Neurotransmitters, Drugs and Brain Function.
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12 Peptides

A. H. DICKENSON

INTRODUCTION

The status of amino acids such as glutamate and GABA as neurotransmitters is well established and widely accepted. However, when peptides are being considered as transmitters, views tend to be more diverse. The definition of a peptide is a chain of amino acids which does not exceed 30 amino acids in length, the arbitrary cut-off before the molecule becomes a protein, which is too bulky to be stored, released and interact with a receptor molecule. One problem with considering peptides as transmitters is that many of the peptides active in the CNS have additional roles elsewhere in the body such as somatostatin controlling insulin and glucagon release and substance P and bradykinin acting on the vasculature. Nevertheless, it is clear that signalling molecules can have roles in many places in the body so there is no reason why a transmitter substance can act as a hormone via the vasculature on a distant site as well as at closer range when released from a nerve terminal to act on an adjacent neuron.

The realisation that peptides can function as neurotransmitters has increased the number of NTs in the brain by at least 20! The increasing number of synthetic agonists and antagonists for the peptide receptors means that function can now be probed and novel therapeutic targets are achieved. It cannot be ignored that the therapeutic effects of morphine and its antagonist naloxone arise from an ability to act on a receptor that is there for the functional effects of endogenous opioid peptide systems. This chapter will consider the life history of a peptide transmitter, comparing it to the classical transmitters such as the excitatory and inhibitory amino acids, acetylcholine and the monoamines and then briefly review the main groups of peptides and their receptors and some of the possible functional aspects of peptides in the CNS. En route, the principle of co-existence that started with the finding of a peptide co-habiting with acetylcholine in parasympathetic neurons will be extended to the CNS — the old 'rule' that a neuron made and used a single transmitter is unlikely to be the case for many brain and spinal cord neurons.

NEUROCHEMISTRY

PEPTIDES — PRODUCTION AND RELEASE

The release (calcium-dependent) and receptor actions of peptides resemble those of the 'classical' transmitters, the receptors being seven transmembrane-spanning receptors

