REVIEW

Central non-glucocorticoid inhibitors of the hypothalamo–pituitary–adrenal axis

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Introduction

The popular perception of stress is that of social or work tensions and pressures that have an overall negative influence often accompanied by increased frequency of illness. However, stress is not all bad. A healthy response to acute stress is crucial for our interaction with the environment and for our ability to deal with everyday challenges, either physical or psychological. The key is to be able to terminate a response to stress when the stressor is no longer present. Failure to terminate a response to acute stress can result in the syndrome of chronic stress which is associated with inappropriately elevated secretion of glucocorticoids, with consequent immunosuppression and predisposition to infection and illness.

Responses of the hypothalamo–pituitary–adrenal (HPA) axis to stress are mediated principally through the neuropeptides corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) which are synthesised in the parvocellular subdivision of the hypothalamic paraventricular nucleus (PVN) (for review see Buckingham *et al.* 1997). CRH and AVP are transported down axonal projections to the median eminence (ME) where they are secreted into the hypophyseal portal blood system to synergistically stimulate the release of adrenocorticotrophin (ACTH) from corticotroph cells in the anterior pituitary, leading to increased secretion of glucocorticoids from the adrenal cortex (Fig. 1). Glucocorticoid feedback at the hippocampal and hypothalamic levels is a well-recognised mechanism for inhibiting basal and stress-induced HPA axis activity (for a review see De Kloet *et al.* 1998), but there is also considerable evidence for the existence of non-glucocorticoid mechanisms of inhibition. In adrenalectomised animal models, basal plasma ACTH concentrations are elevated but not maximally, since stressors such as ether (Rees *et al.* 1971), restraint (Chowdrey *et al.* 1991) and haemorrhage (Darlington *et al.* 1990) are capable of further stimulating ACTH release. In addition, a chronic osmotic stimulus inhibits ACTH release through a mechanism which, because it occurs in adrenalectomised rats, cannot be regulated by glucocorticoids

(Jessop *et al.* 1990). Thus the HPA axis is subject to mechanisms of tonic inhibition which operate independently of glucocorticoids.

Inhibitory agents may act at any or all levels of the HPA axis. Lesioning of selective hypothalamic nuclei can enhance the HPA axis response to some stressors (Herman & Cullinan 1997), providing evidence for endogenous hypothalamic inhibitors acting on CRH and/or AVP expression within the PVN. Observations of pulsatile ACTH secretion after complete lesioning of the PVN (and elimination of CRH from the ME) have revealed a surprising degree of residual pituitary–adrenocortical activity (Boyle *et al.* 1997). Total PVN lesioning was still accompanied by an ACTH response to some cytokines (Kovacs & Elenkov 1995), and electrical lesions that destroyed the entire hypothalamus did not prevent a response to the stress of laparotomy (Witorsch & Brodich 1972). One explanation for these results is that the lesions eliminate not only hypothalamic corticotrophin-releasing factors but also inhibitory factors which may be acting to block the secretion of ACTH from the anterior pituitary. Further evidence for the release of ACTH inhibitors into hypophyseal portal blood was supplied by the report that hypothalamo–pituitary disconnection in sheep was accompanied by increased ACTH and cortisol secretion (Engler *et al.* 1988). Although some of these results could be explained by the existence of novel extrahypothalamic ACTH-releasing factors, cumulative evidence is compelling for the existence of compounds acting on the hypothalamus and/or anterior pituitary that can inhibit the stress response.

The purpose of this review is to distil the literature on non-glucocorticoid inhibitors of the HPA axis (briefly reviewed by Grossman & Tsagarakis 1989 and Engler *et al.* 1994) and to assess the extent to which this information has contributed to our understanding of the biochemical mechanisms of stress control. The emphasis throughout will be on inhibitory agents that have the potential to operate at the hypothalamus or the anterior pituitary, rather than at the adrenal cortex. Compounds directly affecting glucocorticogenesis have been the

Figure 1 Schematic diagram of the hypothalamo-pituitary axis. CRH and AVP are secreted under neural control (A) from the parvocellular neurons of the hypothalamic PVN (B) at the level of the ME into the hypophyseal portal blood system (C) and transported to the corticotrophs of the anterior pituitary (D) where they synergistically stimulate the release of ACTH into the general circulation (E). ACTH inhibitory agents are listed according to their major hypothalamic or anterior pituitary site(s) of action.

subject of a recent review (Ehrhart-Bornstein *et al.* 1998).

