

Neurotensin

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Over the last decade there has been a great expansion of knowledge in the field of peptide hormones. Several of these peptides have been discovered by serendipity during the isolation of other peptide hormones, for example pancreatic polypeptide which was found during the purification of chicken insulin, and glucose-dependent insulin-releasing polypeptide, found as a contaminant of cholecystokinin. Neurotensin was discovered in a similar way during the isolation of substance P from bovine hypothalamus, when it was noticed that certain chromatographic fractions of the tissue extract had the ability to cause marked vasodilatation in the exposed cutaneous areas of anaesthetised rats and, in larger doses, to cause severe cyanosis. Subsequently, it was found that this vasodilatation was associated with a transient hypotension. The active constituent was isolated by Carraway and Leeman in 1973 and given the name neurotensin because of its presence in neural tissue and its ability to affect blood pressure.

After its purification, neurotensin was found to contain 13 amino-acids and to have a molecular weight of 1674. The amino-acid sequence (Fig.) was deduced from analysis of fragments generated by several proteolytic enzymes, as well as from information obtained on the intact peptide (Carraway and Leeman, 1975a).

It has been synthesised using the Merrifield solid phase procedure and the resultant peptide appeared to be identical with the native form, using multiple chemical and biological criteria (Carraway and Leeman, 1975b). There is still some uncertainty about the glutamic acid in position 4, as this may be formed from glutamine during the extraction procedure. The amino-acid sequence of neurotensin suggests a distant relationship with vasopressin and

LH-RH, perhaps reflecting a common ancestral peptide. The similarities to other hypothalamic and gastrointestinal peptides are less striking.

Tissue distribution of neurotensin

CENTRAL NERVOUS SYSTEM

After their initial discovery of neurotensin in bovine hypothalamus, Carraway and Leeman (1976a) developed a specific radioimmunoassay to measure it. Three antisera were raised against different parts of the peptide molecule, and gave very similar results in assays of neurotensin. The immunoreactive substances in acid-acetone extracts of bovine hypothalamus behaved similarly to synthetic neurotensin on gel chromatography and in their reaction with antibody.

Hypothalamic extracts from rat, guinea pig, and rabbit were found to contain similar concentrations of immunoreactive neurotensin, in the range of 45 to 70 pmol/g wet tissue. In the rat, extracts of various parts of the brain showed widely different concentrations, with the hypothalamus, and particularly the median eminence, containing the greatest quantity of neurotensin. High concentrations were also observed in certain components of the limbic system, but in the pons, medulla, and cerebellum the levels were low (Kobayashi *et al.*, 1977). Subcellular fractionation has shown neurotensin to be concentrated in synaptosomal and microsomal subcellular fractions (Uhl and Synder, 1976). ³H- and ¹²⁵I-labelled neurotensin was found to bind to brain cell membranes with high affinity and was only displaced by neurotensin. This specificity suggests interaction with particular neurotensin receptors. The membrane receptors' binding capacity parallels the anatomical distribution of neurotensin immunoreactivity (Kitabgi *et al.*, 1977). Neurotensin has been found in neuronal cell bodies and processes in the brain and in cells of the anterior pituitary by immunofluorescent techniques (Uhl *et al.*, 1977). The facts so far support a neurotransmitter role for brain neurotensin, but further criteria have still to be met before such a role can be proved.

Neurotensin Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ileu-Leu-OH.

Xenopsin Glu-Gly-Lys-Arg-Pro-Trp-Ileu-Leu-OH

Fig. *Amino-acid sequence of neurotensin and its amphibian counterpart, xenopsin*

Neurotensin

GASTROINTESTINAL TRACT

Immunoreactive neurotensin has also been found in tissues outside the central nervous system in several species. In the rat more than ten times as much neurotensin has been found in extracts of small intestine than in brain, the highest concentration being in the jejunoileal section where 76% is present in the mucosa (Carraway and Leeman, 1976b). Neurotensin extracted from bovine small intestine was found to be biologically and chemically identical with the peptide obtained from bovine hypothalamus (Kitabgi *et al.*, 1976). However, a large amount of neurotensin-like immunoreactivity has been extracted from rat stomach, but this was found to result from a smaller peptide, indicating the possible presence of either a related peptide or a breakdown product.

