Opioid peptides and pituitary function

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Introduction

A number of in-vitro model systems have been introduced for the study of the action of morphine-like drugs. Their usefulness depends on their relative simplicity, compared to the brain and central nervous system, and on the good correlation observed between potencies in the models and potencies in the whole animal. For example, the contractions of the isolated guinea-pig ileum and mouse vas deferens to electrical stimulation at low frequencies are inhibited by opiate analgesics due to a reduced release of the motor transmitters acetylcholine (Paton, 1957) and noradrenaline (Henderson, Hughes & Kosterlitz, 1972) respectively, and the order of potencies in these smooth muscle models correlates well with the analgesic potencies of these drugs (see Kosterlitz & Waterfield, 1975).

Observations with these and other model systems lead to the belief that the site of action of opiates included a specific receptor in the true, pharmacological sense; but it seemed unlikely to many workers in the field of opiate research that such apparently selective receptors should have been developed by Nature to interact with the opium alkaloids. Accordingly, the idea that the opiate receptors must be receptors for “an endogenous humoral substance” was proposed by Collier (1972).

Experimental support for this hypothesis came shortly afterwards when Akil, Mayer & Liebskind (1972) reported that analgesia could be produced in rats by electrical stimulation through electrodes implanted in the periaqueductal region of the brain and that this “stimulation-evoked analgesia” was reduced by administering the morphine antagonist drug naloxone. These results were taken to mean that the brain contained an endogenous substance which produced a morphine-like analgesic effect by interacting with the opiate receptor and which could be released by electrical stimulation. It was then that concerted efforts commenced in several centres to isolate the endogenous morphine-like substance.

The discovery of the endogenous opioid peptides

The first attempts to isolate the endogenous ligand for the opiate receptor had been unsuccessful due to the mistaken presumption of chemical similarity to morphine, but in 1975 Hughes reported the isolation from aqueous extracts of brain of a material, ‘enkephalin’, which was peptide in nature and produced morphine-like responses in the in-vitro model systems. This extract of enkephalin was later shown to contain a mixture of two pentapeptides whose structures were H-Tyr-Gly-Gly-Phe-Met-OH (methionine-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (leucine-enkephalin) (Hughes *et al.*, 1975). Hughes *et al.* recognized that the amino acid sequence of methionine-enkephalin was already known as residues 61 to 65 of β -lipotropin (β -LPH), a 91-amino acid peptide of unknown function which had been isolated 10 years earlier from ovine pituitary (Li, Barnafi, Chrétien & Chung, 1965). The significance of this observation was enhanced when it was shown that the so-called ‘C-fragment’ of β -LPH (β -endorphin, β -

LPH₆₁₋₉₁), which itself was present in the pituitary, had the methionine-enkephalin sequence at its N-terminus and also had morphine-like activity (Bradbury, Smyth, Snell, Birdsall & Hulme, 1976; Lazarus, Ling & Guillemin, 1976; Li & Chung, 1976). Other fragments, including α -endorphin (β -LPH₆₁₋₇₆) and γ -endorphin (β -LPH₆₁₋₇₇), have also been isolated from the pituitary and have opioid activity (Guillemin, Ling & Burgus, 1976; Lazarus *et al.*, 1976), although recent evidence suggests that these may be artefacts of extraction or breakdown products of β -endorphin, as C-fragment is now known (Rossier *et al.*, 1977a).

It was originally proposed that methionine-enkephalin might be derived from β -endorphin which in turn was derived from β -LPH (Hughes *et al.*, 1975) and by analogy that similar precursors might exist for leucine-enkephalin (Hughes *et al.*, 1978). Attractive as this hypothesis may be, there is no experimental evidence in its favour (see McKnight, Sosa, Hughes & Kosterlitz, 1978). Although β -endorphin in the pituitary may be derived from β -LPH (Chrétien *et al.*, 1978), the enkephalins in both central and peripheral sites are synthesized locally from as yet unknown precursors (McKnight *et al.*, 1978).

The distributions of the endogenous opioid peptides have been determined by several different techniques and the results suggest that the enkephalins and β -endorphin form two independent systems.

Enkephalins are widely distributed, both in the brain and the periphery. They are particularly concentrated in the corpus striatum and hypothalamus of guinea-pig brain and the myenteric plexus of guinea-pig small intestine. Although the ratio of the contents of methionine- and leucine-enkephalin varies from region to region, methionine-enkephalin is always present in a greater concentration (Hughes, Kosterlitz & Smith, 1977). Immunohistochemical studies have shown enkephalin-like immunoreactive material in laminae I and II of the spinal cord, the substantia gelatinosa of the spinal trigeminal nucleus, the vagal nuclei, the periventricular and periaqueductal areas of the upper medulla and midbrain, the dorso-medial thalamus, the globus pallidus and the central nucleus of the amygdala (Elde, Hökfelt, Johansson & Terenius, 1976; Hökfelt, Elde, Johansson, Terenius & Stein, 1977; Hökfelt, Ljungdahl, Elde, Nilsson & Terenius, 1977; Simantov, Kuhar, Uhl & Snyder, 1977). This immunofluorescence is mainly associated with nerve fibres and terminals which suggests that the enkephalins may be neurotransmitters.