γ -Aminobutyric acid (GABA)

The neurotransmitter GABA is a well-established inhibitor of ACTH release (Makara & Stark 1974), probably through a central action on hypothalamic CRH. GABA agonists are capable of inhibiting serotonin-induced CRH release from hypothalami in culture (Calogero *et al.* 1988a), and *in vivo* injection of the GABA A receptor antagonist bicuculline into the dorsomedial hypothalamus resulted in increased plasma ACTH and corticosterone (Keim & Shekhar 1996). Thus the HPA axis appears to be under tonic GABA inhibition at the hypothalamic level, mediated through GABA A receptors, although GABA B receptors have also been implicated (Hausler *et al.* 1993). GABA is located within CRH-containing neurons in the PVN (Meister *et al.* 1988) and also has the potential to influence CRH expression through GABAergic neurons within the bed nucleus of the stria terminalis and preoptic nuclei which project into the PVN (reviewed by Herman & Cullinan 1997). Evidence that neurosteroid analogues with GABA A receptor agonistic properties can attenuate

HPA axis responses to stress (Reddy & Kulkarni 1996, Patchev *et al.* 1997) may suggest a cooperative stress-protective mechanism operating between GABA and selective neurosteroids. The GABA transaminase inhibitor sodium valproate has been employed clinically to reduce ACTH secretion in some patients with Nelson's syndrome, but this has not been observed in normal subjects (Torpy *et al.* 1995).

β -Endorphin

The HPA axis can probably regulate its own activity through feedback by neuropeptides. Pro-opiomelanocortin (POMC) mRNA is synthesised in the arcuate nucleus of the hypothalamus, and the POMC products ACTH and β -endorphin are transported through axonal projections to the parvocellular subdivision of the PVN (Sawchencko *et al.* 1982, Kiss *et al.* 1984) where they abut perikarya of CRH-containing neurons (Liposits *et al.* 1988). Human studies using the morphine antagonist naloxone have established that endogenous opioids can inhibit HPA axis activity (Grossman & Besser 1982, Torpy *et al.* 1997). In one study in which normal human subjects received an infusion of β -endorphin, an abnormal response

to insulin-induced hypoglycaemia was observed, although no overall inhibition of HPA axis activity occurred (Inder *et al.* 1996). This was probably due to poor penetration of the blood–brain barrier by β -endorphin, in contrast with naloxone which is lipophilic. In horses infused i.v. with a low dose of naloxone, basal plasma concentrations of ACTH and cortisol were elevated (Alexander & Irvine 1995). Opioid inhibition of the HPA axis is most probably exerted at the hypothalamic level, and may be mediated through AVP rather than CRH (Delitala *et al.* 1994), although opioids are capable of inhibiting the release of CRH from rat hypothalami *in vitro* (Calogero *et al.* 1988b, Tsagarakis *et al.* 1989, Tsagarakis *et al.* 1990).

Although the inhibitory effect of endogenous opioids has been well documented in rats and humans, it is interesting to note that earlier work demonstrating a stimulatory naloxone-reversible effect of opioids on HPA axis activity in rats (Buckingham & Cooper 1986) has recently gained renewed support from a paper showing that central injection of β -endorphin stimulated rat plasma ACTH, an effect that could be prevented by naloxone or by injection of β -endorphin antiserum (Yamauchi *et al.* 1997). These apparently paradoxical reports of the effects of opioids within and between species may be reconciled by proposing that, if these actions reflect short loop feedback inhibition of CRH synthesis, then the effects may be concentration-dependent, such that increasing central concentrations of POMC peptide products may stimulate CRH expression to a maximum point beyond which POMC peptides become inhibitory. The observed ability of CRH to either stimulate (Ono *et al.* 1985) or inhibit (Calogero *et al.* 1988b) its own release suggests a mechanism whereby β -endorphin might exert opposing concentration-dependent effects upon the HPA axis, mediated by CRH.

Atrial natriuretic peptide (ANP)

In addition to the integral role of ANP in maintaining electrolyte and fluid homeostasis through inhibition of aldosterone and AVP release, there is considerable evidence that ANP can also act as a corticotrophin-inhibitory factor. ANP was initially reported to inhibit the release of CRH- and AVP-stimulated ACTH from rat anterior pituitary cells (Antoni & Dayanithi 1989). This phenomenon was highly sensitive to pulsatile pretreatment of the cells with CRH and AVP (Antoni & Dayanithi 1990), conditions resembling those occurring *in vivo* in hypophyseal portal blood. There is physiological potential for an action of ANP at the pituitary, since significant amounts of ANP are secreted from the hypothalamus into hypophyseal portal blood (Lim *et al.* 1994) and ANP receptors are located within the anterior pituitary. However, these early promising results could not be reproduced in humans infused with ANP (Ur *et al.* 1991, Wittert *et al.* 1993).