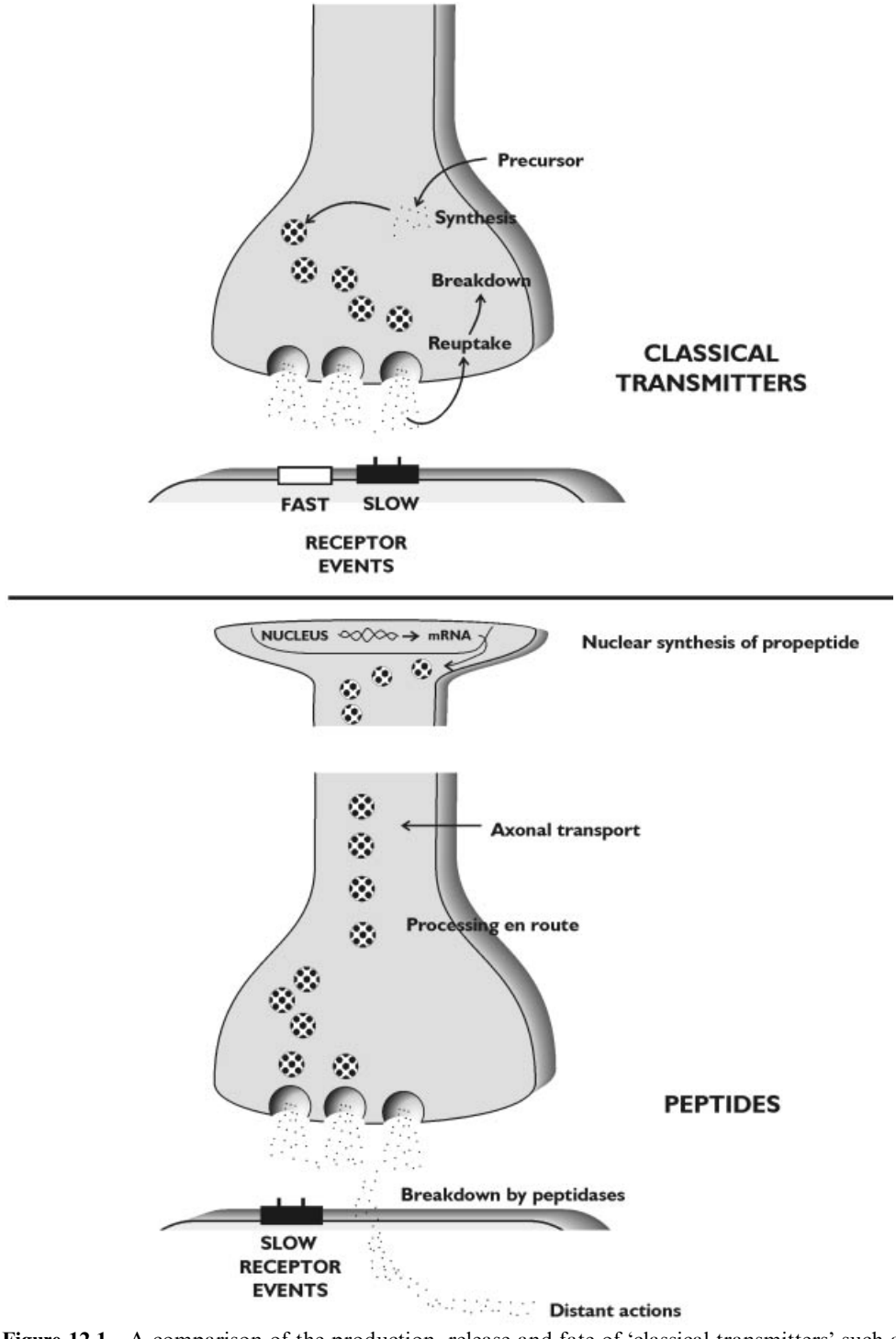

Figure 12.1 A comparison of the production, release and fate of 'classical transmitters' such as glutamate and the monoamines and a peptide. (Most neurons use both classical and peptide transmitters)

coupled to second messengers. As yet there is no evidence that a peptide acts on a receptor that is ionotropic — although it may yet turn out that a peptide receptor can couple to an ion channel, the data points to peptides acting as 'slow' transmitters.

A major difference between the 'classical' transmitters and peptides is that the production of a peptide is quite different since the synthesis of a peptide is in the form of a huge precursor of about 300 amino acids which is produced in the nucleus of the cell and then transported to the terminal being processed en route (Fig. 12.1). The prepropeptide is produced by translation in ribosomes and so occurs only in cell bodies or dendrites while the 'classical transmitters' are produced at the terminal via a short series of enzymatic steps from a simple precursor. The study of the production of the propeptides have revealed a series of principles in that:

- Some propeptides lead to the production of different, in terms of receptor affinities, peptides (substance P and neurokinin A act on neurokinin 1 and 2 receptors, respectively).
- Some propeptides produce multiple copies of similar peptides (met-enkephalin and leu-enkephalin act on the same delta opioid receptor).

The whole process of production of a peptide is sluggish simply because the size of the precursor is so great. Once produced the precursor is packaged into vesicles and then transported down the axon to the terminal. Axonal transport is generally a slow process in that mm–cm/day is rarely exceeded. Thus in a long axon the arrival of the peptide at the release site at the terminal will not be quick. While the precursor is being transported it is processed further by peptidases within the vesicles that cleave the larger parent molecule into smaller fragments. This process continues until the active peptide(s) is produced.

It is easy to speculate that in an active neuron with a rapid firing pattern, the continued release of a peptide may eventually lead to depletion of the peptide occurring. This has been shown in the peripheral nervous system. If this also happens in the CNS it would provide a mechanism whereby the release and resultant receptor effects of a transmitter no longer match the firing pattern and demands of the neuron and so could contribute to long-term adaptations of neurons by a reduction in the time over which a peptide is effective.

The release of some peptides may differ from that of other transmitters, depending on the firing rate of the neurons. The large vesicles needed to store a peptide may need a greater rate of depolarisation for membrane fusion and release of the contents. In the salivary gland the release of vasoactive intestinal polypeptide requires high-frequency stimulation whereas acetylcholine is released by all stimuli. Due to the complexities and problems of access to CNS synapses it is not known if the same occurs here but there is no reason why this should not. In sensory C-fibres a prolonged stimulus appears to be a prerequisite for the release of substance P.

BREAKDOWN

A peptide, once released, is not subject to reuptake like most transmitters, but is broken down by membrane peptidases. There are no known peptide transporters so that reuptake and re-use are not likely. The peptidases are predominantly membrane bound at the synapse and many are metalloproteases in that they have a metal moiety, most often zinc, near the active site. These enzymes are generally selective for particular

amino-acid sequences so that one peptidase may cleave a number of peptides if the amino-acid sequences overlap. A number of peptidases are found in the vasculature, including aminopeptidases and angiotensin-converting enzyme and any peptide with an acidic amino acid near the amino-terminal end of the peptide will be degraded after systemic administration.

