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Review article

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ROLE OF PROSTAGLANDINS IN THE STIMULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS BY ADRENERGIC AND NEUROHORMONE SYSTEMS

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Role of prostaglandins (PGs) in the activation of the hypothalamic-pituitary-adrenal (HPA) axis by the adrenergic agonists, corticotropin-releasing hormone (CRH) and vasopressin (VP) in rats under basal and social stress conditions was investigated. Systemic or intracerebroventricular (icv) pretreatment with indomethacin powerfully reduced the corticosterone response to ivc phenylephrine, an α_1-receptor agonist, significantly diminished the response to clonidine, an α_2-receptor agonist, but did not alter the response to isoprenaline,a β-adrenergic agonist. Consequently, indomethacin considerably reduced the corticosterone response to noradrenaline, an α_1- and α_2-adrenergic agonist, but did not change the response to adrenaline, a predominant β-adrenergic agonist. Thus, prostaglandins considerably mediate the HPA activity stimulated *via* central α_1-and α_2-but not β-adrenergic receptors. Social crowding stress for 3 days did not affect the corticosterone response to ip or icv CRH, but drastically reduced the response to VP. In stressed rats indomethacin did not alter the corticosterone response to CRH but significantly further impaired the diminished by stress corticosterone response to VP. Neither social stress nor endogenous prostaglandins affected the responsiveness of the CRH system. By contrast, both social stress and prostaglandins considerably diminished the HPA response to VP. The above results indicate that both these neurohormone systems have a distinct mode of adaptation and interaction with PG systems during social stress. Interleukins, particularly IL-1β and IL-6, activate the HPA axis. Most immunological stimuli and interleukins also activate both the central and the peripheral noradrenergic systems. Activation of the HPA axis *in vivo* depends on the secretion of CRH, an intact pituitary and the ventral adrenergic bundle innervating the hypothalamic paraventricular nucleus. Interleukins may cross the blood-brain-barrier or be produced in the CNS to stimulate their receptors in brain structures involved in the regulation of the HPA axis.

Key words: *Prostaglandins, indomethacin, adrenergic receptors, adrenergic agonists, hypothalamic-pituitary-adrenal axis, corticotropin-releasing hormone, vasopressin, social stress, neurohormone systems adaptation interleukins.*

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INTRODUCTION

Prostaglandins have been implicated in regulation of a wide variety of tissue functions and may be found in almost all tissues, including the hypothalamus, hippocampus and pituitary gland (1—3). Prostanoids are not stored by cells, but rather are synthesized in response to cell-specific proteolytic or hormonal stimuli. Neurons can take up free arachidonic acid (AA) and store it rapidly by esteryfying it to membrane phospholipids. As a result, only trace levels of free arachidonate may be found in resting cells. Although free AA is thought to be generated either directly or indirectly by the three phospholipases, phospholipase A_2 (PLA_2), C (PLC), and D (PLD), PLA_2 plays a key role in the AA release. It releases AA from the position 2 of membrane phospholipids, such as phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine. Arachidonic acid is further metabolized to prostaglandin H, the precursor of prostaglandins, by the action of prostaglandin H synthase, also called cyclooxygenase (COX) (2, 3).

Several neuromodulators such as amino acids, biogenic amines and peptides stimulate the deacylation of phospholipids causing a release of free arachidonate which can modulate the prostanoid synthesis. Noradrenaline has been shown to stimulate the synthesis and release of PGs in different tissues, such as the kidney, heart and blood vessels, as well as in various rodent brain areas (4—6). Involvement of particular adrenergic receptors in the formation of PGs is still unclear. In some peripheral tissues such as rabbit heart, the synthesis of PGs elicited by adrenergic stimuli is mediated by the activation of β_1-adrenergic receptors (7). The β-adrenergic activation induced by isoproterenol may generate PGE_2 in pulmonary tissues and evoke a direct increase in the arachidonic acid metabolism in lung mast cells. In other tissues such as the rabbit and rat kidney and rabbit aorta, the PGs synthesis is linked to α-adrenergic receptors (5, 8, 9). The coupling between neurotransmitter receptors and PLA_2 appears to involve GTP-binding proteins, in particular the βγ subunits of the heteromeric G proteins. Adrenergic receptors acting by G_i, can induce the Ca^{2+}-dependent translocation of an AA-specific $cPLA_2$ from the cytosol to the cell membrane, and its subsequent activation (1, 2, 4). Noradrenaline induces PGE_2 release in various rodent brain areas, and in higher concentrations noradrenaline elicits $PGF_{2\alpha}$ release. In primary cultures of spinal cord neurons, noradrenaline stimulated the release of AA as well as the turnover of inositol phosphates *via* α_1-adrenergic receptors (10).

Conflicting reports describe various roles of PGE_2 in the mechanism of ACTH and corticosterone secretion (11—14). The circulating PGs can induce ACTH secretion either indirectly *via* stimulation of CRH and/or AVP secretion, or directly, by acting on the pituitary gland. In addition, prostaglandin E_2 can stimulate the corticosterone production by isolated, perfused rat

zona glomerulosa cells of the adrenal cortex (15). On the other hand, according to some authors, PGE_2 neither stimulate nor inhibits ACTH release from the anterior pituitary *in vitro* in response to the ACTH secretagogues CRH and AVP. Inhibition of the PGs biosynthesis by systemic treatment with indomethacin impairs the normal corticosterone secretion in response to acute elevation in the plasma ACTH.

INVOLVEMENT OF PROSTAGLANDINS IN ADRENERGIC STIMULATION OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL AXIS

Watanabe et al. (16) have reported that PGE_2 mediates ACTH release induced in rats by noradrenaline. We recently examined the contribution of PGs to the central stimulation of HPA axis by α_1-, α_2- and β-adrenergic receptor agonists, as well as by noradrenaline and adrenaline in conscious rats. Also the site of action of the PGs linked to the HPA stimulations was investigated by administering indomethacin systemically or intracerebroventricularly prior to selective adrenergic receptor agonists (17). Systemic pretreatment with indomethacin (2 mg/kg) 15 min before icv administration of phenylephrine (30 μg), an α_1-receptor agonist, almost totally blocked the phenylephrine-induced corticosterone response. Also pretreatment with indomethacin (10 μg icv) considerably diminished the corticosterone response to phenylephrine. Indomethacin given by either route elicited a potent diminution of the corticosterone secretion induced by clonide (10 μg icv), an α_2-adrenergic receptor agonist (18). By contrast, indomethacin given by either route was unable to markedly affect the corticosterone secretion stimulated by isoproterenol (20 μg icv), a β-adrenergic receptor agonist. (*Fig. 1*). Systemic or icv pretreatment with indomethacin considerably reduced the increase in corticosterone secretion elicited by noradrenaline (10 μg icv), but did not markedly affect this response to icv adrenaline (10 μg) (17). These results clearly demonstrate that PGs significantly mediate the HPA activity induced by central stimulation of α_1-adrenergic receptors by phenylephrine. Although indomethacin poorly penetrates the blood-brain barrier from the systemic circulation, it may reach hypothalamic CRH neurons through the organum vasculosum of the lamina terminalis, a site almost devoid of the blood-brain barrier (19—21). Indomethacin may also inhibit the PGs synthesis at the median eminence and anterior pituitary. The latter sites contain adrenergic terminals and adrenergic receptors and are readily accessible from the systemic circulation. Although the hypothalamic paraventricular nucleus (PVN) represents the ulimate region mediating the stimulatory effect of adrenergic agonists administered icv, systemic indomethacin probably acts first at the level of CRH and adrenergic terminals in the median eminence situated outside the blood-brain barrier. Stimulation of central α_1-adrenergic receptors by icv

phenylephrine or methoxamine induces release of vasopressin (22, 23) which, according to our recent findings, is almost as potent as CRH in evoking ACTH and corticosterone secretion (24). Catecholaminergic neurons terminate in the vicinity of both CRH and AVP neurons in the regions of PVN where α_1-adrenoceptors were identified by an autoradiography. Brain prostaglandins play a central role in the control of AVP secretion, and AVP has been found to stimulate PGE synthesis in peripheral tissues (25). Vasopressin stimulates the PGE_2 synthesis in the rat adenohypophysis. Therefore our present results strongly suggest that phenylephrine may induce AVP release which, in turn, stimulates the PGs synthesis, and that both AVP and PGs enhance ACTH and corticosterone secretion.