The inability of physiological doses of peripheral ANP to attenuate HPA axis activity in man, together with evidence that ANP can inhibit the release of CRH from rat hypothalami *in vitro* (Ibanez-Santos *et al.* 1990), suggested that, if ANP did influence the HPA axis, the peptide was likely to be acting at the hypothalamic rather than at the pituitary level. This hypothesis proposing a central site of action was almost immediately controverted by the demonstration that central injection of ANP antiserum had no effect upon basal ACTH release (Fink *et al.* 1991, Franci *et al.* 1992) but when administered peripherally resulted in elevated plasma ACTH and corticosterone levels in rats (Fink *et al.* 1991). These observations switched attention back to an inhibitory role for ANP at the anterior pituitary and are supported by data showing a correlation between rat plasma ACTH and ANP during blood volume expansion (Jezova *et al.* 1994) and a potent inhibition by ANP of POMC expression in cultured mouse AtT-20 cells (Tan *et al.* 1994).

Despite numerous studies in support of a physiological inhibitory role for ANP over ACTH release, such a role remains controversial. Continuous perfusion of ANP in an equine pituitary cell system did not alter ACTH responses to pulsatile AVP (Mulligan *et al.* 1997) and ANP perfusion had no effect upon CRH- or AVP-stimulated ACTH release from cultured ovine or rat anterior pituitary cells (Bowman *et al.* 1997). ANP had no effect on basal or CRH-stimulated plasma ACTH in patients with Cushing's or Addison's disease (Ambrosi *et al.* 1994) and did not alter the ACTH response to a morning CRH test (Kellner *et al.* 1995). However, in the latter study, evening plasma ACTH levels were decreased during ANP infusion. The significance of this is that most human studies are performed in the morning during a period when the pituitary may be refractory to any effects of ANP. An evening response to ANP may reflect increased HPA axis sensitivity through facilitation (Dallman *et al.* 1992). Further human studies performed in the evening with ANP during this period of the circadian rhythm might be revealing in this respect, since the work of Antoni & Dayanathi (1990) clearly shows the importance of the relationship between pituitary conditioning and sensitivity to ANP.

In contrast with ANP, C-type natriuretic peptide (CNP) has been shown to stimulate the release of ACTH in sheep (Charles *et al.* 1995) and cortisol in man (Kellner *et al.* 1997). mRNA for CNP receptors has been located within the anterior pituitary (Grandclement *et al.* 1995), and ANP at high concentrations can bind to CNP receptors. Therefore the failure of some groups to show an inhibitory effect of ANP may be due to non-specific binding of ANP to CNP receptors in the pituitary following administration of supraphysiological doses of ANP. ANP acting through CNP receptors may result in activation of the HPA axis, which could mask any inhibitory effects mediated specifically through ANP

receptors. This mechanism would be difficult to explain if both ANP and CNP receptors were mainly expressed in pituitary corticotrophs, since both are linked to the common intracellular pathway of guanylate cyclase. However, CNP receptor mRNA is distributed throughout other anterior pituitary cell types such as lactotrophs and gonadotrophs (Grandclement *et al.* 1995) which may influence corticotroph activity through intercellular cross-talk. This stimulatory effect of ANP acting through CNP receptors may explain the report of enhanced effects on ACTH and cortisol release by an infusion of high-dose ANP, after an initial period of inhibition (Bierwolf *et al.* 1998).

Leptin

The protein leptin, which is produced in adipocytes, is a potent inhibitor of food intake and an important regulator of body weight. Because CRH also has anorectic properties, it has been proposed that the actions of leptin might be mediated via hypothalamic CRH. Subcutaneous infusion of leptin decreased CRH mRNA levels in the PVN of obese mice (Huang *et al.* 1998) and leptin blocked CRH release from perfused rat hypothalami *in vitro* while having no effect on ACTH release from cultured anterior pituitary cells (Heiman *et al.* 1997). Leptin has also been reported to inhibit ACTH and corticosterone increases induced by a restraint stress *in vivo* in mice (Heiman *et al.* 1997). Leptin receptor mRNA is present in the anterior pituitary as well as in the hypothalamus (Raber *et al.* 1997), conferring on leptin the potential to act at both the hypothalamus and pituitary.

