Effects of catecholamines on secretion of adrenocorticotrophic hormone (ACTH) in man

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SUMMARY The hypothalamus receives a rich supply of adrenergic and noradrenergic nerve fibres from the brain stem, terminating in many hypothalamic regions, including the paraventricular nucleus, which is the site of the cell bodies of corticotrophin releasing factor (CRF) neurones in man. Experimental evidence has shown that an α_1 adrenoceptor mechanism stimulates adrenocorticotrophic hormone (ACTH) secretion in man. The site of action of this mechanism seems to be within the blood brain barrier, presumably modulating the secretion of the CRF complex. This mechanism is important in the control of ACTH secretion in some physiological conditions in healthy subjects.

The pioneering work of Harris¹ postulated the existence of a hypothalamic corticotrophin releasing factor (CRF) that is transported in the hypothalamo-hypophyseal portal system to the anterior pituitary gland, and which stimulates the secretion of adrenocorticotrophic hormone (ACTH). This work was crowned by Vale *et al*, who isolated and synthesised² a 41 amino acid peptide from ovine hypothalamus with specific CRF bioactivity. Equivalent peptides have been identified in other species, including man. Immunohistochemical studies have shown this peptide to be widely distributed in the central nervous system, but within the hypothalamus it is localised mostly in the parvocellular neurones of the paraventricular nucleus. The axons extend to the zona externa of the median eminence, which is the site of the first capillary bed in the hypothalamo-hypophyseal portal system.³⁻⁵

Further investigations have shown that other peptides may possess CRF activity. Prominent among these is vasopressin, which has a weak direct stimulant effect on ACTH secretion; but more importantly, it strongly enhances the activity of CRF-41.⁶ It therefore seems that the hypothalamus secretes a CRF "complex" the constituents of which may vary under different circumstances. The paraventricular nucleus contains high concentrations of catecholamines which may influence the activity of the hypothalamo-pituitary adrenal axis (HPAA). In theory peripheral circulating catecholamines may also influence ACTH secretion as activation of the sympathoadrenal system

often accompanies that of the HPAA.

In this review, we summarise the relation of catecholamine systems to the hypothalamus and the pituitary gland and discuss the evidence from human experiments for a regulatory role of these amines in ACTH secretion.

Catecholamine systems, the hypothalamus, and the pituitary gland

The hypothalamus contains the highest concentrations of noradrenaline in the brain.⁷⁻⁹ The origin of this noradrenaline is almost all extrinsic as surgical isolation of the hypothalamus results in a drastic reduction of its noradrenaline content.^{10,11} The noradrenergic innervation of the hypothalamus is derived from parts of the reticular formation in the ventral and dorsal medulla oblongata and the locus ceruleus at the junction of the pons and the midbrain. The axons ascend in the medial forebrain bundle, and some cross the midline.¹²⁻¹⁶ Noradrenergic nerve terminals can be identified in every hypothalamic nucleus, but two of the most densely innervated are the paraventricular and supraoptic nuclei^{12,14,17} and the median eminence.^{14,17-19} The noradrenergic innervation of the paraventricular nucleus is derived largely from the ventral medulla.²¹ By simultaneously using catecholamine histofluorescence and neuropeptide immunocytochemistry, catecholaminergic neurones were observed with their terminals on the cell bodies of peptidergic neurones in hypothalamic nuclei,^{20,22} suggesting a monosynaptic contact.

The hypothalamus also contains high concentrations of adrenaline.⁷ The cell bodies of adrenergic neurones lie within the reticular formation in the upper medulla^{23 24} and project to various parts of the brain, including the diencephalon, in which the highest concentrations are found in the paraventricular and arcuate nuclei and the median eminence.²³⁻²⁷ Using combined immunocytochemistry Mezey *et al*²⁸ showed that adrenergic neurones lie in close proximity to CRF-41 cell bodies in the paraventricular nucleus of rats.