β -Endorphin is more restricted in its distribution with high concentrations in the anterior and intermediate lobes of the pituitary and lower concentrations in a few brain areas around the midline and in the hypothalamus, thalamus, midbrain and pons (Cox, Goldstein & Li, 1976; Bloom *et al.*, 1977; Rossier *et al.*, 1977c; Bloom, Battenberg, Rossier, Ling & Guillemin, 1978).

Physiological significance of the opioid peptides

When the existence of the enkephalins and β -endorphin was firmly established, many researchers were interested to examine whether these peptides produced morphine-like analgesia in the standard tests for anti-nociception in animals. The natural enkephalins had only a small and transient effect, even after intracerebroventricular injection (see Pert, Bowie, Fong & Chang, 1976) while β -endorphin showed a strong and long-lasting anti-nociceptive action (Feldberg & Smyth, 1976; Loh, Tseng, Wei & Li, 1976; Pert, 1976; Tseng, Loh & Li, 1976). In the mouse tail-flick test, β -endorphin was 48 times more potent than morphine, on a molar basis, after intracerebroventricular injection and 3.4 times more potent after intravenous injection (Li, 1977). These observations lead several groups to propose that the enkephalins had no physiological significance but rather that they were breakdown products of β -endorphin, like α - and γ -endorphin. This idea has largely been rejected since the transience of the effects of enkephalins is now known to be due to their high sensitivity to hydrolysis by aminopeptidases which are widely distributed in the central nervous system (Hambrook, Morgan, Rance & Smith, 1976; Meek, Yang & Costa, 1977). Rather, this rapid inactivation of the enkephalins, together with their rapid

onset of action, would favour the hypothesis that they are neurotransmitters since it would obviously be important to terminate the actions of such a substance rapidly. The prolonged action of β -endorphin might then favour a hormonal role for this peptide.

Current evidence suggests that the putative neurotransmitter role of the enkephalins is in the tonic, short-term control of release of other transmitters. Such a role would obviously have many applications. The areas of the central nervous system which are rich in enkephalins are closely associated with dopaminergic, noradrenergic, serotonergic and substance P-containing neuronal systems and have been implicated in sensory transmission, endocrine control, respiration, motor activity and behaviour. Direct evidence in support of this hypothesis of a general control function of enkephalins is accumulating. The enkephalins have been shown to inhibit the release of noradrenaline (Taube, Borowski, Endo & Starke, 1976) and acetylcholine (Subramanian *et al.*, 1977) from rat brain and of substance P from rat trigeminal nucleus (Jessel & Iversen, 1977). This role may also extend to the periphery since the stimulation-induced release of acetylcholine from the guinea-pig ileum and of noradrenaline from the mouse vas deferens is reduced by enkephalin (Waterfield, Smokcum, Hughes, Kosterlitz & Henderson, 1977). The presence of β -endorphin-like immunoreactive material in the midline pathway in the brain (Bloom *et al.*, 1978) suggests that this peptide may have a similar, if somewhat less widespread, control function. The role of β -endorphin in the pituitary has been the subject of more intense speculation.

β -Endorphin may itself be a pituitary hormone. Either β -endorphin or its putative precursor β -LPH is stored within secretory granules (Queen, Pinsky & La Bella, 1976; La Bella, Queen & Senyshyn, 1977) and plasma levels of β -endorphin increase in parallel with those of adrenocorticotrophin (ACTH) during stressful procedures in animals (Rossier *et al.*, 1977b; Guillemin *et al.*, 1977). Whether the release of β -endorphin and ACTH is generally controlled by similar mechanisms is not yet known, but it seems likely that their biosyntheses are closely associated. Recent evidence suggests that they share a common, high molecular weight precursor, "pro-opiocortin" (Mains, Eipper & Ling, 1977; Roberts & Herbert, 1977a, b; Rubinstein, Stein & Udenfriend, 1978). Also, pituitary levels of both increase after adrenalectomy and both increases are reduced by dexamethasone (Guillemin *et al.*, 1977).

Alternatively, or in addition, β -endorphin within the pituitary may play some role in the control of hypophysial hormone release or synthesis. This may be a direct action since opiate receptors are present in the pituitary (Simantov & Snyder, 1977), or may be exerted at the hypothalamic level since the possibility of retrograde transport of hypophysial hormones in the vasculature of the pituitary stalk has recently been demonstrated (Oliver, Mical & Porter, 1977; Bergland & Page, 1978).

Evidence for the control of pituitary function by opioid peptides

The possibility that the opioid peptides may play some part in the control of pituitary hormone release has been considered recently by many groups and many of the well-known effects of morphine and other opiates which pointed to actions on the pituitary are being closely re-examined and re-interpreted in the light of our knowledge of the possibility that such effects may have a physiological significance. In view of the diversity of techniques used, it is hardly surprising that conflicting results have been obtained, but in general the opioid peptides produce responses which are qualitatively similar to those with morphine and are reduced by opiate antagonists. β -Endorphin is usually more potent than the enkephalins, but with enkephalin analogues which are resistant to enzymic hydrolysis potencies comparable to that of β -endorphin are obtained.