In contrast with these observations, centrally injected microgram amounts of leptin increased CRH mRNA in the PVN (Schwartz *et al.* 1996) and increased circulating corticosterone (van Dijk *et al.* 1997). Leptin also stimulated the release of CRH from hypothalamic explants *in vitro* (Costa *et al.* 1997, Raber *et al.* 1997). These discrepancies between different laboratories are not easy to explain. Most of the studies so far have required relatively large amounts of leptin to elicit an effect, and it is not yet known whether such amounts are present within the hypothalamus. On the other hand, if these effects are mediated by circulating leptin, it is not clear how a large protein such as leptin can penetrate the blood-brain barrier to act within the PVN. What is certain is that the pathways controlling food intake are complex and involve many other peptides such as α -melanocyte-stimulating hormone, neuropeptide Y, glucagon-like peptide-1, galanin and melanin-concentrating hormone, all of which may interact with CRH and leptin within the PVN. Reports of stimulatory and inhibitory effects of leptin on the HPA axis may reflect opposing aspects of this multicircuited interaction during obesity or malnutrition, conditions associated with dysfunctional HPA axis activity.

Lipocortin-1 (LC-1)

Lipocortins are a family of glucocorticoid-regulated anti-inflammatory proteins, originally detected in immune tissues and also located within the hypothalamus and pituitary (Strijbos *et al.* 1991, Smith *et al.* 1993). It is well established that LC-1 plays a role in mediating the inhibitory effects of glucocorticoids on ACTH release (Taylor *et al.* 1993) but there is also evidence that LC-1 itself has intrinsic ACTH-modulating potential. It was found to inhibit interleukin (IL)-6-induced CRH release from hypothalami harvested from adrenalectomised rats, while conversely potentiating IL-6-induced AVP release (Loxley *et al.* 1993a). It was also found to attenuate the ACTH response to CRH *in vivo* and inhibit IL-1-induced depletion of CRH in rat hypothalami (Sudlow *et al.* 1996). These data are consistent with an important role for LC-1 at the hypothalamic level in mediating the HPA axis response to immune activation. Preincubation of hypothalami with LC-1 prevented CRH, but not AVP, secretion in response to a range of cytokines (Loxley *et al.* 1993b). This may reflect a more important role for LC-1 governing CRH rather than AVP expression during the HPA axis response to immune activation after inflammation. A 188-residue N-terminal LC-1 fragment in low picogram amounts inhibited ACTH release from rat anterior pituitary fragments (Taylor *et al.* 1993) in which high-affinity LC-1-binding sites are present (Christian *et al.* 1997), and LC-1 inhibited CRH-stimulated ACTH release from mouse pituitary AtT-20 cells (Pompeo *et al.* 1997). Thus LC-1, like glucocorticoids, can inhibit HPA axis activity at both the hypothalamus and pituitary. There are no data at present for the levels of LC-1 in hypophyseal portal blood, but it is clear from the study by Taylor *et al.* (1993) that LC-1 would only need to be secreted from the ME in relatively low amounts to potentially inhibit the release of ACTH.

Somatostatin

Acute restraint stress stimulates growth hormone-releasing hormone inhibitory factor somatostatin (SRIF) secretion into hypophyseal portal blood (Cataldi *et al.* 1994), thus providing the potential for an action of SRIF at anterior pituitary corticotrophs to modulate the HPA axis response to stress. However, human studies using a long acting synthetic analogue of SRIF (sandostatin or octreotide) have largely proved negative. Sandostatin had no effect on CRH-stimulated ACTH or cortisol in normal subjects (Invitti *et al.* 1991), did not alter basal plasma ACTH or cortisol in patients with ACTH hypersecretion (Ambrosi *et al.* 1990) and had no effect upon CRH-stimulated ACTH release in patients with Cushing's disease (Stalla *et al.* 1994). In patients with Nelson's syndrome or ectopic ACTH syndrome characterised by very high circulating concentrations of ACTH, peripheral administration of

sandostatin has been used with some success in the reduction of ACTH secretion (de Herder & Lamberts 1996), and an ACTH-secreting bronchial carcinoid tumour responded to long term treatment with sandostatin (Phlipponneau *et al.* 1994). SRIF inhibited the release of ACTH from human pituitary adenoma cell cultures (Stalla *et al.* 1994).