The dopaminergic innervation of the hypothalamus is almost all intrinsic; surgical isolation of the hypothalamus results in little reduction of dopamine content.^{10 11} In the hypothalamus the tuberoinfundibular dopaminergic tract has its cell bodies in the arcuate nucleus, near the inferior end of the third ventricle. The axons project to the zona externa of the median eminence where they are in close proximity to the first capillary bed of the hypothalamo-hypophyseal portal system, into which dopamine is secreted. Dopaminergic neurones also supply the neural and the intermediate lobes of the rat pituitary.^{12 18} The incerto-hypothalamic tract has its cell bodies mainly in the zona incerta and partly innervates the paraventricular nucleus.²⁹

The mammalian adeno-hypophysis does not receive a catecholaminergic innervation.^{30 31} The neural and intermediate lobes of the rat contain considerable quantities of dopamine and smaller amounts of nor-adrenaline.³⁰ The dopaminergic innervation is derived from neurones with their cell bodies in the arcuate nucleus,³² but the noradrenergic innervation seems to be from peripheral sympathetic nerves accompanying blood vessels.³²

Dopamine is secreted into hypophyseal portal plasma,³³ and its role in the tonic inhibition of prolactin secretion is well established. There is controversy, however, as to whether adrenaline and nor-adrenaline are secreted into hypophyseal portal plasma. Several studies in rats have reported the concentrations of adrenaline and noradrenaline in hypophyseal portal plasma to be no greater than those in peripheral plasma.³³⁻³⁷ More recently, some investigators³⁸⁻⁴⁰ have found that the concentration of adrenaline in plasma from the transected pituitary stalk of anaesthetised rats was 60-90% higher than the adrenaline concentrations in peripheral venous plasma, and interpreted this as indicating a central source of adrenaline secretion into the portal circulation. The source of adrenaline in those experiments may have been partly derived from the severed stalk nerves containing adrenergic fibres innervating the posterior and intermediate lobes, as the concentration of adrenaline in plasma from a single portal vessel in the intact stalk is lower than that in peripheral

plasma.³⁷ Nevertheless, whichever of these views concerning the rat prevails, as the mammalian adeno-hypophysis does not receive a catecholaminergic innervation^{31 32} it is unlikely that the anterior pituitary is exposed to much higher concentrations of adrenaline than those found in peripheral plasma.

The distribution of adrenoceptors in the hypothalamus has been studied using autoradiography and radioligand binding studies on homogenised rat brain membranes. Within the hypothalamus, α_1 and α_2 adrenoceptors are found in several nuclei, including the paraventricular nucleus.⁴¹ The median eminence has a high density of α_2 adrenoceptors but no high α_1 binding.⁴¹ The density of β -adrenoceptor binding sites is lower in the hypothalamus than in the cerebral cortex, and even lower densities are found in the adeno-hypophysis, about 10% of the density in the cortex.⁴² Roughly equal densities of the two β -adrenoceptor subtypes are found in the hypothalamus, but only β_2 adrenoceptors are found in the rat adeno-hypophysis.⁴² The paraventricular nucleus has an intermediate density of β -adrenoceptor binding sites.⁴¹ Within the hypothalamus dopaminergic receptors are found in highest densities in the median eminence (mostly D-1 subtype), but the paraventricular nucleus has an intermediate density of both D-1 and D-2 binding sites.⁴¹ In the adeno-hypophysis only the D-2 receptor subtype is found and helps to regulate prolactin secretion.⁴³

Effects of adrenoceptor stimulation on ACTH secretion

The early studies that assessed the interaction of catecholamines and the HPAA in man examined the effects of administration of adrenaline on various indirect indices of HPAA activity, such as the blood eosinophil count,⁴⁴⁻⁴⁶ 17-hydroxycorticosteroids,⁴⁵⁻⁴⁸ and various early ACTH bioassays,^{49 50} but the results were conflicting. More recently, intravenous infusions of adrenaline^{51 52} and noradrenaline^{53 54} have been reported to have no stimulatory effect on basal plasma cortisol concentrations in man. Muller-Hess *et al*⁵¹ suggested that adrenaline may attenuate the cortisol response to hypoglycaemia induced by insulin, but this is probably attributable to the attenuation of the hypoglycaemic effect of insulin by adrenaline. Other evidence from man includes the observations that amphetamines stimulate the secretion of ACTH and cortisol and that the effect is blocked by thymoxamine but not propranolol, which suggests that it is mediated by α_1 adrenoceptors.^{55 56} Amphetamines have complex pharmacological actions and cause a generalised arousal effect that is correlated with the height of the cortisol peak.^{55 57} so the mechanism by which they stimulate ACTH secretion is uncertain.

