Figure 2 summarizes the potential interactions between systemic cytokine generation, cytokine signaling to the adrenal medulla, and subsequent autocrine, paracrine and hormonal modulation in vivo.

An additional mode of cytokine signaling via the adrenal medulla noted in Figure 2 is through activation of sensory afferents to the CNS. The adrenal medulla is known to be innervated by vagal sensory afferents, albeit the density of innervation varies among mammalian species (Coupland et al. 1989). Sensory afferents mediate neurogenic inflammation (mainly through substance P) leading to cytokine elaboration from mast cells, Kupffer cells, and other innate immunocytes, and also bear receptors for a plethora of neuropeptides (Weihe et al. 1991; Weihe 1991). Cytokine-initiated neuropeptide synthesis and secretion from the adrenal medulla therefore allows the possibility of bi-directional communication between periphery and CNS providing further integration between central and peripheral mechanisms of inflammatory and stress response (Fig. 2). It will be of interest to employ pharmacological as well as genetic approaches to determine if the adrenal medulla, through cytokine-dependent as well as PACAP-dependent regulation of substance P, neurotensin, IL-6, and other informational molecules, acts to coordinate CNS as well as adrenocortical homeostatic responses to stress and inflammation, individually and in combination.

Concluding remarks

Cytokine signaling to chromaffin cells of the adrenal medulla is remarkable for the profound alteration in cellular plasticity that ensues as a result of de novo expression of a cascade of neuropeptides and chemokines with their own signaling capabilities both within the adrenal gland and beyond. The full prospect of how this signaling network may act to control and

integrate immune and stress responses is beginning to come into view, but there is as yet no clear picture as to how this information can be systematically exploited in management of stress-related immunodeficiency, immune hyper-reactivity, altered immune function in ageing, or septic shock. It may be relevant to end on the note that, while many features of cytokine signaling to chromaffin cells are distinct from cytokine signaling between cellular components of the classical immune system, the chromaffin cell may be a paradigm for how cytokines regulate other endocrine organs (see, e.g. (Elenkov 2008)), including the pituitary, enterochromaffin system, and endocrine pancreas, in neuroimmunoendocrine homeo- and allostasis.