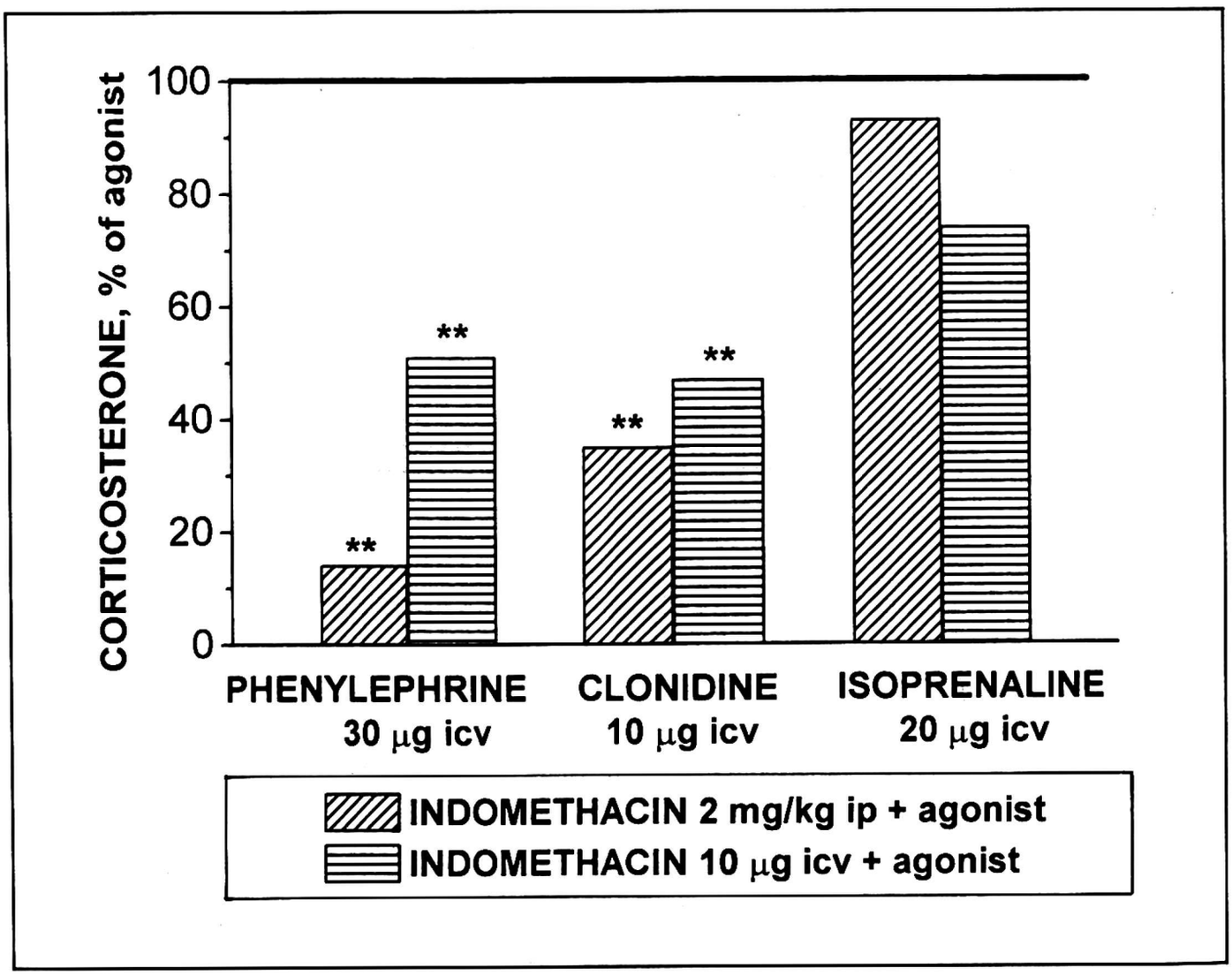

Fig. 1. Effect of indomethacin on corticosterone secretion induced by adrenergic receptor agonists.

The corticosterone response to clonidine (10 μg icv), an α_2-adrenergic agonist, was significantly diminished by systemic or icv pretreatment with indomethacin, though that diminution was somewhat smaller compared with the reduction of the phenylephrine-elicited response (17, 18). It may be expected that activation of α_2-adrenergic receptors, like that of α_1-adrenergic receptors,

also promotes PGs synthesis, since it is associated with increased cellular levels of calcium which are known to activate lipases to release arachidonic acid for the PGs synthesis. Therefore the considerable impairment of the clonidine-induced corticosterone response by indomethacin may be connected with promotion of the PGs synthesis via intracellular calcium ion mobilization (26). Alpha$_2$-adrenergic receptors are known to be mainly involved in stimulation of the PGs synthesis in the isolated rabbit aorta, and clonidine can enhance the biosynthesis of PGs in the isolated perfused rabbit kidney. Hypothalamic α_2-adrenergic receptors, stimulated by icv clonidine, may be responsible, at least in part, for generation of PGs and stimulation of the HPA axis. By contrast PGs are not markedly involved in stimulation of the