In our current investigations of α -adrenergic agonists we have chosen methoxamine, a highly selective agonist at post-synaptic α_1 adrenoceptors^{58,59} that is free of behavioural arousal effects when injected intracerebroventricularly in experimental animals.⁶⁰ Like Nakai *et al.*,⁶¹ we found that intravenous infusions of methoxamine stimulate the secretion of ACTH and cortisol in man (fig 1).⁶² The stimulant effects of methoxamine on ACTH and cortisol secretion were dose dependent and were accompanied by an increase in blood pressure, as may be expected from an α_1 adrenoceptor agonist.⁶² The effects of methoxamine were abolished by concomitant administration of the highly selective α_1 adrenoceptor antagonist thymoxamine,⁵⁹ confirming that they were mediated by α_1 adrenoceptors (fig 1).⁶² When given in large doses in experimental animals, methoxamine may have β -adrenoceptor antagonist activity,⁶³ but this is not the mechanism by which it stimulates the HPA axis in man: the effect was abolished by concomitant administration of thymoxamine, which lacks activity at β -adrenoceptors.⁶⁴ Thymoxamine has weak H-1 antihistaminic action on the guinea pig ileum *in vitro*,⁶⁴ but we have found that thymoxamine does not attenuate the bronchoconstrictor action of intravenously injected histamine,⁶⁵ suggesting that thymoxamine has no H-1 antihistaminic activity in the doses used in man.

To examine whether methoxamine stimulates ACTH secretion by a central effect or by a peripheral action, such as vasoconstriction or an increase in blood pressure, we compared the effects of methoxamine to those of the more hydrophilic noradrenaline.⁶⁶ Noradrenaline is a potent α_1 adrenoceptor agonist⁵⁸ that reaches the pituitary gland and the median eminence after a systemic injection but does not cross the blood brain barrier.⁶⁷⁻⁶⁹ The noradrenaline infusions were designed to raise systolic blood pressure by amounts equivalent to those produced by methoxamine, so that equivalent peripheral α_1 adrenoceptor activation by the two drugs could be compared.⁶² Fig 2 shows that the noradrenaline infusions were not followed by a rise in plasma cortisol above the mean control value, suggesting that the stimulation of the HPA axis by methoxamine is exerted by a central rather than a peripheral mechanism. As the pituitary gland and the median eminence are outside the blood brain barrier and are accessible to circulating noradrenaline,⁶⁷⁻⁶⁹ our inability to stimulate the HPA axis with noradrenaline suggests that the site of the stimulant α_1 adrenoceptors is within the blood brain barrier, presumably modulating the secretion of the CRF complex.

In addition to its hydrophilic properties, noradrenaline differs from methoxamine in its β and α_2 adrenoceptor agonist actions. These receptor

activities of noradrenaline, however, do not account for the differences from methoxamine, as we found that β_1 and β_2 adrenoceptor agonists (prenalterol and salbutamol, respectively), do not have a direct action on ACTH secretion in man; and an α_2 antagonist (yohimbine) does not modify cortisol secretion during noradrenaline infusions.⁶²

The noradrenaline infusions caused a slight inhibition of cortisol secretion compared with saline (fig 2),⁶² as had been described by Wilcox *et al.*⁵³ The mechanism of this inhibitory effect of peripheral

Fig 1 Effects of infusions of saline and methoxamine (0.22 mg/minute) with and without thymoxamine (0.2 mg/kg bolus + 2.2 μ g/kg/minute) on plasma ACTH, immunoreactive β -endorphin (C-terminal LPH), cortisol, heart rate (HR), and blood pressure (BP) in nine normal subjects. Open circles, saline; closed circles, methoxamine; triangles, methoxamine and thymoxamine. (Reproduced by permission of the publishers of *Neuroendocrinology*, S Karger AG, Basel.)

Fig 2 Comparison of effects of methoxamine and noradrenaline on plasma cortisol and systolic blood pressure (expressed as percentage change from mean control $\pm$ SEM). Noradrenaline infusions were given to increase SBP by about 10 and 25% of the mean control value, similar to changes after methoxamine. (Reproduced by permission of the publishers of *Neuroendocrinology*, S Karger AG, Basel.)

adrenoceptor activation on the HPA axis is unclear, but it may be caused by the known inhibitory effect of intravenous infusions of noradrenaline on the secretion of vasopressin,⁷⁰ which is a constituent of the CRF complex.