References

- Ait-Ali D, Samal B, Mustafa T, Eiden LE. Neuropeptides, growth factors and cytokines: A cohort of informational molecules whose expression is up-regulated by the stress-associated slow transmitter PACAP in chromaffin cells. *Cell. Mol. Neurobiol.* 2010a; 30:1441–1449. [PubMed: 21107678]
- Ait-Ali D, Stroth N, Sen JM, Eiden LE. PACAP-cytokine interactions govern adrenal neuropeptide biosynthesis after systemic administration of LPS. *Neuropharmacology.* 2010b; 58:208–14. [PubMed: 19647754]
- Ait-Ali D, Turquier V, Grumolato L, Yon L, Jourdain M, Alexandre D, Eiden LE, Vaudry H, Anouar Y. The proinflammatory cytokines tumor necrosis factor- α and interleukin-1 stimulate neuropeptide gene transcription and secretion in adrenochromaffin cells via activation of extracellularly regulated kinase 1/2 and p38 protein kinases, and activator protein-1 transcription factors. *Mol. Endocrinol.* 2004; 18:1721–1739. [PubMed: 15087472]
- Ait-Ali D, Turquier V, Tanguy Y, Thouenon E, Ghzili H, Mounien L, Derambure C, Jegou S, Salier JP, Vaudry H, Eiden LE, Anouar Y. Tumor necrosis factor (TNF)- α persistently activates nuclear factor- κ B signaling through the type 2 TNF receptor in chromaffin cells: implications for long-term regulation of neuropeptide gene expression in inflammation. *Endocrinology.* 2008; 149:2840–52. [PubMed: 18292192]
- Andreis PG, Tortorella C, Ziolkowska A, Spinazzi R, Malendowicz LK, Neri G, Nussdorfer GG. Evidence for a paracrine role of endogenous adrenomedullary galanin in the regulation of glucocorticoid secretion in the rat adrenal gland. *Int. J. Mol. Med.* 2007; 19:511–515. [PubMed: 17273801]
- Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* 2001; 11:372–7. [PubMed: 11514191]
- Bertini R, Bianchi M, Ghezzi P. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *J. Exp. Med.* 1988; 167:1708–1712. [PubMed: 3259257]
- Bodnar M, Sarrieau A, Deschepper CF, Walker CD. Adrenal vasoactive intestinal peptide participates in neonatal corticosteroid production in the rat. *Am. J. Physiol.* 1997:273.
- Bunn, SJ.; F. C, SD. Proceedings of the Australian Neuroscience Soc. Canberra, Australia: 2009. Interleukin-6 mediated activation of ERK1/2 and STAT3 in adrenal medullary chromaffin cells.
- Coupland RE, Parker TL, Kesse WK, Mohamed AA. The innervation of the adrenal gland. III. Vagal innervation. *J Anat.* 1989; 163:173–81. [PubMed: 2606772]
- Douglas SA, Bunn SJ. Interferon- α signalling in bovine adrenal chromaffin cells: involvement of signal-transducer and activator of transcription 1 and 2, extracellular signal-regulated protein kinases 1/2 and serine 31 phosphorylation of tyrosine hydroxylase. *J Neuroendocrinol.* 2009; 21:200–7. [PubMed: 19207826]
- Douglas SA, Sreenivasan D, Carman FH, Bunn SJ. Cytokine interactions with adrenal medullary chromaffin cells. *Cell Mol Neurobiol.* 2010; 30:1467–75. [PubMed: 21088883]
- Elenkov IJ. Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. *Neurochem Int.* 2008; 52:40–51. [PubMed: 17716784]
- Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.* 2000; 52:595–638. [PubMed: 11121511]