At a central synapse, the termination of action of a peptide relies on these peptidases. Thus, if there is considerable release at any one time, the peptide may saturate the enzyme(s) and so metabolism will not keep pace with release.

Thus the peptide could escape the synapse where it was released and then diffuse through the tissue. The peptide may then act at sites distant from the neuron that released it, and these sites will be determined simply by receptors for the particular peptide. Consequently, volume transmission or non-synaptic effects may be important. This has been shown for lutenising hormone releasing factor (LHRH) in sympathetic ganglion cells where the peptide can act on neurons over distances of many hundreds of microns. In the CNS, the areas of spinal cord where neurokinins can be detected increase markedly when a prolonged intense peripheral stimulus is applied, suggesting saturation of peptidases allowing the intact transmitter to move through the tissue. Finally, one of the neurokinins, substance P, is found with calcitonin gene-related peptide (CGRP) in sensory neurons that terminate in the spinal cord. These peptides potentiate each other's actions by competing for the same peptidase. When CGRP occupies the active site the actions of substance P are greater since there is less degradation.

MANIPULATION OF ENDOGENOUS PEPTIDES

The only approach so far has been centred on the opioids. The enkephalins are rapidly degraded by membrane-bound peptidases. The synthesis of peptidase inhibitors has been a successful strategy so that kelatorphan, a mixed peptidase inhibitor, inhibiting at least two of the important breakdown enzymes (aminopeptidase N/M and neutral endopeptidase) affords almost complete protection to the enkephalins. The spinal application of the inhibitor produces a reduction of nociceptive responses of cells with the pool of enkephalins protected by the inhibitor likely to be derived from both a segmental release and from descending pathways activated by the stimulus. The recent reports of a systemically active mixed peptidase inhibitor, RB101, is the next stage towards the clinical application of this novel approach to pain relief. Interestingly, the side-effect profile of RB101 appears to be unlike that of morphine in that it does not cause physical and psychological dependence, suggesting that the receptor activation produced by endogenous opioids is unlike that of exogenous drugs. This is to be expected as the peptide transmitter release and consequent receptor activation will only occur following physiological events — the inhibitors only act when the peptide is released. The potential problems with this approach are that of the two enzymes involved (aminopeptidase N/M and neutral endopeptidase), neither is selective for the enkephalins and inhibition of the former could also increase levels of angiotensin, endothelin, CCK and substance P, among others.

PLASTICITY IN PEPTIDES

The processing of the peptide en route from the cell body to the terminal can result in different products being released from the same gene product. Since the precursor is

Table 12.1

	Inflammation	Neuropathy
Glutamate levels	Normal	Normal
Substance P	Upregulation	Downregulation
CCK	Downregulation	Upregulation
Neuropeptide Y	Normal	Upregulation
Galanin	Normal	Upregulation

produced in the nucleus, gene induction and suppression can change the peptide content of neurons. Nerve growth factor appears important in this, as does NMDA-induced calcium influx. There is much evidence for an induction of early onset protoncogenes in neurons elicited by neuronal activity and *c-fos* and *c-jun* are protein markers of these events. When a gene is switched on or off after neuronal activity then some peptides will always be present in neuronal systems and others appear as a result of damage and/or dysfunction to neurons. Thus the pharmacology of a neuron will change as a consequence of pathological changes. This is best illustrated by consideration of sensory C fibres after peripheral inflammation or nerve damage, two conditions that commonly contribute to pain in patients (Table 12.1).