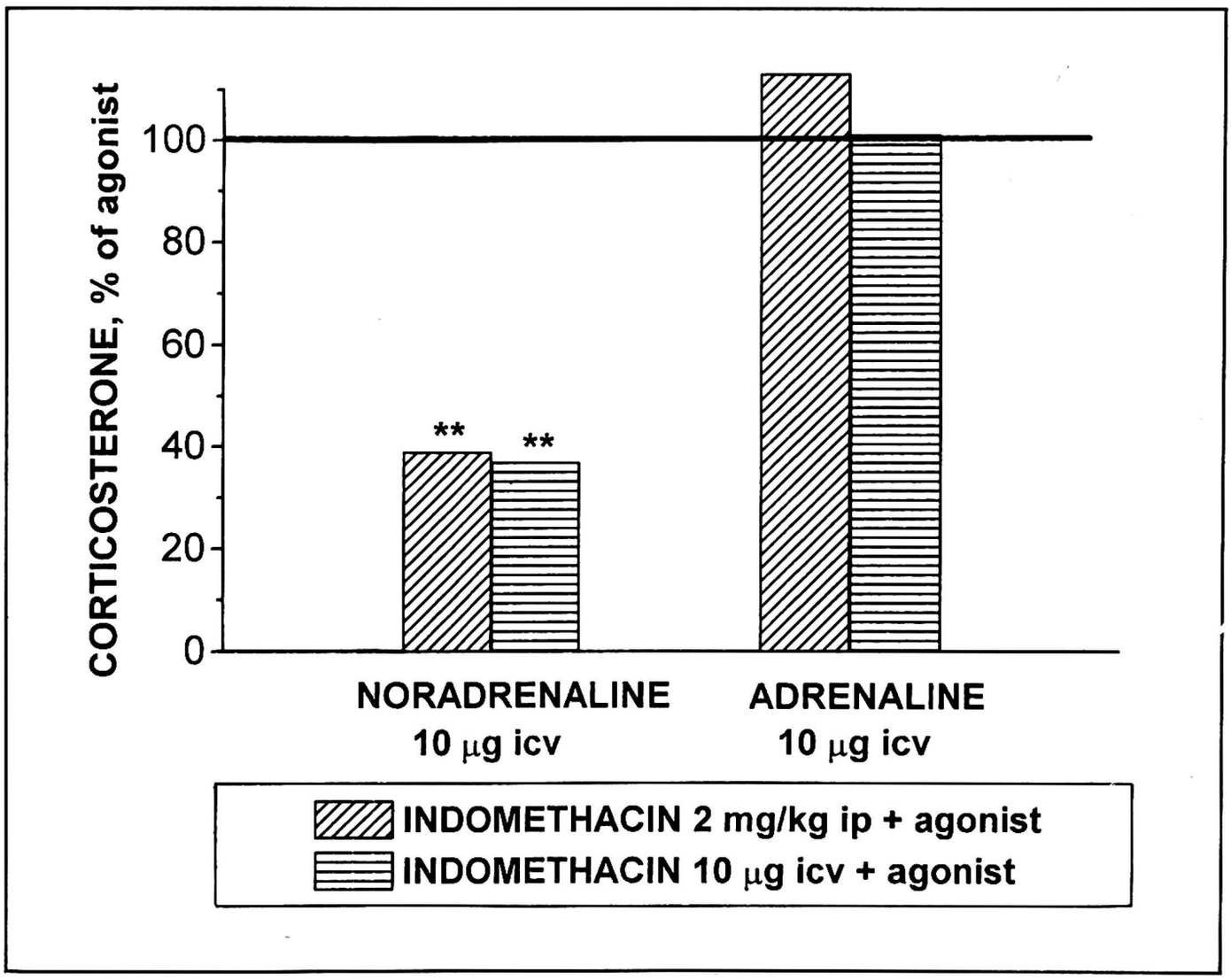

Fig. 2. Effect of indomethacin on corticosterone secretion elicited by noradrenaline and adrenaline.

HPA axis by β-adrenergic receptor agonist isoproterenol since indomethacin does not significantly alter the isoproterenol-elicited corticosterone response. The β-adrenoceptor activation by isoproterenol, is known to generate PGs in isolated pulmonary tissues and airway mucosa, lung mast cells and cardiac tissue. We have also shown that PGs mediate the HPA response to icv

noradrenaline but not to adrenaline, since icv or ip pretreatment with indomethacin considerably inhibits the rise in the corticosterone secretion elicited by noradrenaline, but did not markedly affect such a response to adrenaline (*Fig. 2*). Noradrenaline stimulates the CRH release from *in vitro* superfused rat hypothalamus mainly *via* α_1- and α_2-adrenergic receptors (27). On the other hand, adrenaline is known to stimulate ACTH secretion mainly *via* β-adrenoceptors located in the brain (28). The results obtained with adrenaline and isoproterenol consistently show a lack of involvement of PGs in the HPA axis stimulation by the β-adrenergic agonist (*Fig. 3*). Activation of α_1- and α_2-adrenoceptors on the hypothalamic paraventricular nucleus CRH secreting neurons by icv administration of an adrenergic agonist may promote the synthesis and release of PGs which mediate the stimulatory effect of α_1- and α_2-adrenergic agonists on the CRH and ACTH secretion. Watanabe et al (29) demonstrated that the ACTH responses induced by intrahypothalamic injection of PGE_2 were significantly though not totally suppressed by systemic pretreatment with anti-CRH antibody, which indicates that CRH is, at least in part, involved in the ACTH response to PGE_2. A portion of this response could be mediated by induction of AVP from the pituitary gland or/and from the magnocellular part of the hypothalamic PVN and supraoptic nucleus (30). Involvement of PGs in the anterior pituitary corticotrops seems unlikely in the case of adrenergic agonists given icv. Adrenergic α_2-receptors have not been found on these cells, and the possibility of a direct stimulation of pituitary α_1-adrenoceptors by icv phenylephrine and noradrenaline reaching the anterior pituitary *via* the portal circulation to promote the PGs synthesis, and ACTH secretion, seems to be of negligible significance. It is still far less likely that icv adrenergic agonists in the doses used can reach, *via* peripheral circulation, the adrenal cortex at concentrations sufficient to directly stimulate the PGs synthesis and corticosterone secretion.

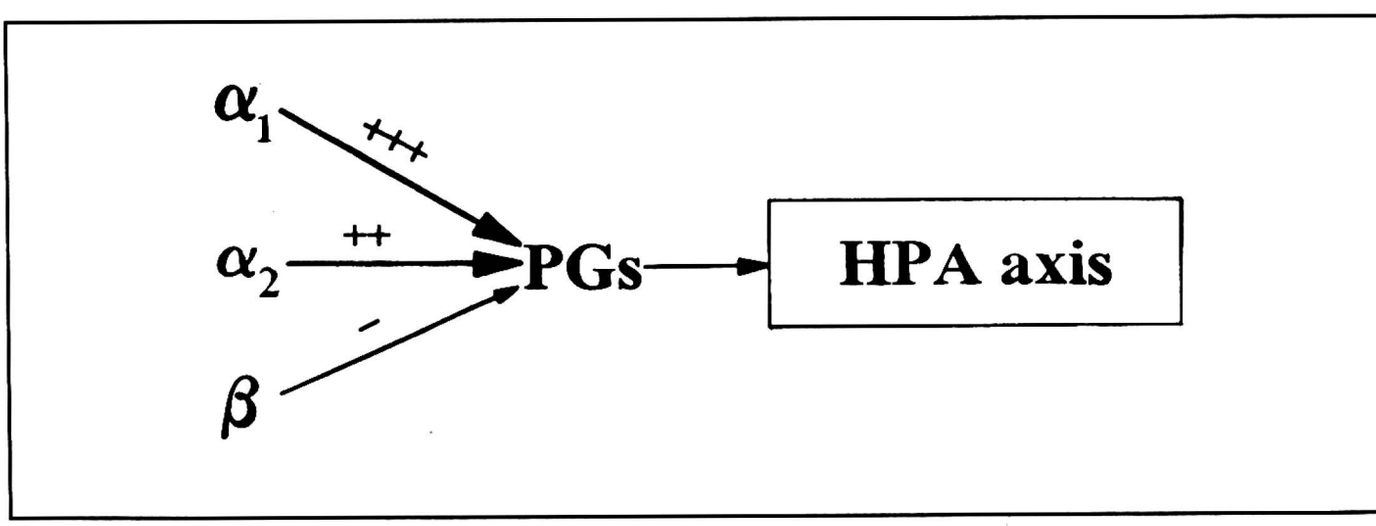

Fig. 3. Schematic intensity of involvement of prostaglandins in corticosterone secretion induced by icv adrenergic receptor agonists: phenylephrine (α_1), clonidine (α_2) and isoprenaline (β).