In another experiment a different approach was used to examine the site of action of α_1 adrenoceptors in stimulating ACTH secretion. We studied a group of patients with hypopituitarism caused by diseases of the hypothalamus or the pituitary stalk, such as craniopharyngiomas. These patients respond to an intravenous injection of synthetic ovine CRF-41 by an increase in ACTH and cortisol secretion but they do not respond to hypoglycaemia induced by insulin with a rise in plasma cortisol. This indicates that the cause of their hypoadrenalism is a failure of the synthesis or delivery of CRF, rather than a primary defect in ACTH reserve. If the site of action of the stimulant α_1 adrenoceptors in stimulating ACTH secretion is on the hypothalamus or its central connections—that is, within the blood barrier—then these patients would be expected to have no ACTH or cortisol response to an infusion of methoxamine. Fig 3 shows the result of a study in one such patient who shows no cortisol response to hypoglycaemia induced by insulin or methoxamine but responds normally to synthetic ovine CRF-41. This is consistent with the view that the site of action of the stimulant α_1 adrenoceptors is likely to be on the hypothalamus or its central connections, rather than directly on the pituitary gland.

Some data from experimental animals are consis-

tent with the view that central adrenergic mechanisms stimulate the HPA axis. Thus in cats corticosteroid secretion is stimulated by the implantation of noradrenaline into hypothalamic areas but not into the pituitary gland.⁷¹ In rats the depletion of hypothalamic noradrenaline by stereotaxic injection of the neurotoxin 6-hydroxydopamine into the medial forebrain bundle is followed by a reduction of ACTH secretion.⁷² Other experimental evidence, however, has been collected on the rat and the dog and has been interpreted as showing an inhibitory effect of central α adrenoceptors on ACTH secretion.⁷³ The systemic administration of β -adrenoceptor agonists stimulates ACTH secretion in the rat.⁷⁴ Most of these apparently conflicting data may be explained on the basis of the technical approaches and the pharmacological properties of the compounds used.

Adrenaline has been reported to enhance the stimulant effect of synthetic ovine CRF-41 on ACTH secretion by cultured rat adenohypophyseal cells in vitro.^{75,76} We therefore investigated the effects of increases in plasma concentrations of adrenaline within the normal range on the activity of CRF-41 in man in vivo.⁷⁷ Intravenous infusions of adrenaline that increased plasma adrenaline concentrations to 4.33 (SEM 0.82) nmol/l had no stimulant effect on ACTH or cortisol secretion basally or after the injection of oCRF-41 (fig 4). The plasma adrenaline concentrations during the adrenaline infusions were at the upper limit of the range that has been observed in normal subjects and patients in a variety of physiological and pathological situations, such as

Fig 3 Effects on plasma cortisol of hypoglycaemia induced by insulin (blood glucose nadir < 2.2 mmol/l), synthetic ovine CRF-41 (100 μ g bolus at 0 minutes), and methoxamine (20 mg intravenously from 0 to 90 minutes) in patient with craniopharyngioma causing hypothalamic deficiency. Shaded areas are 95% confidence limits of plasma cortisol response to identical dose of methoxamine in 11 normal subjects.

Fig 4 Dose-response relation of plasma ACTH to bolus doses of synthetic ovine CRF-41 during infusions of saline (open circles) and adrenaline (3 µg/minute, closed circles) in six normal subjects. Mean SEM values are shown. (Reproduced by permission of the publishers of *The Journal of Endocrinology*.)

cold exposure,⁷⁸ near maximal exercise,⁷⁹ acute myocardial infarction⁸⁰ in patients in an intensive care unit⁸¹ and in septicæmic patients who are not shocked.⁸² Higher concentrations of plasma adrenaline are observed only in extremes of pathological situations, such as impending death⁸¹ or severe septicæmia with shock,⁸² and after the pharmacological stimulus of hypoglycaemia induced by insulin. It was therefore concluded that circulating adrenaline has no physiological role in stimulating ACTH secretion either basally or in the presence of CRF-41. This conclusion is compatible with the previous findings that peripheral adrenergic stimulation with noradrenaline, prenalterol, and salbutamol was not associated with activation of the pituitary adrenal axis. The possibility that hypophyseal portal plasma may contain somewhat higher concentrations of adrenaline derived from a central source is controversial and has been discussed here. Milsom *et al*⁸³ studied the effects of adrenaline, noradrenaline, and clonidine and found no enhancement of the action of synthetic ovine CRF-41 by any of these substances.