The location of neurotensin in canine ileum was shown by an immunofluorescent technique to be restricted to discrete endocrine cells in the mucosa (Orci *et al.*, 1976). These are most numerous in the ileal mucosa, and possess microvilli and secretory granules 300 nm in diameter grouped at the vascular pole. These observations have since been confirmed in many other species including man (Sundler *et al.*, 1977; Helmstaedter *et al.*, 1977b; Polak *et al.*, 1978). Embryological studies indicate that neurotensin cells are first identifiable in the ileal and jejunal mucosa in man as early as 12 to 13 weeks' gestation. In more mature embryos they are even more widely distributed than in the adult (Helmstaedter *et al.*, 1977a).

PLASMA

Carraway and Leeman (1976b) found neurotensin levels of about 50 pmol/l in bovine, rabbit, rat, and human plasma. This finding has been confirmed in man by Blackburn and Bloom (1979), who also showed that the substance in plasma, like that extracted from brain, behaved on a Sephadex G50 SF column and reacted with antibody similarly to the synthetic bovine peptide, suggesting considerable chemical similarity between the two species. Present information suggests fasting plasma levels in man to be of the order of 20 to 50 pmol/l. That the ileum is one source of the plasma neurotensin was suggested by Rosell *et al.* (1978) who found, in anaesthetised dogs, a large rise in plasma neurotensin immunoreactivity in the venous drainage from an ileal loop, the lumen of which was perfused with buffer solution.

Biological actions of neurotensin

ACTIONS ON THE VASCULAR SYSTEM

Neurotensin was initially discovered because of its ability to cause marked vasodilatation, and this

property was used in a bioassay during its initial separation from substance P. Since it is active when added to smooth muscle preparations *in vitro*, causing contraction of isolated guinea pig ileum and rat uterus and relaxation of rat duodenum, it can be classified as a kinin (Carraway and Leeman 1973). However, its ability to induce cyanosis in the anaesthetised rat is both striking and unique. This latter response is thought to be the result of peripheral stasis of blood because the partial pressures of oxygen and carbon dioxide in arterial blood are unchanged (Carraway and Leeman, 1973). At the same time there is also a marked increase in vascular permeability to proteins with consequent haemoconcentration. A rise in the haematocrit occurs within a few minutes after the intravenous injection of neurotensin in the anaesthetised rat, and probably reflects the movement of protein and water from the intravascular to the extravascular compartment.

Neurotensin is a potent hypotensive agent in the anaesthetised rat, the threshold intravenous dose being about 100 pmol/kg. The hypotensive effect exhibits acute tachyphylaxis, that is a second equal dose administered within 60 min of the first dose produces no effect, whereas a second dose given several hours later is effective. This activity is not significantly altered by adrenalectomy, hypophysectomy, or previous administration of atropine, phenoxybenzamine, or propranolol, and is therefore likely to represent a direct effect of neurotensin on the vasculature (Carraway and Leeman, 1973). Similarly the contraction of rat isolated gastric fundus strips caused by neurotensin could not be blocked by atropine, hexamethonium, methysergide, or morphine (Rökæus *et al.*, 1977). Moreover, the tachyphylaxis seen in the contractile response of guinea pig ileum did not inhibit responses to acetylcholine, histamine, serotonin, or DMPP, thus further supporting the view that neurotensin may act on a specific receptor site.