CO-EXISTENCE OF PEPTIDES WITH OTHER TRANSMITTERS

The nature of a peptide makes it easy to raise specific antibodies to amino-acid sequences unique to that particular peptide. On this basis peptides can be mapped accurately in neuronal tissues. When this type of analysis was applied to autonomic neurons, there was hardly a neuron that did not contain a peptide in addition to noradrenaline or acetylcholine. As the peripheral nervous system is easily accessible and end-points are simple to measure (salivation, blood pressure, etc.) the functional consequences of this co-existence have been demonstrated. Thus, VIP and acetylcholine interact to produce the full integrated salivation and vasodilatation that occur following stimulation of the parasympathetic supply to the salivary glands. Stimulation of the nerve causes secretion and vasodilatation — the former is muscarinic since it is blocked by atropine. When administered exogenously the same pattern is seen; ACh causes secretion and VIP dilates. When co-applied, both secretion and vasodilatation are potentiated and this may partly be due to VIP increasing muscarinic binding of ACh, an effect that has also been seen in the cortex. Noradrenaline and neuropeptide Y also have similar interactive effects on the vasculature. Thus, the vasoconstriction produced by both sympathetic stimulation and exogenous noradrenaline is enhanced in the presence of NPY. Co-existence of more than one neurotransmitter in a neuron is likely to be common in CNS neurons but only a small proportion of the billions of neurons have been investigated.

The consequences of co-existence are considerable since the two or more transmitters can:

- Synergise postsynaptically
- Oppose postsynaptically
- Alter release
- Alter breakdown

The combinations of co-existence within a single neuron that have been seen in the CNS are:

(1) Different peptides from the same gene product (met and leu enkephalin, substance P and neurokinin A). The former two act on the same receptor, the delta opioid receptor, whereas the latter act on different receptors, the neurokinin 1 and 2 receptors. Despite this, the receptors for the neurokinins produce the same direction of effect, a slow depolarisation, even though their distribution differs.
(2) Two different peptides (TRH and enkephalin, substance P and CCK) coming from different precursors. These combinations are intriguing in that TRH is excitatory yet enkephalins are inhibitory — complex postsynaptic effects can be envisaged. Substance P is excitatory and CCK acts on two receptors, A and B, with the former being the predominant CNS form.
(3) Co-existence of one or more peptides with a non-peptide transmitter within a single neuron. For example, dopamine and CCK are found together in some but not all of the monoamine neurons and there is a complex interaction involving mutual control of release. This could have implications for therapy for Parkinson's disease, psychoses, addiction, etc., all areas where dopamine is manipulated while the co-existing peptide has, as yet, not been attacked. In another instance, 5-HT and substance P can be found together in supraspinal pathways projecting to the spinal cord. Here the inhibitions produced by activation of these pathways are due to 5-HT yet drug-induced depletion of the monoamine leads to excitatory effects on sensory neurons that are likely to be due to the peptide. Furthermore, as in the periphery, noradrenaline and neuropeptide Y co-exist in some neurons and in C-fibres glutamate and substance P are found together — in this case, 90% of substance P-containing cells have glutamate alongside. Interestingly, as described in Chapter 10, the long slow peptide depolarisations elicted on release of the peptide allow glutamate to activate the NMDA receptor by removal of the Mg^{2+} block. Here substance P plays a permissive role determining which receptor(s) the amino acid can activate.
(4) Different non-peptides, e.g. GABA and 5-HT, are found in some of the descending monoamine neurons forming bulbospinal pathways, as well as substance P and enkephalin. This case of four peptides is the most seen thus far and it suffices to say that in theory, the target neuron could be exposed to information via two fast ionotropic receptors ($GABA_A$ and $5\text{-}HT_3$) and a mix of slow inhibitory and excitatory effects via the remaining 5-HT receptors, the NK1 receptor, the $GABA_B$ receptor and the delta opioid receptor.

Figure 12.2 shows some of the ways in which the co-existence of peptides with classical transmitters can interact at a synapse.

FUNCTION OF PEPTIDES

The peptides will now be considered individually in some detail. It must be noted that the large molecular size of the peptides means that they are even less likely to cross the blood–brain barrier than classical transmitters and the instability of peptides means that full functional studies require non-peptide agonists and antagonists. Whereas nature has provided morphine and medicinal chemists have made naloxone, tools are lacking for many other peptides.

