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Three tachykinins in mammalian brain

Two polypeptide precursors to the neuropeptide substance P have recently been identified. One of them (β-protoparathymin) contains amino acid sequences corresponding not only to substance P but also to substance K, a novel, related peptide. A third substance P-like peptide, neuromedin K, has recently been isolated from spinal cord. The existence in vertebrates of three members of the tachykinin family of peptides may account for pharmacological observations suggesting the presence of more than one type of substance P receptor in the nervous system.

The tachykinin family of peptides (Fig. 1) is characterized by the common amino acid sequence- Phe-X-Gly-Leu-Met-NH₂ at the C terminus, where X is a hydrophobic or aromatic residue. Substance P, the best-known member of the family, was until recently the only tachykinin known to occur in mammals, all the other tachykinins having been isolated from octopod salivary glands or from amphibian skin. However, a number of lines of evidence suggested that there might be other tachykinins present in the vertebrate nervous system.

Using a variety of antisera to the amphibian peptide physalaemin, Lazarus and colleagues were able to demonstrate the widespread occurrence in mammals with physalaemin-like immunoreactivity. Immunological, chemical and pharmacological analysis of the physalaemin-like material in a small-cell carcinoma from human lung demonstrated that it was similar or identical in its properties to amphibian physalaemin. Keen and colleagues demonstrated the biosynthesis in dorsal root ganglia of substance P together with a related peptide which was immunoprecipitated by C-terminal but not by N-terminal substance P antisera. Analysis of the incorporation of different radiolabelled amino acids into the novel peptide indicated that it differed from substance P in containing no proline residues and in containing the amino acids phenylalanine and methionine in the ratio 1/1 instead of 2/1.

Evidence for the existence of novel tachykinins in mammalian tissue was complemented by evidence for the presence of more than one type of tachykinin receptor in vertebrates. This concept, first suggested by Erspamer and colleagues who defined the responses of a variety of pharmacological test systems in terms of two types of tachykinin receptor. All the tachykinins display approximately equal potency at one type of receptor (the SP-P receptor), whereas the second type of receptor (the SP-E receptor) is considerably more sensitive to eleidoin and kassinin than to the other tachykinins. Searching for an endogenous ligand to the SP-E receptor, Maggio and colleagues developed a radioimmunoassay for kassinin, the most potent ligand at SP-E receptors. They found that extracts of spinal cord contained abundant kassinin-like immunoreactivity which they attributed to a novel peptide. This peptide they named 'substance K' to indicate its apparent immunochmical similarity to kassinin.

Kimura et al. were at this time searching for novel neuropeptides in porcine spinal cord, using as an assay their activity on the isolated guinea-pig ileum. Four fractions exhibiting ileum-contracting activity were identified by Sephadex G-15 chromatography: two of the fractions contained substance P and its oxidized derivative; the remaining

Viruses and the pathogenesis of multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease which produces primary demyelination of the CNS. The diagnosis of MS is based on clinical criteria and supported by adjunctive laboratory measurements. Both environmental and host influences have a role in the disease. It has a very uneven distribution, with high prevalence (30 to 80 cases per 100,000) in the temperate zones of North America and Europe and diminishing prevalence in southern latitudes. Geographical clustering and apparent epidemics of MS have also been reported.

Based on studies of migration from high- to low-prevalence areas, there is evidence for exposure to an environmental agent in childhood or early adolescence years before MS becomes clinically manifest in adult life. In addition to this environmental influence there is evidence of a host susceptibility factor, since the prevalence of MS is increased approximately 12-20-fold among individuals with primary relatives who have MS, and certain HLA types (antigens encoded in the major histocompatibility complex on chromosome 6) are observed in increased frequency among persons with MS. Patients exhibit an enhanced, or perhaps an unsuppressed, in situ immune response within the CNS with excessive synthesis and secretion of antibody; however, the major portion of antibody present has an unknown specificity.

Attempts to unify these observations and other results of a variety of investigational activities have not yet led to the identification of an etiologic agent or pathogenic mechanism for MS. Given
Physalaemin
Substance P
Kassinin
Neurokinin α (Substance K)
Neurokinin β (Neuromedin K)
Eledoisin

Fig. 1. Structures of the tachykinins referred to in this article showing sequence homologies.

two fractions contained novel peptides, ‘neurokinin α’ and ‘neurokinin β’, which were isolated and sequenced. Neurokinin β proved to be identical in structure to neuromedin K, a spinal cord peptide isolated independently by Kangawa et al. using similar methodologies. Neurokinin α proved to be the same as the substance K first described by Maggio et al., and almost certainly corresponds to the substance P-like peptide first described by Keen et al.