There is more convincing evidence for the effects of SRIF in rats: it inhibited CRH-stimulated ACTH release from isolated pituitary cells (Lamberts *et al.* 1989) and inhibited CRH release from hypothalami *in vitro* (Tizabi & Calogero 1992). Thus SRIF is effective at both the hypothalamic and anterior pituitary levels in rodents, but the absence of evidence for an effect of SRIF in normal human subjects suggests that this peptide plays little part in regulating the human HPA axis.

Prepro-thyrotrophin-releasing hormone (TRH)(178–199)

On the basis of the original observation that ACTH release from the mouse tumour AtT-20 cell line was attenuated in cells transfected with prepro-TRH cDNA (Redei *et al.* 1995a), it was proposed that a peptide fragment of prepro-TRH could act as an inhibitor of ACTH at the anterior pituitary. In sequence deletion studies, the fragment prepro-TRH(178–199) was shown to inhibit basal and CRH-stimulated ACTH release from anterior pituitary cell cultures (Redei *et al.* 1995b). Intravenous injection of prepro-TRH(178–199) significantly decreased plasma ACTH and corticosterone responses to restraint stress whereas centrally injected prepro-TRH(178–199) was ineffective (McGivern *et al.* 1997). Thus prepro-TRH(178–199) has emerged as a leading contender for the role of principal corticotrophin-inhibitory factor at the anterior pituitary (Redei *et al.* 1998). However, the data derived from these elegant studies are not supported by evidence from another group who found no effects of prepro-TRH(178–199) on basal or CRH-stimulated ACTH release from cultured anterior pituitary cells (Nicholson & Orth 1996). Given the similar *in vitro* methodology employed by the two groups, it is difficult to evaluate the reasons for this discrepancy, and final judgement on the efficacy of prepro-TRH(178–199) as a corticotrophin-inhibitory factor awaits independent confirmation.

Substance P (SP)

SP, a member of the neurokinin peptide family, is found in the ME and PVN in significant amounts and has been implicated in a wide variety of neuroendocrine functions (Jessop *et al.* 1992). Intracerebroventricular injection of SP in rats resulted in decreased circulating ACTH (Chowdrey

et al. 1990) while SP inhibited the release of CRH from rat hypothalami *in vitro*, but not from isolated ME tissue (Faria *et al.* 1991), indicating a role within the PVN. Injection i.c.v. of an SP antagonist peptide stimulated circulating concentrations of ACTH and corticosterone and increased CRH mRNA in the parvocellular subdivision of the PVN (Larsen *et al.* 1993), demonstrating that central endogenous SP tonically inhibits the synthesis and release of CRH. SP also exerts a tonic inhibitory influence over the HPA axis response to the chronic stress of osmotic stimulation (Larsen *et al.* 1993) and to the chronic inflammatory stress of adjuvant-induced arthritis (Chowdrey *et al.* 1995), since in both those studies parvocellular CRH mRNA was increased following administration of an SP antagonist. SP is involved in terminating the response to acute restraint stress, an action that is mediated centrally through the NK1 receptor (Jessop *et al.* 1998). This may be a direct inhibitory action on CRH expression through NK1 receptors in the parvocellular subdivision of the PVN or indirectly via a neurotransmitter-mediated pathway (Fig. 2). SP can stimulate the release of GABA (Sakuma *et al.* 1991) and thus may exert an inhibitory effect on CRH expression indirectly through GABA. Another pathway could involve serotonergic nerve terminals which abut directly on to CRH-containing cell bodies in the PVN (Liposits *et al.* 1987). Centrally injected SP was found to inhibit the release of serotonin in the PVN (Culman *et al.* 1995) and also serotonin-stimulated release of corticosterone (Saphier *et al.* 1994). Thus SP could endogenously inhibit stress-induced expression of CRH through inhibition of serotonin release in the PVN.