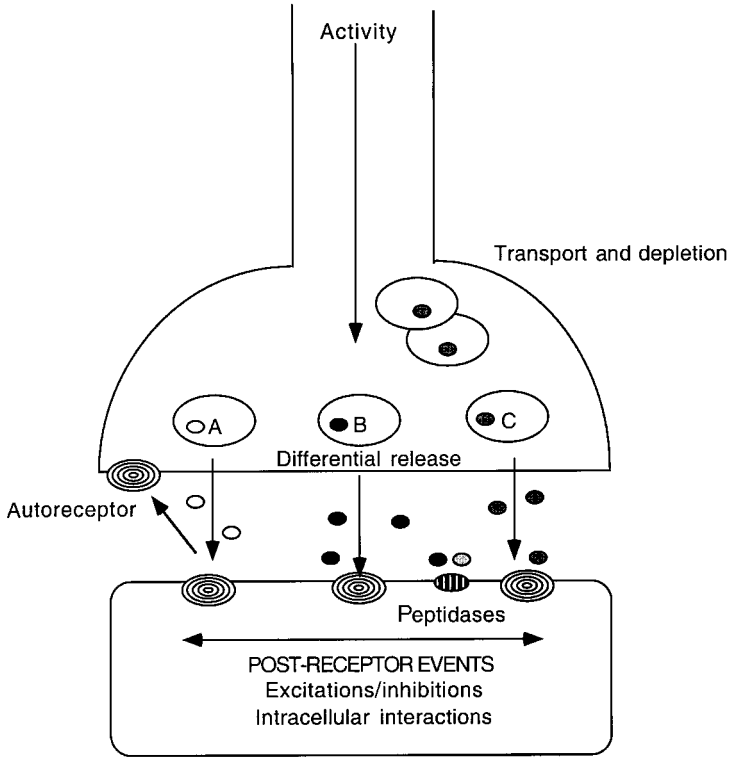

Figure 12.2 A hypothetical synapse where co-existence of peptides and 'classical transmitters' occurs. A is a 'classical transmitter' whereas B and C are peptides. The slow synthesis of peptides and the need for axonal transport may mean that in active neurons, the 'classical transmitter' may be released under all conditions, but the peptide(s) may require higher intensities of stimulation for release and be depleted if the neuron continues to fire for long periods. Competition for peptidases can lead to changes in levels of two co-released peptides. At the postsynaptic site, the receptor mechanisms of the co-existing transmitters can also produce complex changes in neuronal activity

OPIOID PEPTIDES

Despite the use of opium for thousands of years, it was only in the 1970s that the existence of opioid receptors became a reality and subsequently endogenous opioids were identified soon after as the transmitters at these receptors. To date, four receptors have been identified, the mu, delta, kappa and ORL-1 receptors. In 1992 the amino-acid sequence of the delta-opioid receptor was determined by expression cloning, and based on the expected homology to the cloned receptor, the mu and kappa receptors were also cloned. This method was extended to search for novel members of the opioid receptor gene family, and the cDNA encoding of a previously unrecognised receptor protein — the orphan opioid receptor was identified in humans, rats and mice. This new receptor, the hORL1 (human Opioid Receptor-Like 1), exhibited substantial sequence identities with opioid receptors and, once stably transfected into cells, mediated inhibition of adenylyl cyclase. The effect was abolished by diprenorphine, an opiate antagonist. However, since naloxone, the universal opioid antagonist, has low affinity

for this receptor, previous functional studies that have used this, or indeed other selective antagonists, to probe opioid function have probably failed to manipulate the nociceptin/orphanin FQ system. The functional roles of nociceptin/orphanin FQ thus remain somewhat elusive and contradictory at present, yet much is known about the other opioid peptides since there are a plethora of selective agonists and antagonists at the receptors.

Mechanisms of action

The mu, delta and kappa opioid receptors are coupled to G^o and G^i proteins and the inhibitory actions of the opioids occur from the closing of calcium channels (in the case of the κ receptor) and the opening of potassium channels (for μ, δ and ORL-1). These actions result in either reductions in transmitter release or depression of neuronal excitability depending on the pre- or postsynaptic location of the receptors. Excitatory effects can also occur via indirect mechanisms such as disinhibition, which have been reported in the substantia gelatinosa and the hippocampus. Here, the activation of opioid receptors on GABA neurons results in removal of GABA-mediated inhibition and so leads to facilitation.

The four opioid receptors display *in vivo* binding preference for mu-endorphins and endomorphins, delta-enkephalins, kappa-dynorphin and ORL1-nociceptin/orphanin FQ (Table 12.2).

These peptides are not completely selective for each type of receptor since the opioid peptides show a degree of sequence homology, although modified synthetic agonists are more selective. Investigation of mu receptor-mediated controls has been hampered by the lack of an endogenous ligand for the receptor in many areas, and in particular, within the spinal cord. Very recently, two peptides (endomorphin-1 and -2) have been isolated with high affinity and selectivity for μ-opioid receptors, making it likely that they are the natural endogenous ligands for the receptor for morphine itself. Table 12.3 summarises the endogenous and synthetic agonists and the antagonists of the four opioid receptors.