- Eskay RL, Eiden LE. Interleukin-1 alpha and tumor necrosis factor alpha differentially regulate enkephalin, vasoactive intestinal polypeptide, neurotensin, and substance P biosynthesis in chromaffin cells. *Endocrinology*. 1992; 130:2252–2258. [PubMed: 1372239]
- Gadient RA, Lachmund A, Unsicker K, Otten U. Expression of interleukin-6 (IL-6) and IL-6 receptor mRNAs in rat adrenal medulla. *Neurosci Lett*. 1995; 194:17–20. [PubMed: 7478202]
- Goldstein DS. Adrenal Responses to Stress. *Cell Mol Neurobiol*. 2010; 30:1433–1440. [PubMed: 21061156]
- Gwosdow AR. Mechanisms of interleukin-1-induced hormone secretion from the rat adrenal gland. *Endocr Res*. 1995; 21:25–37. [PubMed: 7588387]
- MacEwan DJ. TNF receptor subtype signalling: differences and cellular consequences. *Cell. Signal*. 2002; 14:477–92. [PubMed: 11897488]
- Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL. Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. *J. Biol. Chem*. 2004; 279:32869–81. [PubMed: 15155767]
- Marshall CJ. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell*. 1995; 80:179–85. [PubMed: 7834738]
- Morita K, Miyasako T, Kitayama S, Dohi T. Interleukin-1 inhibits voltage-dependent P/Q-type Ca²⁺ channel associated with the inhibition of the rise of intracellular free Ca²⁺ concentration and catecholamine release in adrenal chromaffin cells. *Biochim Biophys Acta*. 2004; 1673:160–9. [PubMed: 15279887]
- Path G, Bornstein SR, Ehrhart-Bornstein M, Scherbaum WA. Interleukin-6 and the interleukin-6 receptor in the human adrenal gland: expression and effects on steroidogenesis. *J Clin Endocrinol Metab*. 1997; 82:2343–9. [PubMed: 9215317]
- Rosmaninho-Salgado J, Alvaro AR, Grouzmann E, Duarte EP, Cavadas C. Neuropeptide Y regulates catecholamine release evoked by interleukin-1beta in mouse chromaffin cells. *Peptides*. 2007; 28:310–4. [PubMed: 17207896]
- Rosmaninho-Salgado J, Araujo IM, Alvaro AR, Mendes AF, Ferreira L, Grouzmann E, Mota A, Duarte EP, Cavadas C. Regulation of catecholamine release and tyrosine hydroxylase in human adrenal chromaffin cells by interleukin-1beta: role of neuropeptide Y and nitric oxide. *J Neurochem*. 2009; 109:911–22. [PubMed: 19309436]
- Schultzberg M, Andersson C, Unden A, Bartfai T. Interleukin-1 in adrenal chromaffin cells. *Neuroscience*. 1989; 30:805–810. [PubMed: 2788829]
- Tachikawa E, Kondo Y, Takahashi M, Kashimoto T, Yanagihara N, Toyohira Y, Izumi F. Interferon-alpha reduces catecholamine secretion from bovine adrenal chromaffin cells stimulated by acetylcholine. *Naunyn Schmiedeberg's Arch Pharmacol*. 1997; 356:699–705. [PubMed: 9453454]
- Thommesen L, Laegreid A. Distinct differences between TNF receptor 1- and TNF receptor 2-mediated activation of NFkappaB. *J. Biochem. Mol. Biol*. 2005; 38:281–289. [PubMed: 15943902]
- Tortorella C, Neri G, Nussdorfer GG. Galanin in the regulation of the hypothalamic-pituitary-adrenal axis (Review). *Int J Mol Med*. 2007; 19:639–47. [PubMed: 17334639]
- Toth IE, Hinson JP. Neuropeptides in the adrenal gland: distribution, localization of receptors, and effects on steroid hormone synthesis. *Endocr Res*. 1995; 21:39–51. [PubMed: 7588403]
- Toyohira Y, Yanagihara N, Minami K, Ueno S, Uezono Y, Tachikawa E, Kondo Y, Kashimoto T, Izumi F. Down-regulation of the noradrenaline transporter by interferon-alpha in cultured bovine adrenal medullary cells. *J Neurochem*. 1998; 70:1441–7. [PubMed: 9523560]
- TweeD D, Sambamurti K, Greig NH. TNF-alpha inhibition as a treatment strategy for neurodegenerative disorders: new drug candidates and targets. *Curr Alzheimer Res*. 2007; 4:378–85. [PubMed: 17908040]
- Weihe E, Nohr D, Michel S, Muller S, Zentel HJ, Fink T, Krekel J. Molecular anatomy of the neuro-immune connection. *Int. J. Neurosci*. 1991; 59:1–23. [PubMed: 1774130]
- Weihe F, Nohr D, Muller S, Muchler M, Friess H, Zentel HJ. The Tachykinin Neuroimmune Connection in Inflammatory Pain. *Ann NY Acad Sci*. 1991; 632:283–295. [PubMed: 1719872]

Yanagihara N, Minami K, Shirakawa F, Uezono Y, Kobayashi H, Eto S, Izumi F. Stimulatory effect of IL-1 beta on catecholamine secretion from cultured bovine adrenal medullary cells. *Biochem Biophys Res Commun.* 1994; 198:81–7. [PubMed: 8292052]

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1. Signaling pathways for cytokine regulation of gene expression in adrenal medullary chromaffin cells