At about this time, Nawa et al. conquered one of the great challenges in substance P research by elucidating the nucleotide sequences of cloned cDNAs for two types of bovine substance P precursor.

The techniques used were similar to those which have been successfully employed to identify precursors to a growing number of neuropeptides and hormones. Briefly, mRNA from bovine striatum (a region of the brain especially rich in substance P-synthesizing cell bodies) was isolated and transcribed into cDNA. The cDNA was used to generate a ‘library’ of bacterial clones containing plasmid DNA into which sequences complementary to striatal mRNA had been inserted. Clones containing substance P-related nucleotide sequences were recognized by the ability of their DNA to hybridize with synthetic DNA probes (oligonucleotides with a sequence predicted by the amino acid sequence of the substance P molecule). A number of positive clones were identified and their cDNA inserts sequenced; two precursor molecules to substance P – α- and β-preprotachrhynin (α- and β-PPT) – were identified.

The structures of α- and β-PPT are illustrated schematically in Fig. 2. α- and β-PPT are identical, except that a 54-nucleotide segment of the cDNA sequence of β-PPT is missing in α-PPT. Thus, β-PPT is a polypeptide of 130 amino acids (mol. wt 15 076), whereas α-PPT lacks 18 consecutive amino acids present in the β-PPT sequence and has a methionine residue in place of the arginine residue at the boundary of the deleted region. The 19-amino acid sequence unique to β-PPT contains the sequence of the substance K molecule. The other structural features of the two PPT molecules are similar to those found in the sequences of other peptide precursors. Like all other polypeptides

Fig. 2. Schematic representation of the structures of α- and β-preprotachrhynin (α- and β-PPT), showing the locations of substance K, substance P and the putative signal peptide within the two polypeptides. The region of β-PPT which is deleted in the α-PPT sequence is indicated by dashed lines. Basic amino acids corresponding to probable processing sites are shown. ‘Amide’ represents the sites of amidation for substance P and substance K. (Modified from Ref. 9, with permission.)
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... destined for secretion from the cell, the α- and β-PPT sequences begin with a 15-30 amino acid 'signal sequence' (largely composed of hydrophobic amino acids), which promotes attachment of the newly synthesized polypeptide to the endoplasmic reticulum and its secretion into the intracellular space. This sequence is cleaved off by a membrane-bound protease during or immediately after synthesis: the exact length of the signal sequence in the PPT molecule has not yet been determined. Immediately preceding the sequence of substance K in the precursor is a pair of basic amino acids (Lys-Arg). A trypsin-like protease is thought to cleave the precursor after the second basic residue to liberate the N terminus of the substance K molecule. The N terminus of the substance P sequence (Arg-Pro) is preceded in the precursor by a single basic amino acid (arginine). Although the double basic amino acid 'signal' for proteolytic processing is again present, the Arg-Pro bond is selectively resistant to trypsin-like proteases and instead the cleavage occurs between the two arginine residues. Immediately following the sequences of substance P and substance K is the sequence Gly-Lys-Arg - a pair of basic amino acids indicating a site of proteolytic processing, preceded by a glycine residue which is thought to be the donor of the amide group found at the C terminus of both peptides. Other biologically active peptides may be liberated from the PPT sequences. In particular the sequence Arg-Arg-Arg-Lys at the C terminus of both precursors could be a processing site, liberating peptides corresponding to residues 72-108 of α-PPT and residues 111-126 of β-PPT. These peptides may possess some, as yet undefined, biological activity.

The presence of two precursors for substance P in brain has exciting implications. The two PPT molecules may arise from two closely similar but separate genes. Alternatively they may arise from a single gene by alternative splicing events. The vast majority of eukaryotic genes consist of precisely defined regions coding for sections of mature mRNA (exons) interspersed with regions (introns) which are either deleted during the synthesis of mRNA precursors or 'spliced' out of the mRNA sequence during its maturation. The portion of the β-PPT mRNA that is missing from α-PPT may correspond to a single exon, and both precursor mRNAs may be generated from a common gene product by different splicing events. A similar variable splicing mechanism enables a single gene (the calcitonin gene) to code for different products (calcitonin and calcitonin-related peptide) in the thyroid and hypothalamus, respectively. The origin of the two PPT molecules will finally be understood (no doubt very soon) when the structure of the PPT gene(s) is established.