Morphine acts on the mu receptor, and so do most of the clinically used opioid drugs. The detailed structure of these receptors has been described and we now have a reasonable understanding of their relative roles in physiological functions and in different pain states.

The best-understood sites of action of morphine are at spinal and brainstem/midbrain loci, producing both the wanted and unwanted effects of the opioid. The spinal actions of opioids and their mechanisms of analgesia involve (1) reduced transmitter release from nociceptive C-fibres so that spinal neurons are less excited by incoming painful messages, and (2) postsynaptic inhibitions of neurons conveying information from the spinal cord to the brain. This dual action of opioids can result in a

Table 12.2 Amino-acid sequences of endogenous opioid peptides

Nociceptin	Phe–Gly–Gly–Phe–Thr–Gly–Ala–Arg–Lys–Ser–Ala–Arg–Lys–Leu–Ala–Asn–Gln
Dynorphin A (1–17)	Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg–Pro–Lys–Leu–Lys–Trp–Asp–Asn–Gln
Endomorphins 1 and 2	Tyr–Pro–Trp–Phe; Tyr–Pro–Phe–Phe
***Leu*- and *met*-enkephalin**	Tyr–Gly–Gly–Phe–Leu/Met

Table 12.3

	Mu (μ)	Delta (δ)	Kappa (κ)	ORL1
Endogenous ligands	β-endorphin Metorphamide Endomorphins	Methionine Enkephalin Leucine Enkephalin β-endorphin	Dynorphin A1–13 Dynorphin A1–8 Dynorphin B	Nociceptin/ orphanin FQ
Synthetic ligands	Morphine DAGO	DPDPE SNC-80 DSTBULET	U50488H Bremazocine Pentazocine	None so far
Antagonists	Naloxone β-FNA	Naltrindole Naloxone	Naloxone nor-BNI	[Phe$^1\Psi$(CH_2–NH)-Gly^2]NC (1–13)NH_2

total block of sensory inputs as they arrive in the spinal cord and is the basis for the spinal analgesic effects of these drugs. At supraspinal sites, morphine can act to alter descending pathways from the brain to the cord which involve noradrenaline and serotonin and these pathways then act to reduce spinal nociceptive activity. In addition, these sites form a link between emotions, depression and anxiety, and the level of pain and analgesia in a patient.

An intriguing area of research on opioids has been the accumulating evidence for plasticity in opioid controls. The degree of effectiveness of morphine analgesia is subject to modulation by other transmitter systems in the spinal cord and by pathological changes induced by peripheral nerve injury. Thus in neuropathic states, pain after nerve injury, morphine analgesia can be reduced (but can still be effective) and tactics other than dose-escalation to circumvent this will be briefly discussed in Chapter 21.

Finally, there is little or no clinical evidence that morphine causes psychological dependence or drug-seeking behaviour, tolerance or problematic respiratory depression in patients. These events simply do not occur when opioids are used to control pain. The reason is likely to be that the actions of morphine and the context of its use in a person in pain are neurobiologically quite different from the effects of opioids in street use. These actions of opioids are described in more detail in Chapter 23.

TACHYKININS

These are a family of peptides which include substance P, isolated in 1931 but only sequenced in 1971. This peptide has been extensively studied since it was the first major peptide to be extracted from brain but only now are useful antagonists becoming available. Two closely related peptides were then isolated from mammalian tissues and can be added to a number of other tachykinins, many of which are found in amphibians. The name tachykinins originated from the vasoactive effects of substance P but the nomenclature has been resolved into calling the three major mammalian peptides substance P, neurokinin A (NKA) and neurokinin B (NKB) with the corresponding receptors being numbered 1 to 3. The order of potencies at the three receptors as follows:

NK1 receptor: SP > NKA > NKB
NK2 receptor: NKA > NKB > SP
NK3 receptor: NKB > NKA > SP

The peptides have the following amino-acid sequences:

SP: Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met
NKA: His–Lys–Thr–Asp–Ser–Phe–Val–Gly–Leu–Met
NKB: Asp–Met–His–Asp–Phe–Phe–Val–Gly–Leu–Met

A single gene gives rise to substance P but it can be produced from three different mRNAs derived from alternative splicing (α, β and γ prepro-tachykinins). Neurokinin A has the same gene and shares two of the same mRNAs as substance P whereas neurokinin B has a different gene. High levels of the tachykinins are found in many parts of the CNS including the caudate, nucleus accumbens, olfactory bulb, colliculus and spinal cord. Both the peptide itself, substance P and the NK1 receptor have been 'knocked-out' and these mice are being extensively studied in order to probe the function of this peptide. Early indications suggest that the peptide and its receptor play important roles in pain (see Chapter 21), inflammation and possibly stress, emesis, anxiety, depression and reward. This is generally backed up by studies with antagonists, although some of the early NK1 receptor antagonists lacked specificity and several blocked calcium channels.