The present data clearly show that *in vivo* PGs potently mediate the stimulation of the HPA axis transduced by α_1- and α_2-, but not β-adrenergic receptors. Prostaglandins can mediate adrenergic stimulation at a hypothalamic and/or a pituitary levels. Although in isolated perfused rat zona glomerulosa cells PGE_2 can directly stimulate the steroidogenesis and corticosterone secretion (15), during an *in vivo* activation of the central adrenergic system, the adrenal cortex may be excluded as a site of a direct stimulatory action of PGs on the corticosterone secretion.

THE ROLE OF PROSTAGLANDINS IN THE PITUITARY-ADRENOCORTICAL RESPONSE TO INTERLEUKIN-1

Interleukin-1 is the predominant immune cytokine secreted from activated monocytes and macrophages. Interleukin-1 is one of the key mediators of complex changes in the immune, physiological and behavioral functions induced by infection or tissue injury, collectively called as the acute phase response. It is generally accepted that there is a bidirectional network between the neuroendocrine and immune systems. The immune system, through cytokines, such as IL-1, IL-6 and the tumor necrosis factor-α, and the HPA axis through the CRH release by the hypothalamus and a subsequent increase in the ACTH and corticosteroid secretion, are functionally linked in expressing a coordinated response to immune challenges (31).

Arachidonic acid metabolites or eicosanoids can mediate the interleukins (ILs) modulation of the rat HPA axis (29, 32—34). The effect of IL-1 on CRH released by isolated fragments of the hypothalamus is blocked by indomethacin, suggesting mediation of eicosanoid pathways in this process. In the rat, indomethacin totally abolished the effect of IL-1 on the HPA axis (16, 29, 35). Interleukin-1 injected iv, ip or icv caused, a significant increase in circulating ACTH levels. Because of its high molecular weight IL-1 given systemically may access to the brain through structures devoid of the blood-brain barrier, such as the median eminence, subfornical organ, organum vasculosum lamina terminalis (OVLT) or area postrema. Katsuura et al (20) reported that IL-1β can possibly enter the brain through the OVLT, where it binds and stimulates its receptors on astrocytes and induces the release of PGE_2. Liberated PGE_2 may reach and activate neurons in the medial preoptic area (MPOA) which send stimulatory signals to CRH neurons in the PVN. Indomethacin or a PGE antagonist, microinjected into the OVLT or POA, suppressed or abolished that response. Komaki et al (36) observed that iv administered IL-1β induced a significant and a greater rise in PGE_2 in the OVLT and the MPOA, than in the PVN and suggested that the OVLT and MPOA may be the primary sites of activation of the HPA axis by circulating

IL-1β. It is accepted, however, that central administration of IL-1β primarily activates directly the PVN to increase the CRH and ACTH secretion. To identify brain sites at which IL-1β exerts stimulation of the HPA axis c-fos protein expression as an index of cellular activation was used in order to determine possible differences between the effects of peripheral and central injection of IL-1β on the activation of specific brain areas. It appears that the stimulatory effect of blood-borne IL-1β on the ACTH secretion does not cause an immediate increase in the brain c-fos protein suggesting that peripherally administered IL-1β acts at the level of median eminence-to induce the release of CRH from nerve terminals (37). Also *in vivo* data indicate CRH secretion in the median eminence after iv administration of IL-1β (38). Watanobe et al (33) found that PGE_2 may be a trigger in the PVN for the activation of CRH and AVP neurons, and thereby ACTH secretion which follows IL-1β injection.

A role of the cytokine IL-6 in the neuroendocrine systems is suggested by several recent studies (39). Interleukin-6 has many similarities to IL-1, both structurally and functionally. There appear to be multiple pathways regulating IL-6 production, one of which involves induction of IL-6 by IL-1 and IL-2 (40). In the rat anterior pituitary gland the presence and expression of a IL-6 binding site as well as the presence of a IL-6 binding site on human gonadotrophs have been demonstrated, suggesting the important role of IL-6 in the pituitary hormone release in both these species (41). Interleukin-6 has been reported to stimulate also the release of LH, FSH, PRL and GH from the anterior pituitary gland. Interleukin-1 and IL-6 are involved in the acute phase response and activation of the HPA axis. Interleukin-6 has been reported to be a more potent secretagogue for ACTH by AtT-20 pituitary tumor cells than CRH. Interleukin-6 may function as a paracrine mediator of the anterior pituitary hormone release. Also iv injection of IL-6 increases the plasma ACTH *via* CRH release, since a CRH antibody CRH blocked the rise in ACTH induced by IL-6 as by IL-1 (40).

Also in hypothalamic explants *in vitro* the cytokines IL-1β and IL-6 stimulate directly the release of CRH (42). These cytokines significantly increase the release of PGE_2 from the incubated hypothalamus, and this effect was antagonized by indomethacin which indicates that IL-1β and IL-6 specifically stimulate the PGE_2 release possibly implicating this PG in CRH secretion (43). The release of IL-6 from rat anterior pituitary cells *in vitro* has been found to be stimulated by both IL-1β and arachidonic acid (44).

The potential role of peripheral and brain noradrenergic neurons in the stimulation by interleukins of the HPA axis is not clear. Interleukins can increase circulating catecholamine levels which, however, is not a major mechanism of the IL-1-induced activation of the HPA axis, since the blockade of adrenergic receptors does not alter the stimulatory effect of IL-1. Systemic administration of the human recombinat IL-1β accelerated the noradrenaline

turnover in the hypothalamus and some peripheral organs. Indomethacin abolishes the IL-1-induced hypothalamic NA turnover. This action of IL-1 is mediated through PGD_2 production to activate noradrenergic neurons in the hypothalamus, whereas PGE_2 mediates, increased NA turnover in the spleen (45, 46). It appears that the IL-1-induced activation of the HPA axis *in vivo* depends upon the ventral noradrenergic bundle which innervates the CRH-containig neurons in the hypothalamic PVN (47). On the other hand, Shintani et al (48) using an *in vivo* brain microdialysis reported that IL-1β acted directly on the anterior hypothalamus to augment the release of NA without involvement of PG or elevation of the CRH levels. Besides CRH, interleukin-1 can also release vasopressin, a secretagogue of ACTH. Using electron microscopic immunocytochemistry to directly assay the IL-1 induced depletion of secretory vesicles from the identified VP-expressing and VP-deficient CRH neurosecretory axons, Whitnal et al (49) found that IL-1 induced the release of VP from neurosecretory axons in the portal capillary of the extrenal zone of the median eminence. Activation of the VP-system by an inflammatory mediator involves mechanisms different from these mediating the stress response.

Of different prostanoids, PGE_2 has been repeatedly reported to be the most important PG mediating the rat HPA stimulated by interleukin-1 (20, 29, 33, 36, 50—53). However, some authors suggest PGE_1, $PGF_{2\alpha}$ and PGD_2 as a mediator of the IL-1 effect on the ACTH secretion (46, 54, 55). Watanobe et al (55) using icv administration examined the effects of neutralizing antibodies against PGE_1, PGE_2 and $PGF_{2\alpha}$ on the ACTH response induced by icv injection of IL-1β. They demonstrated that not only PGE_2 but also PGE_1 and $PGF_{2\alpha}$ can mediate the IL-1β stimulation of ACTH secretion in the rat brain.