Observations that axonal terminals in the ME of the rat and primate stained strongly for SP (Hökfelt *et al.* 1978, Larsen 1992) raised the possibility that significant amounts of SP are released into hypophyseal portal blood to act as an ACTH inhibitor directly on corticotrophs of the anterior pituitary. However, concentrations of SP are very low in hypophyseal portal blood of sheep (Clarke *et al.* 1993), rat (Lim *et al.* 1990) and monkey (Eckstein *et al.* 1980), and SP in physiological doses had no effect on ACTH release from dispersed anterior pituitary cells *in vitro* (Chowdrey *et al.* 1990). Therefore, in the absence of evidence that SP plays any significant physiological role at the level of the anterior pituitary, it is probable that it modulates HPA axis activity by inhibiting the expression of CRH within the PVN. Consequent reduction in glucocorticoid secretion is consistent with a potential pro-inflammatory role for central SP, as has been demonstrated for peripheral SP in a number of inflammatory diseases (Levine *et al.* 1993). Therefore, by implication, the central anti-inflammatory effects of substance P antagonists currently being evaluated as anti-depressants (Kramer *et al.* 1998) may confer an advantage compared with the pro-inflammatory effects of selective serotonin re-uptake inhibitors now widely prescribed (Harbuz *et al.* 1998).

Figure 2 Possible mechanisms of SP inhibition of CRH expression in the hypothalamus. SP projections from the brain stem (BS), lateral hypothalamus (LH) and arcuate nucleus (AN) may directly inhibit axonal transport of CRH from cell bodies within the PVN and/or release of CRH from the ME into hypophyseal portal blood. SP may also inhibit CRH expression indirectly through stimulation of GABAergic or inhibition of serotonergic inputs to CRH-containing cells within the PVN.

Oxytocin (OT)

OT is synthesised in the magnocellular neurons of the hypothalamus and released from the posterior pituitary in response to a variety of stressors. When last reviewed by Grossman & Tsagarakis in 1989, the literature suggested stimulatory effects of OT on ACTH release in rodents and inhibitory effects in humans. Support for the latter has been reinforced by a report that CRH-induced ACTH release was completely inhibited by an OT infusion in normal human subjects (Page *et al.* 1990), which suggests a direct effect on the pituitary since OT probably does not cross the blood–brain barrier in significant amounts. An inhibitory effect of OT in rats has also been reported in a model permitting rapid automated blood sampling in which the corticosterone response to a noise stress was attenuated by central infusion of OT, but not AVP (Windle *et al.* 1997). Although a direct pituitary effect cannot be ruled out in the latter study, the likelihood is that OT is acting centrally on CRH expression within the PVN, possibly in response to reduced noradrenergic tone, since noradrenaline secretion from the PVN was reduced during lactation (Toufexis *et al.* 1998). HPA axis activity is attenuated during lactation coincident with a period when central levels of OT are elevated (Lightman & Young

1989). However, evidence for a stimulatory role for OT persists, with the observation that OT stimulated pulsatile release of ACTH from superfused anterior pituitary cells harvested from female rats (Link *et al.* 1993).

Monoxide gases

Nitric oxide (NO) and carbon monoxide (CO) have attracted considerable attention as potential modulators of the HPA axis. They are synthesised widely throughout the central nervous system, and mRNAs for their respective rate-limiting enzymes NO synthase (NOS) and haem oxygenase have been located within the PVN (Grossman *et al.* 1994, Vincent *et al.* 1994). NO is generally believed to inhibit AVP release from the hypothalamus (Yasin *et al.* 1993a, Grossman *et al.* 1997) but there has been little agreement among investigators on the influence of NO over CRH release. In studies on hypothalamic explants, addition of NOS substrate inhibited potassium- or IL-1-induced release of CRH (Costa *et al.* 1993), while other investigators have reported a stimulatory effect of NO on CRH *in vitro* in response to IL-1 (Brunetti *et al.* 1993, Sandi & Guaza 1995) or IL-2 (Karanth *et al.* 1993). *In vivo* data are strongly supportive of an inhibitory role for

endogenous NO in mediating the HPA axis response to immune activation, since inhibition of NOS activity resulted in an enhanced release of ACTH and corticosterone in response to lipopolysaccharide or IL-1 injected i.v. (Rivier & Shen 1994, Rivier 1995, Kim & Rivier 1998) but not i.c.v. (Rivier & Shen 1994, Lee & Rivier 1998), and a NOS inhibitor further elevated inflammation-induced ACTH secretion (Turnbull & Rivier 1996). In contrast, blocking NO synthesis attenuated the HPA axis response to footshock or water avoidance stress (Rivier 1994), suggesting that NO may be a stimulatory agent in mechanisms in the PVN that mediate acute physical or psychological stress. It has been proposed that, depending upon whether the stressor is non-immune or immune, NO may exert either a stimulatory effect on the synthesis of CRH within the PVN or an inhibitory effect upon its release from the ME (Rivier 1998). These mutually opposing influences may explain the failure of the non-immune stressors of restraint or hypertonic saline to further stimulate the HPA axis in the chronic immunological stress of adjuvant-induced arthritis (Harbuz *et al.* 1997).