Coupling of the receptors is very similar with all three coupling to Gq and increasing IP3/DAG and in a number of neuronal systems it has been shown that the receptors produce slow depolarising responses via the closing of potassium channels.

CHOLECYSTOKININ

Cholecystokinin (CCK) and gastrin are members of a family that share a similar C-terminal sequence (Gly–Trp–Met–Asp–Phe), the biologically active end of the molecule. CCK is the predominant form in the CNS although it also occurs in the periphery where much of the body's gastrin is found. Early problems on the localisation of CCK stemmed from the overlap of antibodies with CGRP. There is now consensus that CCK-8 is the main transmitter in the CNS although larger forms, CCK-58 and 33, and smaller versions have been located (CCK-7, 5 and 4). Interestingly, CCK-8 is sulphated at the tyrosine residue:

CCK-8: Asp–Tyr–SO_3–Met–Gly–Trp–Met–Asp–Phe

This peptide itself has no selectivity for the two CCK receptors, CCK-A and B, which have so far been established to stimulate IP3/DAG while, like substance P, can close potassium channels to increase neuronal activity. The CCK-B receptor is thought to predominate in the CNS but species differences may make this interpretation difficult. It has a wide distribution in the CNS but is also found in the gut whereas the CCK-A receptor is more restricted but is found in the hypothalamus, hippocampus and in the brainstem. There are high levels of the natural peptide, CCK-8 in cortex, hippocampus, hypothalamus, ventral tegmentum, substantia nigra, brainstem and spinal cord. CCK is one of the most abundant peptides in the brain and CCK co-exists with dopamine, substance P, 5-HT and vasopressin. Interestingly, in the dopamine areas, CCK co-exists in the mesolimbic pathways but in the nigrostriatal projections, the peptide and

monoamine neurons form closely apposed but separate groups. This may be the basis for the complex interactions described for dopamine and CCK with regard to release and effects of the two transmitters — both inhibitions and enhancements of function and release/levels have been reported. Nevertheless, there is good evidence that CCK potentiates some of the motor roles of dopamine although this has yet to be demonstrated clinically.

A negative interaction between CCK, acting at the CCK-B receptor, and mu opioids has been consistently reported by electrophysiological, neurochemical and behavioural approaches. The analgesic effects of morphine are attenuated by CCK and potentiated by antagonists at the CCK-B receptor such as L-364,718 (devazepide). The mechanism is thought to involve calcium in that intracellular calcium is mobilised by CCK whereas mu receptor activation, by hyperpolarisation of the neuron, will reduce calcium entry through voltage-operated calcium channels. Thus CCK causes a physiological antagonism of the actions of morphine but this can only occur where the receptors are co-localised. This appears to be restricted to neurons in spinal cord and possibly brainstem which are associated with analgesia since antagonists potentiate only morphine analgesia, not respiratory depression. It is noteworthy that devazepide is only one of a number of recently developed non-peptide antagonists at the CCK-B receptor — agents that will allow CCK-B receptor function to be probed more fully. CCK causes satiation and inhibits feeding in animals and although this central action occurs after peripheral administration of the peptide, it is thought to happen through activation of peripheral vagal pathways to the hypothalamus. This nicely ties in with the established roles of CCK and gastrin in normal digestion.

Finally, the peptide can induce anxiety and panic in normal and anxious volunteers. Some synthetic CCK-B receptor antagonists are chemically similar to the benzodiazepine anxiolytics. Again, the clinical role of CCK manipulation in anxiety remains to be resolved.

NEUROPEPTIDE Y

Neuropeptide Y (NPY) is a large 36 amino-acid peptide found in large amounts in the brain. Although the central administration of NPY in animals causes a number of biological effects which range from control of CRF, increased food intake, anxiolysis, changes in memory and circadian rhythms, the five receptors for the peptide and the lack of any really selective tools for the Y1, 2, 4, 5 and 6 receptors (what happened to the Y3 site!) makes appraisal of this peptide as a drug target rather difficult. A selective antagonist exists only for the Y1 receptor. The marked vasoconstrictor actions of the peptide in the periphery will mean that systemic therapy will require receptor subtype selective agents.