EFFECT ON BLOOD GLUCOSE, INSULIN, AND GLUCAGON

One of the most interesting actions of neurotensin originally found by Carraway and Leeman (1973) was its ability to produce a dose-dependent hyperglycaemia in fed, anaesthetised rats. The hyperglycaemia was associated with a fall in liver glycogen content and a seven-fold increase in liver glycogen phosphorylase A, and occurred, though to a small degree, even in hypophysectomised and adrenalectomised animals (Carraway *et al.*, 1973). Since neurotensin does not release glucose from liver slices directly, a mediator for its hyperglycaemic effect has been postulated, with pancreatic glucagon

as the most likely possibility. Brown and Vale (1976) injected neurotensin into anaesthetised rats and found within 5 min a rise in plasma glucagon and a fall in plasma insulin levels associated with hyperglycaemia. Somatostatin, which inhibits the release of glucagon and insulin from the pancreas, but has no direct effect on glucose production by the liver, abolished the neurotensin-induced hyperglycaemia, again suggesting that neurotensin is not acting directly on the liver. On a molar basis neurotensin was approximately 30 times more active in raising blood glucose than glucagon itself. Histamine produced similar effects on plasma glucagon and glucose in the rat and these responses to histamine and neurotensin were inhibited by the histamine H-1 receptor antagonist, diphenhydramine (Brown *et al.*, 1976).

Ukai *et al.* (1977), unlike Brown and Vale (1976), reported that neurotensin released insulin as well as glucagon. The controversy about the differential effect of neurotensin on glucagon and insulin release may in part be explained by differences in experimental animals or the anaesthesia used, and the degree of hyperglycaemia induced. The problem is unfortunately not resolved by work on isolated tissue preparations. Patton *et al.* (1976) found that neurotensin suppressed insulin release but increased glucagon release from isolated canine pancreas. Preliminary work on isolated rat islet tissue, however, indicates that neurotensin causes (1) at low glucose concentrations, rapid release of insulin, glucagon, and somatostatin, and (2) at high glucose concentrations, an inhibition of both insulin and somatostatin release (Dolais-Kitabgi, 1978). The apparent inconsistencies will presumably be resolved over the next few years but it is clear that neurotensin may well have an important role in glucose homeostasis and in the modulation of pancreatic endocrine functions.

EFFECT ON GASTRIC FUNCTION AND MOTILITY

An intravenous infusion of neurotensin in dogs was found to produce considerable inhibition of penta-gastrin-stimulated, but not of histamine-stimulated, gastric acid secretion. The dose of neurotensin was such that this effect was unlikely to be secondary to hyperglycaemia (Andersson *et al.*, 1976). Subsequently, it was found that neurotensin caused a rise in plasma gastrin levels, greatest in antral venous blood and independent of the pituitary (Ishida, 1977).

Neurotensin and the analogue Gln⁴-neurotensin have been found to have very similar biological actions *in vivo* and *in vitro*. Gln⁴-neurotensin was found to inhibit spontaneous motor activity in isolated gastric pouches in dogs (Andersson *et al.*,

1976). Vagally innervated antral pouches were more sensitive to this inhibitory effect than the vagally denervated fundic pouches. Doses as low as 6.3 ng/kg/min, lower than infusion rates which cause significant changes in blood pressure or hyperglycaemia, were effective. Neurotensin is found in large amounts in the small intestine, which raises the possibility that neurotensin may be involved in the enteric regulation of gastric secretion and motility.

EFFECT ON THE HYPOTHALAMIC-PITUITARY ENDOCRINE SYSTEM

Intravenous neurotensin has been shown to cause a rise in plasma glucocorticoids in the rat, which is abolished by previous hypophysectomy and significantly diminished by pretreatment with morphine (Carraway and Leeman, 1976a). The latter implies that neurotensin may be acting as a non-specific stress, only secondarily causing the release of endogenous corticotrophin-releasing factors. In rats, increased secretion of pituitary gonadotrophins (Makino *et al.*, 1973), growth hormone, and prolactin (Rivier *et al.*, 1977) has also been shown.

CENTRAL ACTIONS

In the rat, the injection of neurotensin directly into the CNS through an intracisternal cannula abolished the animal's normal cold adaptation reflexes and, in cold-exposed animals, resulted in hypothermia. This effect was dose-related and could not be produced by systemic administration of the peptide (Nemeroff *et al.*, 1977).