Like those for NO, initial reports on the actions of CO have been inconsistent, and two *in vitro* studies using rat hypothalamic tissue have shown opposite effects. Increasing substrate levels for haem oxygenase inhibited IL-1-stimulated production of CRH and AVP (Pozzoli *et al.* 1994), an effect that was reversed by inhibition of haem oxygenase activity, while a haem analogue exerted a stimulatory effect on CRH release (Parkes *et al.* 1994). NO and CO selectively modulate the release of CRH and AVP from rat hypothalami in response to lipopolysaccharide (Kostoglou-Athanassiou *et al.* 1998), providing evidence for a counter-regulatory mechanism controlling the HPA axis response to immune activation. In the one *in vivo* study reported so far, blocking of central CO production decreased footshock-stimulated ACTH release (Turnbull *et al.* 1998), providing evidence for a stimulatory role for CO.

Discrepancies between reports on the effects of NO and CO, both *in vitro* and *in vivo*, are not really surprising since it is exceptionally difficult to obtain consistent results when dealing with compounds with biological half-lives that can be measured in seconds. Inconsistent results between laboratories may be due to different experimental methodology or sources of cytokines, doses of which are notoriously difficult to standardise. Differences between data generated *in vitro* and *in vivo* may reflect the isolated responses of tissues *in vitro* removed from neuronal influences. The uniform conclusion that can be derived from the literature is that the actions of NO, CO, cytokines, CRH and AVP are all integrated within the hypothalamus. Thus central NO and CO can act as agents in an important homeostatic mechanism to control the HPA axis response to stimulatory effects of cytokines released peripherally during acute or chronic inflammation. This

area of investigation is of great importance in elucidating the anti-inflammatory role of the HPA axis during immune activation. Differential and cooperative actions of NO and CO in paradigms of psychological or physical stress will prove a fascinating area of study, and it will be of interest to see whether the inhibitory effects of any other agents listed in this review are mediated through NO and/or CO.

Endothelins (ETs)

ETs are peptides with potent vasoconstrictor activity, originally isolated from endothelial tissue and also located in the brain and pituitary. ET-1, the principal mammalian form, did not affect basal release of CRH from rat hypothalamic explants (Yasin *et al.* 1994) and had no effect upon potassium-stimulated CRH release from rat hypothalami, but did inhibit CRH-induced ACTH release from pituitary cell cultures (Calogero *et al.* 1994). In contrast with these inhibitory effects, ET-1 stimulated basal ACTH secretion in rats (Calogero *et al.* 1994, Malendowicz *et al.* 1997). It has been reported that ET-1 given i.v. augmented basal and CRH-stimulated ACTH release in normal human subjects (Vierhapper *et al.* 1993), possibly because of increased blood pressure associated with ET-1 infusion, since both the increased ACTH and blood pressure were reversed by the calcium channel antagonist nifedipine (Vierhapper 1996). These data are difficult to interpret, since a cocktail dose of all synthetic hypothalamic releasing hormones was given to each subject, and multiple confounding interactions at the anterior pituitary cannot be discounted. Overall, there is no convincing evidence for a specific role for ET-1 in regulating the HPA axis. ET-1 stimulated the basal release of AVP from rat hypothalami *in vitro* (Shichiri *et al.* 1989, Yasin *et al.* 1994), but this is likely to be from magnocellular neurons which mediate the neurohypophysial response to alterations in blood pressure.

Adrenomedullin (ADM)

The potent vasodilator and hypotensive agent ADM was first reported to inhibit basal and CRH-stimulated ACTH release from pituitary cells *in vitro* (Samson *et al.* 1995). Plasma concentrations of ACTH and cortisol in the sheep were significantly reduced following i.v. ADM infusion (Parkes & May 1995). Infusion of ADM i.c.v. actually increased circulating ACTH and cortisol in the sheep (Charles *et al.* 1998), although these data are difficult to interpret since cortisol levels were elevated nearly an hour before a significant increase was observed in plasma ACTH. Parkes & May (1995) found no effects of i.c.v. infusion of ADM on plasma ACTH and cortisol. Thus ADM appears to exert a specific inhibitory action at the

pituitary level over HPA axis activity. The N-terminal peptide fragment of pro-ADM also inhibited basal ACTH release (Samson 1998).