In our investigation of the interaction of adrenaline and CRF-41 the adrenaline infusions were associated with a transient inhibition of ACTH secretion basally and a slowing of the rate of rise of ACTH secretion after the injection of CRF-41.⁷⁷ This is compatible with the previously described inhibition of cortisol

secretion by noradrenaline described above, and localises the site of action of this effect to the pituitary gland.

The effects of clonidine on the activity of the HPA axis have also been examined, with conflicting results: the drug has been reported both to inhibit^{84,85} and to have no effect^{86,87} on corticotroph activity. This may perhaps be due to the drug's complex pharmacology, as it stimulates both α_1 and α_2 adrenoceptors, the latter having the effect of inhibition of noradrenaline release from nerve endings. Clonidine also causes hypotension, which is a potent stimulus to ACTH secretion,^{88,89} and sedation,⁶⁰ which would be expected to depress the HPA axis.

The physiological importance of the stimulant α_1 adrenoceptors

1 THE ROLE OF α_1 ADRENOCEPTORS IN DETERMINING THE 24 HOUR CORTISOL SECRETORY PATTERN⁹⁰

A group of normal subjects were given 24 hour intravenous infusions of the α_1 adrenoceptor agonist methoxamine, the α_1 antagonist thymoxamine, and saline under double blind conditions. During waking hours, the methoxamine infusion was accompanied by higher concentrations of plasma cortisol than saline, while the converse held with thymoxamine (fig 5). In contrast, the nocturnal surge in cortisol secretion was unaffected by these adrenergic manipulations. The results suggest that an α_1 adrenoceptor mechanism maintains cortisol secretion during waking hours but not at night. There is evidence from experimental animals that the nocturnal activity of the HPA axis may be mediated by serotonergic and cholinergic mechanisms.

2 THE ROLE OF α_1 ADRENOCEPTORS IN CORTISOL SECRETION AFTER FOOD

Food ingestion stimulates cortisol secretion in man by an unknown mechanism.⁹¹ In rats feeding increases the turnover of noradrenaline in the hypothalamus,⁹² so we investigated the role of α_1 adrenoceptors in the mediation of cortisol secretion after eating.⁹³ A group of normal subjects was given three hour intravenous infusions of saline, methoxamine, and thymoxamine, and a standard meal was eaten 60 minutes after the start of the intravenous infusions. Methoxamine enhanced and thymoxamine attenuated the ACTH and cortisol responses to the meal without affecting nutrient absorption (fig 6). ACTH is found in the gastrointestinal tract as well as in the pituitary gland.^{94,95} To determine whether the source of this ACTH secretion is the pituitary gland or the gastrointestinal tract four patients with recent onset of ACTH deficiency and normally responsive adrenal

Fig 5 Effects of intravenous infusions of methoxamine (triangles), thymoxamine (open circles), and saline (closed circles) on 24 hour pattern of plasma cortisol in six normal subjects. C, coffee; L, lunch; T, tea; S, supper; D, drink; B, breakfast. (Reproduced by permission of the publishers of *Clinical Endocrinology*, Oxford: Blackwell Scientific Publications.)

Fig 6 Effects of methoxamine ($1 \mu\text{g/kg/minute}$), thymoxamine ($0.15 \text{ mg/kg bolus} + 2.5 \mu\text{g/kg/minute}$), and saline on ACTH and cortisol responses to food ingestion in six normal subjects. Infusions were given continuously throughout study and lunch was given at 60 minutes. Triangles, methoxamine; closed circles, saline; and open circles, thymoxamine. (Reproduced by permission of the publishers of *Clinical Endocrinology*, Oxford: Blackwell Scientific Publications.)