Some reports indicate that interleukins can directly stimulate the adrenal gland to secrete corticosterone. Although interleukins enhance the hypophyseal ACTH release mainly by stimulating hypothalamic CRH secretion, IL-1β was found to elevate dose-dependently the corticosterone blood concentration in hypophysectomized rats, without inducing any significant increase in the level of circulating ACTH. The secretory effect of IL-1β was completely blocked by both the α-helical-CRH and the CRH-inhibiting peptide, two competitive inhibitors which were able to abolish corticosterone response of adrenal slices to CRH and ACTH, respectively (56). Also interleukin-6 was shown to stimulate corticosterone release from primary cultures of rat adrenal zona glomerulosa cells (57). This immune cytokine may play an important paracrine role in integrating signals derived from the immune and endocrine systems. Interleukin-1 can induce corticosterone secretion from the incubated rat adrenal gland directly by a local release of adrenaline. This stimulatory effect is transmitted by α- but not β-adrenergic receptors (58). Although there is scant evidence that IL-1 can cross the blood-brain barrier, many effects of IL-1, such

as fever, anorexia and modulation of the neuroendocrine function, suggest an action of the circulating IL-1 at regulatory sites within the hypothalamus. Accumulating evidence suggests that IL-1 may originate within the central nervous system. In acute rat hypothalamic explants interleukin-1, released by stimulation by PGE_2 and lipopolysaccharide, may specifically stimulate the release of hypothalamic PGE_2 which, in turn, may mediate communication between the circulating and CNS-derived IL-1 (51). In the functional interactions between the immune and CNS systems participate the same mediators and receptors. Interleukins have been shown to be powerful regulators of activity of both these systems and IL-1 receptors have been found in the murine nervous structures, hippocampus and frontal cortex and in the neuroendocrine structure, anterior pituitary (59).

INVOLVEMENT OF PROSTAGLANDINS IN CORTICOTROPIN-RELEASING HORMONE AND VASOPRESSIN-INDUCED PITUITARY-ADRENOCORTICAL RESPONSE

Multiple hypothalamic and peripheral factors control the activity of the HPA axis. (60) CRH synthesized by pericarya in the hypothalamic parvocellular paraventricular nuclei is known to play an integral and obligatory role in regulation of the pituitary ACTH secretion and proopriomelanocortin gene expression (61, 62). Arginine vasopressin is regarded as an intrinsically weaker secretagogue acting sinergistically in stimulating ACTH secretion (30). However, we have recetly shown that after systemic administration AVP is almost as potent as CRH in stimulating ACTH and corticosterone secretion (24). Therefore AVP can no longer be considered to be of minor importance to the control of ACTH release (30, 63, 64). It has been established that AVP and CRH coexist in the same parvocellular pericarya of the PVN, and that AVP is present in half of the CRH axons while the other half of the CRH axons contain little or no detectable AVP (65). Brain prostaglandins play a central role in the control of AVP secretion (25) and AVP has been found to stimulate the PGE synthesis in peripheral tissues.

The mutual involvement of CRH and AVP in regulation of the hypothalamic-pituitary-adrenal axis during various stressful circumstances has not been satisfactorily elucideted as yet. Single and repeated exposure of rats to immobilization stress can significantly upregulate the AVP as well as the CRH mRNA generation in the hypothalamic CRH neurosecretory system (66, 67). Moreover, in stressed rats the number of AVP-expressing parvicellular CRH neurons increased more potently than AVP-deficient CRH neurons (66—69). Also chronic psychosocial stress increases the AVP, but not the CRH, content in the zona externa of median eminence

(70). We have found recently that only the AVP neuropeptide system that stimulates the HPA axis undergoes a significant desensitization during chronic social crowding stress in rats while the CRH system fully retains its responsiveness (71, 72). The latter finding is in contrast to some earlier reports which show significant diminution of the HPA response to CRH following prolonged stimulation both in vitro and *in vivo* (73). The stimulatory action of CRH and AVP on the anterior pituitary corticotroph receptors depends on and may be modulated by different neuromodulators which are known to be released under stressful circumstances. The possibility has been recently suggested that the ACTH response during physical or psychosocial stress is mediated by prostaglandins. Pretreatment with indomethacin, an inhibitor of PGs synthesis, significantly supresses the increase in plasma ACTH induced by swimming or cage-swith stress (74). The mechanism by which stress stimulates PGs synthesis is not known at present.

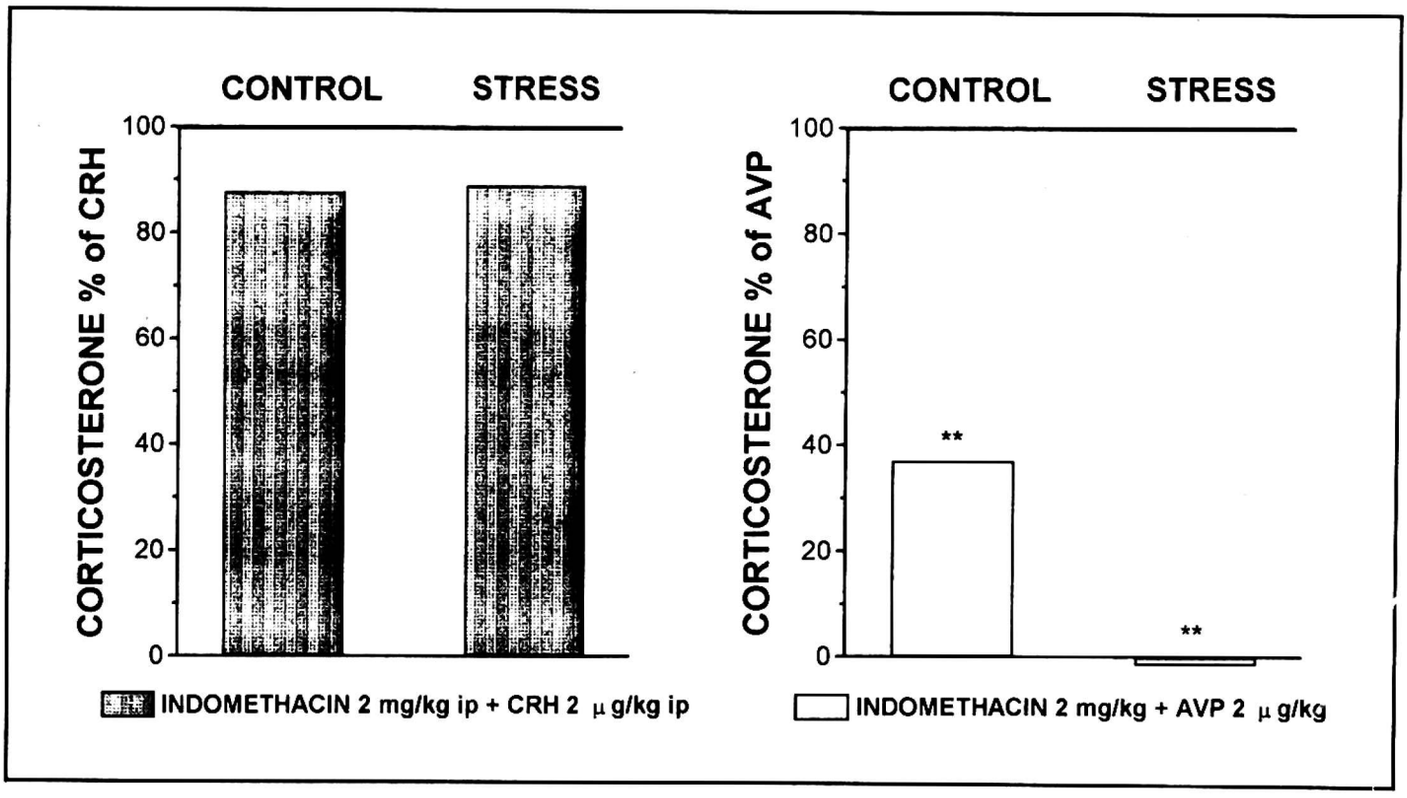

Fig. 4. Effect of indomethacin on corticosterone secretion induced by corticotropin-releasing hormone (CRH) and vasopressin (AVP) in rats unstressed or exposed to 3 days social crowing stress.