Bisset, Chowdrey & Feldberg (1978) have observed an antidiuretic effect with leucine-enkephalin, D-Ala² D-Leu⁵-enkephalin and β -endorphin in water-loaded rats similar to that first

described with morphine in the dog (de Bodo, 1944). These authors showed that the response to leucine-enkephalin was reduced by naloxone and that for the stable enkephalin analogue at least, the antidiuresis was accompanied by an increased excretion of vasopressin-like material in the urine. Weitzman, Fisher, Minick, Ling & Guillemin (1977) found increased levels of arginine vasopressin in the blood of rabbits after intravenous injection of β -endorphin, but van Wimersma Greidanus *et al.* (1979) have claimed that in both normal and water-deprived rats morphine and β -endorphin produce decreases in plasma levels of arginine vasopressin with a corresponding increase in α -melanocyte stimulating hormone. Weitzman *et al.* (1977) concluded that the release of vasopressin they observed with β -endorphin was not due to a direct action on the pituitary since no release could be shown *in vitro* with isolated neural lobes. No information is currently available on possible effects of opiates and opioid peptides on oxytocin release. Clearly, a well controlled and comprehensive study is required before it can be determined whether enkephalins or β -endorphin are involved in the control of release of posterior pituitary hormones.

More information is available on the effects of opiates and opioid peptides on the release of anterior pituitary hormones. Most attention has been directed towards growth hormone (GH) and prolactin and there is general agreement that the enkephalins and various enkephalin analogues and β -endorphin, like morphine, produce increased release of both hormones and that these increases are inhibited by opiate antagonists, naloxone and naltrexone (see Cusan *et al.*, 1977; Rivier, Vale, Ling, Brown & Guillemin, 1977; Shaar, Frederickson, Dininger & Jackson, 1977). There is one report of release of prolactin from cultured pituitary cells by enkephalin (Lien, Fenichel, Garsky, Sarantakis & Grant, 1976) but more recent evidence contradicts this and suggests that the release of both GH and prolactin by morphine and opioid peptides is not due to a direct action on the pituitary (Rivier *et al.*, 1977; Shaar *et al.*, 1977). Grandison & Guidotti (1977) and Guidotti & Grandison (1978) concluded that endogenous opioids control the release of prolactin by inhibiting dopaminergic mechanisms in the mediobasal hypothalamus. Their evidence for this is: (1) that naltrexone administration reduces the serum levels of prolactin in intact rats and in rats with deafferentation of the hypothalamus; (2) that dopamine, but not naltrexone, reduces release of prolactin from pituitary halves *in vitro*; (3) that intrahypothalamic injection of naltrexone reduces, and of β -endorphin increases, serum prolactin levels; (4) that after dopamine receptor blockade by haloperidol or depletion of dopamine stores by reserpine, serum prolactin levels are increased and that these elevated levels are undiminished by naltrexone. The observation that administration of an opiate antagonist alone reduces the serum concentration of prolactin has been confirmed by other groups who also showed that GH was similarly affected (Bruni, van Vugt, Marshall & Meites, 1977; Shaar *et al.*, 1977). This is the most convincing evidence for a physiological role of endogenous opioid peptides in the tonic control of the release of GH and prolactin.

Similar evidence suggests that the release of luteinizing hormone (LH) may be under some form of control by enkephalins or β -endorphin in the mature male rat (Bruni *et al.*, 1977; Blank, Panerai & Friesen, 1979). In the female rat naloxone administration produces an increase in serum LH at various stages before puberty but not in the adult. Significantly, oestradiol administration abolishes the naloxone-induced increase in the prepubertal female (Blank *et al.*, 1979).

The results with follicle-stimulating hormone (FSH) are less clear. Morphine has been shown to block the preovulatory surge of FSH as well as of LH in the rat (Pang, Zimmerman & Sawyer, 1977) but Bruni *et al.* (1977) have reported that although naloxone does increase serum FSH levels in the male rat, morphine does not produce any significant decrease. Conversely, while morphine and methionine-enkephalin decreased levels of thyroid stimulating hormone, an action blocked by naloxone, naloxone alone did not increase serum levels of this hormone.

In conclusion, the possibility that opioid peptides are involved in the control of pituitary hormone release must now be considered in spite of the comparative paucity of well controlled and systematic studies. The evidence for such a role is best for GH, prolactin and LH, since

opiate antagonists decrease serum levels of GH and prolactin and increase levels of LH. It remains to be seen whether future studies can produce unequivocally firm evidence that the control of the release of other pituitary hormones is under similar control and whether this action is independent of the various hypothalamic hypophysiotrophic hormones and is generally due to interactions with hypothalamic aminergic neuronal mechanisms.

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