Others

Isolated observations have appeared of a few other compounds with HPA axis inhibitory activity. Melatonin selectively inhibited AVP, but not CRH, release from hypothalamic explants (Yasin *et al.* 1993b). The thymic peptide thymosin- α -1 potently inhibited the release of hypothalamic CRH both *in vivo* (Milenkovic & McCann 1992) and *in vitro* (Milenkovic *et al.* 1992), while stimulating the release of pituitary ACTH. An early report that melanin-concentrating hormone has ACTH inhibitory activity was not supported by data from Navarra *et al.* 1990.

Concluding comments

So why the need for such a wide range of inhibitors of HPA axis activity in addition to negative feedback from glucocorticoids? A teleological argument can be advanced that, because termination of the stress response is so important, multiple inhibitory mechanisms have evolved as a protective network should one pathway fail. There is, however, another explanation for what might seem to some to be physiological overelaboration. It is instructive to examine the complex interaction of all the components, peptides and neurotransmitters, that coordinate HPA axis responses to various stressors, and to consider whether it is likely that glucocorticoids alone, acting through only two types of receptor, can provide the complex range of controls required to terminate every type of stress, of which there is a considerable range. Although the release of CRH and AVP from the hypothalamus is considered to be the final common pathway in the stress response, it has become increasingly apparent over the last decade that different types of stress are characterised by altered contributions of CRH, AVP and probably other compounds as well, depending on whether the stress is physical, psychological, immunological, acute or chronic (Harbuz *et al.* 1997). These responses may in turn reflect differential stimulatory input to the hypothalamus. Thus each type of stress may have its own unique HPA axis response, defined by the timing and ratio of CRH/AVP release and contributions of other hypothalamic factors. Therefore, if selective stimulatory mechanisms are involved in mediating different types of stress at the hypothalamic level, it seems reasonable to propose that selective mechanisms of termination also exist. Discrepancies between reports on the efficacy of a particular compound may reflect the fact that certain inhibitors are of more importance in some types of stress than in others. No one compound has yet

Figure 3 Putative example of stress-specific inhibition by SP. In the chronic inflammatory stress of adjuvant-induced arthritis, parvocellular CRH mRNA was decreased ($\downarrow$) and AVP mRNA was increased ($\uparrow$) in the presence of increased SP immunoreactivity. SP levels were elevated in the ME while CRH secretion was decreased and AVP secretion was increased. This is consistent with SP acting as a specific inhibitor of CRH, but not AVP, during this type of chronic stress. Secretion of both ACTH and corticosterone was elevated. The acute stressors of restraint or i.p. hypertonic saline failed to further stimulate corticosterone in this model whereas i.p. endotoxin strongly stimulated corticosterone secretion, suggesting that central SP may be influential in mediating acute physical or psychological, but not acute immunological, stress. Based upon data from Chowdrey *et al.* (1995) and Harbuz *et al.* (1997).

been established beyond doubt as the ACTH inhibitor and it is unlikely that one will be. Consistent with the proposal that each type of stress evokes a unique hypothalamic response, and that the site of the response is also the principal point of inhibitory control, is the cumulative evidence that most inhibitors act at the hypothalamus rather than at the pituitary (Fig. 1). Examination of the literature reveals no apparent second messenger system common to all non-glucocorticoid inhibitors whereby the actions of these agents might be integrated at the post-receptor level. Thus different mechanisms of control over CRH, AVP and ACTH expression may operate both extra- and intra-cellularly depending upon the type of stress. Likely examples of stressor-specific inhibitors are OT, which is probably responsible for attenuation of the stress response during lactation, and SP, which is associated with decreased CRH expression during the chronic inflammatory stress of adjuvant-induced arthritis (Fig. 3).

In conclusion, specific mechanisms utilising stimulatory and inhibitory neuropeptides and neurotransmitters may have evolved to modulate each type of stress. The concept of stressor-specific inhibitory compounds can be tested with the availability of a new generation of antagonists to many of these compounds and by advances in oligonucleotide antisense technology allowing selective disruption of neuropeptide expression. The progress we make towards understanding the biochemical mechanisms that control stress must represent our best chance of developing therapies to counteract the deleterious effects of chronic stress that currently exact such a toll in modern society.

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