A stereotyped response to stressful stimuli includes the activation of the central adrenergic system and release of noradrenaline in brain structures involved in regulation of the HPA axis. We have recently found that in nonstressed rats the rise in corticosterone secretion elicited by systemic administration of CRH, was not altered by ip pretreatment with indomethacin which suggests that PGs are not substantially involved in the ACTH/corticosterone response to the corticotroph CRH receptor stimulation

(75). These results exclude a possibility of direct stimulation by PGs of the adrenal steroidogenesis and corticosterone secretion. (*Fig. 4*). Also, the stimulation of corticosterone secretion elicited by icv CRH was not markedly changed by icv indomethacin. Although CRH given icv can stimulate the central adrenergic system (76), which in turn, may induce PGs secretion and activation of HPA axis (17, 18) we have not found any significant CRH interference with central adrenergic receptors or the hypothalamic noradrenaline level (71). Watanabe et al. demonstrated that PGE_2 mediated the release of ACTH stimulated by icv noradrenaline in rats (16). Our present results suggest that stimulation of the central adrenergic system and PGs synthesis by CRH to markedly activate the secretion of CRH and ACTH seems unlikely. Our data show that PGs are not significantly involved in the stimulation of the ACTH and corticosterone secretion by CRH within a medium to submaximum range. Only during the maximal hormonal response induced by CRH, moderate mediation of PGs may appear, yet this may reflect an unphysiological mechanism (77).

Social crowding stress did not affect the corticosterone response to ip CRH nor did pretreatment with indomethacin substantially change that response, indicating that PGs are not involved in corticosterone secrection in sressed rats (75). Thus neither the CRH nor the PG systems become desensitized during social crowding stress which dramatically impaired the responsiveness of the HPA axis to stimulation of central β-adrenergic and AVP receptors (72, 78). Social crowding stress did not impair the HPA responsiveness to stimulaton of the central α_1-adrenergic receptor (78) which is known to stimulate both the CRH and PGs release. Vasopressin is known to mediate the stimulatory effect of central α_1-adrenergic receptor on the ACTH secretion. Although the release of endogenous AVP depends on hypothalamic α_1-adrenergic receptors, the activation of ACTH secretion by AVP does not involve α_1-adrenergic receptors on pituitary corticotrophs, since the AVP-evoked corticosterone response is significantly diminished by pretreatment with propranolol, a β-adrenergic antagonist, but not by prazosin, an α_1-adrenergic antagonist (24, 71). The present data show that PGs significantly mediate the stimulatory action of AVP on the HPA axis under both basal and social stress conditions. (*Fig. 4*). The rise in corticosterone secretion induced by systemic administration of AVP was significantly reduced by ip pretreatment with indometacin. This suggests that the action of AVP is directed towards anterior pituitary corticotrophs, since indomethacin does not penetrate easily the blood-brain barrier. The increase in corticosterone secretion by icv AVP may be elicited by a leakage of the peptide from the cerebral ventricles to portal vessels with delivery to the anterior pituitary. This may account for significant supression of the icv AVP-induced HPA response by systemic pretreatment with indomethacin. On the other hand, a still stronger reduction of the icv AVP-elicited corticosterone

response by central pretreatment with indomethacin also suggests a hypothalamic site of action of both these agents. Therefore the present data indicate that PGs are not involved in the pituitary-adrenocortical response elicited by direct homologous stimulation of pituitary CRH receptors under both basal and social stress circumstances. On the other hand, PGs are significant mediators of the stimulatory action of AVP on the HPA axis, and both the hypothalamus and the anterior pituitary may be involved in this interaction. Social crowding stress does not affect the significant participation of the PGs system in the transduction of AVP hypophysiotropic signals found in nonstressed animals. Our results show for the first time a distinct role of PGs in stimulation and adaptation of the CRH and the AVP regulatory systems.

REFERENCES

1. Katsuki H, Okuda S. Arachidonic acid as a neurotoxic and neurotrophic substance. *Prog Neurobiol* 1995; 46: 607—636.
2. Piomelli D. Arachidonic acid. In: Psychopharmacology: The fourth generation of progress. FE Bloom and DJ Kupfer (ed). New York, Raven Press Ltd, 1995, pp. 595—607.
3. Wolfe LS, Horrocks LA. Eicosanoids. In: Basic neurochemistry: Molecular, Cellular and Medical Aspects. GJ Siegel et al (ed.) New York, Raven Press Ltd, 1994, pp: 475—490.
4. Schaad NC, Magistretti PJ, Schorderet M. Prostanoids and their role in cell-cell intractions in the central nervous system. *Neurochem Int* 1991; 18: 303—332.
5. Cooper CL, Malik KU. Prostaglandin synthesis and renal vasoconstriction elicited by adrenergic stimuli are linked to activation of alpha-1 adrenergic receptors in the isolated rat kidney. *J Pharmacol Exp Ther* 1985; 233: 24—31.
6. Cooper CL, Malik KU. Mechanism of action of vasopressin on prostaglandin synthesis and vascular function in the isolated rat kidney: effect of calcium antagonists and calmodulin inhibitors. *J Pharmacol Exp Ther* 1984; 229: 139—147.
7. Shaffer JE, Malik KU. Enhancement of prostaglandin output during activation of beta-1 adrenoceptors in the isolated rabbit heart. *J Pharmacol Exp Ther* 1982; 223: 729—735.
8. Van Praag D, Minkin E, Farber SJ. Inhibition of eicosanoid formation by prazosin in the rabbit renal cortex. *Prostagland Leuk Med* 1986; 24: 227—240.
9. Nebigil C, Malik KU. Prostaglandin synthesis elicited by adrenergic stimuli in rabbit aorta is mediated via alpha-1 and alpha-2 adrenergic receptors. *J Pharmacol Exp Therapeut* 1990; 254: 633—640.
10. Axelrod J. Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. *Biochem Soc Transactions* 1990; 18: 503—507.
11. Thompson ME, Hedge GA. Corticosterone secretion after inhibition of prostaglandin synthesis by indomethacin. *Am J Physiol* 1981; 240: E131—E135.
12. Sobel DO. Characterization of PGE_2 inhibition of corticotropin releasing factor-mediated ACTH release. *Brain Res* 1987; 411: 102—107.
13. Knepel W, Nutto D, Vlaskovska M, Kittel C. Inhibition by prostaglandin E_2 of the release of vasopressin and beta-endorphin from rat pituitary neurointermediate lobe or medial basal hypothalamus *in vitro*. *J Endocrinol* 1985; 106: 189—195.
14. Thoren M, Stenfors C, Aperia B, Mathe AA. Hypothalamic-pituitary-adrenal axis interaction with prostaglandins. *Prog Neuro-Psychopharmacol Biol Psychiat* 1990; 14: 319—328.