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...these data and the absence of a consistent abnormality in all patients with MS, a combination of influences and factors has been postulated. The most common hypothesis is that the patient with MS is genetically susceptible in some manner, possibly by way of an immunogenetic abnormality related to the major histocompatibility complex, and that a particular environmental exposure, presumably through an infectious agent such as a virus, leads to the destruction of CNS myelin. However, explanations for recurrences, unpredictable progression and marked variations in intensity of the disease have not yet been approached by these theories.

There are few chronic neurological diseases of humans which may be convincingly demonstrated to result from virus infection. These include progressive multifocal leukoencephalopathy due to a papovavirus, subacute sclerosing panencephalitis resulting from rubella, progressive rubella panencephalitis, and several post-infectious encephalomyelitides. In none of these is the pathology exactly that of MS, nor have any of these agents been identified in MS tissue. In fact the most current techniques of virus isolation or recognition of virus genomic material have not shown positive or specific results in MS tissue. It is true that a number of claims have been made about isolation of viruses from MS tissue, but in no case have these claims been confirmed.

Animals afford a more controllable situation in which to study CNS myelin injury, and several diseases have been popular models for studies of both viral causes and autoimmunity as they might relate to MS. One of these is experimental allergic encephalomyelitis (EAE), an autoimmune response to a component of CNS myelin called myelin basic protein, injected with complete Freund's adjuvant which appears to be an essential but not understood facilitator. Myelin basic protein, which has a monomeric molecular weight of 18 500 and comprises approximately 30% of CNS myelin proteins, has been extensively studied because of this role in provoking EAE. Susceptibility to EAE varies among different animal species and strains. In rodents, the Lewis rat is the most susceptible. Cellular immunity against myelin basic protein plays the primary role in the immunopathogenesis of acute EAE. Thus, EAE may be passively transferred to unimmunized recipients with mononuclear cells from lymphoid tissue of sensitized animals.
Exposure of cells from sensitized animals to myelin basic protein in vitro enhances the disease-producing effects of the cells transferred from donor to recipient. The acute form of EAE differs from MS in being more intense, usually monophasic, and pathologically showing more CNS inflammation than demyelination. However, there are now several disease models which permit the induction of a subacute or chronic form of EAE which resembles the clinical course of MS more closely, exhibiting periods of exacerbation and remission, and which also exhibits pathology more similar to that of MS. At least in the guinea-pig, the age of the animal is important for the development of chronic, as opposed to acute, EAE; juvenile guinea-pigs are the most susceptible to induction of the chronic form.

Viral diseases of animals which have been studied as possible models of MS include visna in sheep, canine distemper, Thielers's murine encephalomyelitis, and CNS infections produced by the JHM strain of mouse hepatitis virus. In particular, the latter two examples have recently been studied in depth because they offer models which allow close scrutiny. Thielers's murine encephalomyelitis virus, a picornavirus, infects oligodendrocytes which then bear the virus antigen and serve as targets for a host immune response against the affected cells. The JHM strain of mouse hepatitis virus is a coronavirus which selectively destroys oligodendrocytes in its natural host, the mouse. The CNS effects of the JHM strain have recently been given a fresh look by ter Meulen and co-workers, who have provided evidence of subacute demyelination in a fraction of 4-5-week-old Lewis rats inoculated intracranially with the virus. A prominent feature of this demyelination was a perivascular mononuclear cell infiltration which resembled in some respects the pathology of EAE. Following this lead, it was then shown that lymphocytes from these animals had been sensitized to myelin basic protein. Furthermore, if these lymphocytes were exposed to myelin basic protein in vitro, they acquired the ability to transfer subacute demyelination passively into recipients. Control studies indicate that virus or myelin basic protein alone is neither being transferred nor causative in this passively transferred disease. Thus, this model provides preliminary information that a protracted virus infection of an unnatural and immature host may lead to subsequent demyelination and sensitization to a myelin component. It is tempting to speculate that the infection by J11M virus generates an autoimmune response against myelin basic protein, an immunogenic product of the oligodendrocyte, because the virus can manage only an abortive or chronic infection in a young unnatural host unable to support a full-blown, acute infection.

The availability of this model should be very useful in further assessing the events intervening between a virus infection and the appearance of inflammatory demyelination. Whether this will provide incisive clues as to the etiology of MS remains to be seen, but at least it ties together two events that have been considered to be causally related to the pathology seen in MS. Identification of the portion of the myelin basic protein molecule which contains the immunogenic determinants for the JHM-infected animals, whether these animals are sensitized to other myelin proteins more important in the pathogenesis of demyelination, whether humoral factors as well as cellular factors may be involved, and whether the age of the animal or other temporal events influence the intensity and course of the disease may provide important new insights into the cause of MS.