BIOLOGICALLY ACTIVE SITE

Analysis of the structural requirements for biological activity of neurotensin shows that the biologically active region lies primarily in the carboxy-terminal pentapeptide (Carraway and Leeman, 1975c). The partial sequences and analogues of neurotensin so far examined all display a similar potency for the known pharmacological actions of the peptide. It is interesting that the peptide xenopsin, isolated from the skin of the frog *Xenopus laevis*, has a structure similar to the carboxy-terminal octapeptide of neurotensin (Fig.) and, although it lacks a corresponding amino terminal segment, possesses many of its biological properties (Araki *et al.*, 1973).

Physiological role

The possible physiological role of neurotensin in man is unknown and can only be speculative at present. Its presence in both the brain and the gastrointestinal tract supports the hypothesis that peptide-producing cells of the APUD series are derived from specialised ectoderm and programmed for ultimate neuroendocrine function (Pearse, 1976).

That neurotensin may have a neurotransmitter or neuromodulator role in the CNS is suggested by its distribution in synaptosomal fractions which appear to contain receptor-like structures, and by its presence in neuronal cell bodies and synaptosomes in many regions of the brain. Further support comes from the release of neurotensin from slices of rat hypothalamus by depolarising concentrations of potassium in the medium, the release also being calcium-dependent (Iversen *et al.*, 1978). The micro-iontophoretic application of neurotensin was found to produce an inhibition of the regular discharge pattern of cells in the locus coeruleus of rat brain (Young *et al.*, 1978). This action accords with the presence locally of neurotensin immunoreactivity and functional neurotensin receptors, and suggests a neurotransmitter role.

The possibility of a role in postdigestive physiology is also speculative, though observations in pathological states are suggestive. It has recently been found that there is a small, but statistically significant, rise in plasma neurotensin-like immunoreactivity in man after both an oral glucose load and a normal mixed meal (Blackburn *et al.*, 1978; Blackburn and Bloom, 1979). In view of this and of the known vasoactive properties of the peptide, changes in plasma neurotensin were studied in 19 patients with the dumping syndrome. Two control groups comprising 20 symptom-free, age and sex matched postoperative subjects and 20 preoperative peptic ulcer patients, respectively, were also studied as controls. The patients with dumping symptoms were clearly distinguished from the two control groups not only by a large rise in pulse rate and haematocrit after oral glucose, but also by a simultaneous rise in plasma neurotensin immunoreactivity which was five times greater than that seen in the controls (Blackburn *et al.*, 1978). The significance of this is unknown and the pathophysiology of the dumping syndrome remains ill understood. Other putative peptide hormones are known to be raised in dumping, for example enteroglucagon (Thomson and Bloom, 1976), and clearly a number of possible aetiological factors are present which may all contribute to the symptomatology of this condition.

Another pathological situation where a large rise in plasma neurotensin has been found is in patients who have undergone jejunoileal bypass for morbid obesity. The effect of a standard breakfast was studied in 19 patients with morbid obesity ($225 \pm 7\%$ ideal weight), 21 patients who had undergone jejunoileal bypass with subsequent weight loss ($181 \pm 8\%$ ideal weight), and 16 age and sex matched normal controls ($106 \pm 3\%$ ideal weight). The patients with morbid obesity had an augmented

insulin response reflecting the exaggerated glucose release, but no significant difference in neurotensin release compared to normal subjects. However, in patients who had undergone jejunoileal bypass, insulin release was dramatically diminished, while that of neurotensin was increased eight-fold (Besterman *et al.*, 1978). In view of the effect of neurotensin on insulin release in experimental animals, it is tempting to speculate about the significance of this finding. In both pathological situations, neurotensin may be involved in controlling disordered motility of the bowel and in glucose homeostasis. Alternatively, the high levels found may simply be a reflection of the rapid transit of food to the neurotensin-rich terminal ileum, and thus be a secondary phenomenon in both instances.

For the present, it is impossible to extrapolate from the variety of known biological and probable pharmacological actions of neurotensin in experimental animals to a precise physiological role of this peptide in man. However, it seems likely that neurotensin may have both a neuroregulatory role and a more classical endocrine role concerned with postdigestive physiology. It is to be expected that many of the current questions posed concerning the role of neurotensin will be answered within the next few years.

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