Ligand binding to cytokine-specific receptors (TNF-R2, IL-1R, IL-6R and IFN α R) located on the chromaffin cell membrane leads to the activation of multiple, potentially interacting, intracellular pathways. Evidence suggests that each of the illustrated cytokines stimulate MAP-kinase activity. This may involve a single member of the MAP-kinase family, as appears to be the case for IL-6 activation of ERK1/2, or dual activation of ERK1/2 and p38 as occurs following exposure of the chromaffin cells to TNF- α . Activated MAP-kinases may then act upon cytosolic targets such as tyrosine hydroxylase (not illustrated here) and a variety of nuclear transcription factors including AP-1. In addition to the promotion of MAP-kinase activity each cytokine-mediated signaling pathway contains direct transcriptional regulators, NF- κ B (p50/p52) in the case of TNF- α or STAT1/STAT2 and STAT3 in the case of IFN- α and IL-6, respectively. As suggested by our existing data describing the profile of TNF- α signaling in chromaffin cells it is likely that these different cytokine-activated pathways have distinct temporal profiles. A critical remaining issue is the investigation of potential interactions both within and between the various cytokine-specific signaling pathways. As discussed in the body of this review recent microarray data indicates that each of the above cytokines increases the expression of an array of genes in the chromaffin cell. While detailed analysis of these data is still ongoing, three functional distinct gene classes are readily apparent. First, each cytokine increases the expression of a specific sub-set of genes the products of which act as immediate, and often inhibitory, regulators of that specific pathway; TNF- α for example increases I κ B expression while IL-6 increases that of SOCS3 (not depicted in this figure). Secondly, in agreement with cytokine signaling in other cell types each primary cytokine increases the expression of other cytokines, most notably TNF- α , increases IL-6 mRNA levels probably through NF- κ B activation and results in a potential paracrine regulation of the adrenal gland. Such an action may serve to facilitate the controlled propagation of the inflammatory signal through the adrenal tissue. Finally, each cytokine increases the expression of several neuropeptides, which are known to be secretory products of the adrenal medulla. In the case of TNF- α these include VIP, GAL and secretogranin II. The potential role of such neuropeptides is considered in the text and depicted schematically in Figure 2. The solid lines indicate signaling pathways deduced from published data; the dashed arrows indicate pathways

confirmed by non-published data; the dotted lines indicate potential pathways deduced from non-published data. ACh, Acetylcholine; AChR, Acetylcholine receptor.

\$watermark-text

\$watermark-text

\$watermark-text

Figure 2. Autocrine, paracrine and hormonal cellular communication mediated by cytokines in the adrenal medulla: Implications for integration of inflammation and stress signaling in vivo Cytokines including $\text{TNF-}\alpha$, IL-1, IL-6 and $\text{IFN}\alpha$, secreted by a variety of immunocytes, interact with several types of target cell including the chromaffin cells of the adrenal medulla. Following the activation of specific signaling pathways (as summarized in Figure 1) the chromaffin cells increase expression of genes encoding neuropeptides and additional inflammatory mediators such as cytokines and chemokines. While the adrenal medullary cytokines are likely to be involved in the controlled propagation of the inflammatory response, the role of the secreted neuropeptides is less clear. As outlined in the text some neuropeptides may act as autocrine regulators within the adrenal medulla or as paracrine signals to the adjacent adrenal cortex. These latter two actions may have an anti-inflammatory role through enhancement of glucocorticoid output from the adrenal cortex. Other potentially important targets of the increased adrenal medullary neuropeptide output include the immune system or sensory afferents (vagus nerve) known to be present within the adrenal medulla, and as discussed in the text, responsive to a variety of neuropeptides. Finally, previous work from our laboratory has demonstrated powerful synergistic interactions between the inflammatory cytokines, particularly $\text{TNF-}\alpha$, and the adrenal medullary synaptic transmitter PACAP released from the splanchnic nerve. It is likely therefore that cytokines not only act directly on the chromaffin cells to regulate their gene expression, and thus secretory output, but can also influence, or at least interact with, their synaptic regulation. Such a dual action could potentially allow cytokines to differentially influence basal compared to stress-activated adrenal medullary output. Although the figure summarizes data obtained in several species, not all interactions should be considered as 'pan-specific' without actual evidence in the species of interest (Ehrhart-Bornstein, et al. 1991; Ehrhart-Bornstein, et al. 2000). ACh, acetyl choline; ACTH, adrenocorticotrophic hormone; CNS, central nervous system; SNS, sympathetic nervous system; NPs, neuropeptides; PVN, paraventricular nucleus.