15. Hindawi RK, Al-Dujaili EAS, Padfield PL. The role of prostaglandins in aldosterone and corticosterone secretion by isolated perfused rat zona glomerulosa cells. *Prostagland Leuk Med* 1985; 20: 121—128.
16. Watanobe T, Morimoto A, Morimoto K, Nakamori T, Murakami N. ACTH release induced in rats by noradrenaline is mediated by prostaglandin E_2. *J Physiol* 1991; 443: 431—439.
17. Bugajski J, Gądek-Michalska A, Borycz J, Głód R, Bugajski JA. Effect of indomethacin on the pituitary-adrenocortical response to adrenergic stimulation. *Life Sci* 1996; 59: 1157—1164.
18. Bugajski J, Gądek-Michalska A, Borycz J, Głód R, Ołowska A. The role of prostaglandins and the hypothalamic and hippocampal histamine in the clonidine-induced pituitary--adrenocortical response. *J Physiol Pharmacol* 1996; 47: 487—495.
19. Goldstein GW, Betz AL. The blood-brain barrier. *Sci Amer* 1986; 254: 74—83.
20. Katsuura G, Arimura A, Koves K, Gottschall PE. Involvement of organum vasculosum of lamina terminalis and preoptic area in interleukin 1β-induced ACTH release. *Am J Physiol* 1990; 258: E163-E171.
21. Ermisch A, Ruhle H-J. Peptide-blood-brain barrier interactions: kinetics and dynamics. *J Control Release* 1992; 21: 93—104.
22. Al-Damluji S, Thomas R, White A, Besser M. Vasopressin mediates alpha-1 adrenergic stimulation of adrenocorticotropin secretion. *Endocrinology* 1990; 126: 1989—1995.
23. Brooks DP, Share L, Crofton JT. Central adrenergic control of vasopressin release. *Neuroendocrinology* 1986; 42: 416—420.
24. Bugajski J, Gądek-Michalska A, Ołowska A, Borycz J, Głód R, Bugajski AJ. Adrenergic regulation of the hypothalamic-pituitary-adrenal axis under basal and social stress conditions. *J Physiol Pharmacol* 1995; 46: 297—312.
25. Brooks DP, Share L, Crofton JT. Role of brain prostaglandins in the control of vasopressin secretion in the conscious rat. *Endocrinology* 1986, 118: 1716—1722.
26. Wijkander J, Sundler R. Regulation of arachidonate-mobilizing phospholipase A_2 by Ca^{2+} and by phosphorylation via protein kinase C. *J Lipid Mediators Cell Signalling* 1994; 10: 23—24.
27. Joanny P, Steinberg J, Zamora AJ, Conte-Devolx B, Millet Y, Olivier C. Corticotropin-releasing factor release from *in vitro* superfused and incubated rat hypothalamus. Effect of potassium, norepinephrine, and dopamine. *Peptides* 1989; 10: 903—911.
28. Tilders FJH, Berhenbosch F, Vermes I. Circulating epinephrine stimulates ACTH secretion in rats via beta-adrenoceptors located in the brain. Neuroendocrine Perspectives E.E. Muller EE, Mac Leod RM. (EDs(. 1986; 5: 329—333. Elselvier Sciences Publishers B. V. (Biomedical Division) Amsterdam.
29. Watanobe T, Morimoto A, Sakata Y, Murakami N. ACTH response induced by interleukin-1 is mediated by CRF secretion stimulated by hypothalamic PGE. *Experimentia* 1990; 46: 481—484.
30. Antoni FA. Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Front Neuroendocrinol* 1993; 14: 76—122.
31. Rivier C. Effect of peripheral and central cytokines on the hypothalamic-pituitary-adrenal axis of the rat. *Ann NY Acad Sci* 1993; 697: 97—105
32. Rivier C, Vale W. Stimulatory effects of interleukin-1 on adrenocorticotropin secretion in the rat: Is it modulated by prostaglandins? *Endocrinology* 1991; 129: 384—388.
33. Watanobe H, Takebe K. Effects of intravenous administration of interleukin-1-beta on the release of prostaglandin E_2, corticotropin releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats: estimation by push-pull perfussion. *Neuroendocrinology* 1984, 60: 8—15.
34. Lyson K, McCann SM. Involvement of arachidonic acid cascade pathways in interleukin-6-stimulated corticotropin-releasing factor release *in vitro*. *Neuroendocrinology* 1992; 55: 708—713.

35. Katsuura G, Gottschall PE, Dahl RR, Arimura A. Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats. Possible involvement of prostaglandins. *Endocrinology* 1988; 122: 1773—1779.
36. Komaki G, Arimura A, Koves K. Effects of intravenous injection of IL-1β on PGE_2 levels in several brain areas as determined by microdialysis. *Am J Physiol* 1992; 262: E246—251.
37. Rivest S, Torres G, Rivier C. Differential effects of central and peripheral injection of interleukin-1β on brain c-fos expression and neuroendocrine functions. *Brain Res* 1992; 587: 13—23.
38. Watanobe H, Sasaki S, Takebe K. Evidence that intravenous administration of interleukin-1 stimulates corticotropin releasing hormone secretion in the median eminence of freely moving rats: estimation by push-pull perfusion. *Neurosci Lett* 1991; 133: 7—10.
39. Zhou D, Kusnecov AW, Shurin MR, DePaoli M, Rabin BS. Exposure to physical and psychological stressors elevates plasma interleukin 6: Relatioship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology* 1993; 133: 2523—2530.
40. Smith EM. Hormonal activities of cytokines. In: Chem Immunol. K Ishizaka, PJ Lachmann, R Lerner, BH Waksman (ed). Basel, Karger 1992; 52: 154—169.
41. Ohmichi M, Hirota K, Koike K et al. Binding sites for interleukin-6 in the anterior pituitary gland. *Neuroendocrinology* 1992; 55: 199—203.
42. Navarra P, Pozzoli G, Brunetti L, Ragazzoni E, Besser M. Grossman A. Interleukin-1β and interleukin-6 specifically increase the release of prostaglandin E_2 from rat hypothalamic explants in vitro. *Neuroendocrinology* 1992; 56: 61—68.
43. Ohgo S, Nakatsuru K, Ishikawa E, Matsukura S. Interleukin-1 (IL-1) stimulates the release of corticotropin-releasing factor (CRF) from superfused rat hypothalamo-neurohypophyseal complexes (HNC) independently of the histaminergic mechanism. *Brain Res* 1991; 558: 217—223.
44. Spangelo BL, Wright RM. Arachidonic acid stimulates interleukin 6 release from rat anterior pituitary cells in vitro: comparison with peritoneal macrophages. *Prog Neuro Endocrin Immunol* 1992; 5: 235—244.
45. Terao A, Oikawa M, Saito M. Cytokine-induced change in hypothalamic norepinephrine turnover: involvement of corticotropin-releasing hormone and prostalandins: *Brain Res* 1993; 622: 257—261.
46. Terao A, Kitamura H, Asano A, Kobayashi M, Saito M. Roles of prostaglandins D_2 and E_2 in interleukin-1-induced activation of norepinephrine turnover in the brain and peripheral organs of rats. *J Neurochem* 1995; 65: 2742—2747.
47. Barbanel G, Gaillet S, Mekaouche M et al. Complex catecholaminergic modulation of the stimulatory effect of interleukin-1β on the corticotropic axis. *Brain Res* 1993; 626: 31—36.
48. Shintani F, Kanba S, Nakaki T et al.Interleukin-1β augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. *J Neurosci* 1993; 13: 3574—3581.
49. Whitnall MH, Perlstein RS, Mougey EH, Neta R. Effects of interleukin-1 on the stress-responsive and-nonresponsive subtypes of corticotropin-releasing-hormone neurosecretory axons. *Endocrinology* 1992; 131: 37—44.
50. Weidenfeld J, Crumeyrolle-Arias M, Haour F. Effect of bacterial endotoxin and interleukin-1 on prostaglandin biosynthesis by the hippocampus of mouse brain: Role of interleukin-1 receptors and glucocorticoids. *Neuroendocrinology* 1995; 62: 39—46.
51. Navarra P, Pozzoli G, Becherucci C, Preziosi P, Grossman AB, Parente L. Prostaglandin E_2 and bacterial lipopolysaccharide stimulate bioactive interleukin-1 release from rat hypothalamic explants. *Neuroendocrinology* 1993; 57: 257—261.
52. Morimoto A, Mukarami N, Nakamori T, Sakata Y, Watanobe T. Possible involvement of prostaglandin E in development of ACTH response in rats induced by human recombinant interleukin-1. *J Physiol* 1989; 411: 245—256.