Fig 7 Effect of lunch on plasma cortisol in six normal subjects (shaded area mean $\pm$ 2 SD) and four patients with pituitary ACTH deficiency and responsive adrenal glands. Left panel shows plasma cortisol responses to physiological dose of ACTH in four patients. (Reproduced by permission of the publishers of *Clinical Endocrinology*, Oxford: Blackwell Scientific Publications.)

glands were given the same standard meal. There was no ACTH or cortisol response in any of these patients, indicating that the source of the secretion was the pituitary gland (fig 7).⁹³ In conclusion, cortisol secretion after the ingestion of food is mediated by central stimulant α_1 adrenoceptors which modulate the secretion of pituitary ACTH.

3 THE CORTISOL RESPONSE TO HYPOGLYCAEMIA

Hypoglycaemia is not physiological in man, but several studies have examined the effects of α and β -adrenoceptor antagonists on the cortisol response to this pharmacological stimulus. Nakai *et al*⁶¹ reported that phentolamine blunted the cortisol response to hypoglycaemia induced by insulin, but their findings could not be confirmed by several other groups.^{96–99} It therefore seems at this stage that the HPA response to hypoglycaemia, like the nocturnal surge of cortisol secretion, is not mediated by α_1 adrenoceptors.

Nakai *et al*⁶¹ reported that propranolol enhanced the pituitary-adrenal response to hypoglycaemia induced by insulin but their results were not confirmed by other investigators.^{97–98, 100} As described above, we found no stimulant effect of peripheral β -adrenoceptor activation on ACTH secretion basally or after CRF-41 injection in man. As yet no clear evidence exists for a role of β -adren-ergic mechanisms in the control of ACTH secretion in man. In rats, however, β_2 adrenoceptors stimulate the secretion of the pro-opiomelanocortin-derived peptides from the intermediate lobe,¹⁰¹ which is a vestigial organ in man.¹⁰²

The effects of dopaminergic mechanisms on the secretion of ACTH

Dopamine inhibits the secretion of pro-opiomelanocortin-derived peptides from the rat intermediate lobe.¹⁰³ The effect may be of physiological relevance, as dopaminergic antagonists stimulate the secretion of the pro-opiomelanocortin-derived peptides in rats and dogs in vivo,^{104–106} suggesting the existence of a tonic inhibitory dopaminergic mechanism. In man dopamine has no effect on the secretion of the pro-opiomelanocortin-derived peptides by anterior pituitary tissue in vitro.¹⁰⁷ In healthy subjects dopamine has no important effect on the secretion of ACTH or cortisol under basal conditions^{53, 108–110} or during hypoglycaemia induced by insulin.¹⁰⁸ Recently we found that dopamine has no major effect on the ACTH response to CRF-41 (Al-Damluji *et al*, unpublished observations), but a slight inhibitory action was evident, presumably resulting from the rise of plasma noradrenaline concentrations during the dopamine infusions. This is similar to the inhibitory effect of peripheral adrenoceptor stimulation described above. Dopamine does not cross the blood brain barrier after systemic administration.¹¹¹ Its precursor, l-dopa, does but may produce ambiguous results as it is converted to noradrenaline and adrenaline as well as dopamine and may interfere with the synthesis of other neurotransmitters such as serotonin.¹¹² A useful substitute is bromocriptine, a dopamine agonist that exerts strong central dopaminergic effects when administered systemically.¹¹³ Metoclopramide is a dopamine antagonist that has prominent central effects when given parenterally.¹¹⁴ Neither bromocriptine nor metoclopramide had any

effect on basal plasma cortisol¹¹⁵ in normal subjects, and bromocriptine has no effect on the cortisol response to hypoglycaemia.¹¹⁶ On the basis of the available evidence dopaminergic mechanisms do not seem to have a role in the control of ACTH secretion in man.

Conclusion

An anatomical relation seems to exist between the central adrenergic and noradrenergic systems and the hypothalamo-pituitary adrenal axis. It is possible to show by pharmacological means that α_1 adrenoceptors stimulate ACTH secretion in man. The site of action of these stimulant α_1 adrenoceptors is probably within the blood brain barrier, and they presumably act by modulating the secretion of the CRF complex. This mechanism is important in the control of ACTH secretion under some circumstances in man. There is no evidence as yet of important β -adrenergic or dopaminergic effects on ACTH secretion in man.

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