53. Watanobe H, Takebe K. Effects of intravenous administration of interleukin-1-beta on the release of prostaglandin E_2, corticotropin-releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats: estimation by push-pull perfusion. *Neuroendocrinology* 1994; 60: 8—15.
54. Cambronero JC, Rivas FJ, Borrell J, Guaza C. Role of arachidonic acid metabolism on corticotropin- releasing factor (CRF)-release induced by interleukin-1 from superfused rat hypothalami. *J Neuroimmunol* 1992; 39: 57—66.
55. Watanobe H, Sasaki S, Takebe K. Role of prostaglandins E_1, E_2 and $F_{2\alpha}$ in the brain in interleukin 1β-induced adrenocorticotropin secretion in the rat. *Cytokine* 1995; 7: 710—712.
56. Andreis PG, Neri G, Belloni AS, Mazzocchi G, Kasprzak A, Nussdorfer GG. Interleukin-1β enhances corticosterone secretion by acting directly on the rat adrenal gland. *Endocrinology* 1991; 129: 53—57.
57. Judd AM, MacLeod RM. Adrenocorticotropin increases interleukin-6 release from rat adrenal zona glomerulosa cells. *Endocrinology* 1992; 130: 1245—1254.
58. Gwosdow AR, O'Connell NA, Spencer JA et al. Interleukin-1-induced corticosterone release occurs by an adrenergic mechanism from rat adrenal gland. *Am J Physiol* 1992; 263: E461—E466.
59. Haour F, Marquette C, Ban E et al. Receptors for interleukin-1 in the central nervous and neuroendocrine systems. Role in infection and stress. *Ann Endocrinol* 1995; 56: 173—179.
60. Whitnall MH. Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. *Prog Neurobiol* 1993; 40: 573—629.
61. Levin N, Blum M, Roberts JL. Modulation of basal and corticotropin-releasing factor-stimulated proopiomelanocortin gene expression by vasopressin in rat anterior pituitary. *Endocrinology* 1989; 125: 2957—2966.
62. Ur E, Grossman A. The neuroregulation of corticotropin secretion. In: The pituitary gland, H Imura (ed). New York, Raven Press Ltd 1994, pp: 309—330.
63. Franci CR, Anselmo-Franci JA, Kozłowski JA, McCann SM. Action of endogenous vasopressin and oxytocin on anterior pituitary hormone secretion. *Neuroendocrinology* 1993; 57: 693—699.
64. Herman JP, Adams D, Prewitt C. Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology* 1995; 61: 180—190.
65. Whitnall MH, Smyth D, Gainer H. Vasopressin coexists in half of the corticotropin-releasing factor axons present in the extrenal zone of the median eminence in normal rats. *Neuroendocrinology* 1987; 45: 420—424.
66. Bartanusz V, Aubry J-M, Jezova D, Baffi J, Kiss JZ. Up-regulation of vasopressin mRNA in paraventricular hypophysiotropic neurons after acute immobilization stress. *Neuroendocrinology* 1993; 58: 625—629.
67. Bartanusz V, Jezova D, Bertini LT, Tilders FJH, Aubry JM, Kiss JZ. Stress-induced increase in vasopressin and corticotropin-releasing factor expression in hypophysiotropic paraventricular neurons. *Endocrinology* 1993; 132: 895—902.
68. Whitnall MH. Stress selectively activates the vasopressin-containing subset of corticotropin-releasing hormone neurons *Neuroendocrinology* 1989; 50: 702—707.
69. De Goeij DCE, Binnekade R, Tilders FJH. Chronic stress enhances vasopressin but not corticotropin-releasing factor secretion during hypoglycemia. *Am J Physiol* 1992; 263: E394—E399.
70. De Goeij DCE, Dijkstra H, Tilders FJH. Chronic psychosocial stress enhances vasopressin but not corticotropin releasing factor, in the external zone of the median eminence of male rats: relationship to subordinate status. *Endocrinology* 1992; 131: 847—853.
71. Bugajski J, Gądek-Michalska A, Ołowska A, Borycz J, Bugajski AJ. Effect of corticotropin-releasing hormone on the pituitary-adrenocortical activity under basal and social stress conditions. *J Physiol Pharmacol* 1994; 45: 593—601.

72. Bugajski J, Borycz J, Głód R, Bugajski AJ. Crowding stress impairs the pituitary-adrenocortical responsiveness to the vasopressin but not corticotropin releasing hormone stimulation. *BrainRes* 1995; 681: 223—228.
73. Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol* 1994; 15: 321—350.
74. Morimoto A, Watanade T, Morimoto K, Nakamori T, Murakami N. Possible involvement of prostaglandins in psychological stress induced responses in rats. *J Physiol* 1991; 443: 421—429.
75. Bugajski J, Ołowska A, Gądek-Michalska A, Borycz J, Głód R, Bugajski AJ. Effect of indomethacin on the CRH- and VP-induced pituitary-adrenocortical response during social stress. *Life Sci* 1996; 58: PL 67—72.
76. Lavicky J, Dunn AJ. Corticotropin releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assesed by microdialysis. *J Neurochem* 1993; 60: 602—612.
77. Bugajski J, Gądek-Michalska A, Borycz J, Głód R, Bugajski AJ. The corticosterone response to cholinergic and CRH receptor stimulation in rats exposed to social crowding stress. *Inflamm Res* 1995; 44: Suppl 1, S42—S43.
78. Bugajski J, Gądek-Michalska A, Borycz J. Social crowding stress diminishes the pituitary-adrenocortical and hypothalamic histamine response to adrenergic stimulation. *J Physiol Pharmacol* 1993; 44: 447—456.

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