VASOPRESSIN

Vasopressin is closely related to oxytocin and both peptides are cyclic in that they contain a disulphide bridge. Although much is known about the peripheral actions of the peptides the extent of our current knowledge of their possible CNS function is that vasopressin appears to act as a cognitive enhancer and has positive effects on learning processes in animals. Vasopressin acts on three receptors, V1a and b and a V2 receptor.

Again, the existence of non-peptide antagonists at these receptors will soon lead to a better understanding of the central roles of this peptide.

SOMATOSTATIN

Somatostatin exists in two forms, a 14 and a 28 amino-acid form called SRIF-14 and SRIF-28, respectively. Both are widely distributed in the CNS and the peptide produces inhibitory effects on neurons via G-protein-coupled opening of potassium channels. The original receptor division was twofold with a SRIF-1 and -2 receptor division but it is now clear that what are now known as sst2, 3 and 4 subtypes make up the former receptor while the sst1 and 4 receptors are the original SRIF-2 site.

Few antagonists exist at present but the distribution of the peptide with high levels in cortex, hippocampus, amyglada and spinal cord may give some clues to potential functions of the peptide.

SRIF given directly to the spinal cord is antinociceptive which would be expected from an inhibitory peptide, although some studies suggest toxic rather than physiological effects of the peptide. By contrast, the peptide appears to promote convulsions — here its role may be through disinhibition. A well-established central role in the control of growth hormone release has given rise to hopes of treatment of agromegaly and in other contexts, motor actions and increases in sleep times in animals suggest a number of roles of this peptide. There are reports of reduced brain levels of the peptide in Alzheimer's disease (Chapter 20).

NEUROTENSIN

Neurotensin is a peptide with well-established digestive functions which is also found in CNS neurons. There are two receptors (1 and 2) and a paucity of agonists apart from

Table 12.4 Potential roles of peptides

Peptide	Receptor(s)	Function	Potential indication
Opioids	Mu, delta, kappa, ORL1	Pain, anxiety, mood, reward	Chronic pain, addiction
Tachykinins	NK1–3	Inflammation, anxiolysis	Headache, anxiety
Cholecystokinin	A and B	Anxiogenesis, satiation, dopamine function, pain	Panic, eating disorders, pain, Parkinsonism, psychoses
Neuropeptide Y	Y1–6	Obesity, mood, neuronal excitability	Eating disorders, depression, epilepsy
Vasopressin	V1 and 2	Learning, memory	Amnesias
Somatostatin	Sst1–5	Analgesia	Pain
Neurotensin	Nt1 and 2	Temperature, analgesia, pain, dopamine function	Pyresis, pain, Parkinsonism, psychoses
CGRP	1 and 2	Cardiovascular, inflammation, anorexia	Headache, pain, eating disorders
Galanin	GalR1 and 2	Sensory transmission, feeding	Pain, eating disorders

the peptide itself, although selective NT1 receptor antagonists exist. The 13 amino-acid peptide has been implicated in analgesia, thermoregulation and interactions with dopamine function in the nigrostriatal and mesolimbic pathways.

CGRP

Calcitonin gene-related peptide (CGRP) is a product of the calcitonin gene with a distinct mRNA which is formed from alternative splicing in a tissue-specific manner. Thus CGRP is the main product in the CNS whereas calcitonin is found in the thyroid. The peptide is excitatory but whether there is a single receptor or two remains a point of dispute. There are large amounts of CGRP and substance P in fine sensory nerves and the two peptides are released into the periphery by antidromic stimulation where they contribute to the wheal and flare via vasodilatation through complex interactions. There is evidence that this effect of CGRP is via a potentiation of the vascular effects of SP while at the central terminals of sensory nerves the effects of substance P are enhanced by competing with it for a common peptidase so that the metabolism of SP is reduced. Hence there is interest in the potential of CGRP antagonists as therapies for inflammation and headache, although there are no useful compounds of this class at present. Other actions of CGRP have been reported such as altered food intake and thermoregulatory effects.

GALANIN

Galanin is a 29 amino-acid peptide, one amino acid longer in humans than in rats, which acts on three known receptors, GalR1–3, all of which are G-protein-linked receptors, in common with all peptides. There is a lack of any antagonist and apart from a truncated version of galanin having some GalR2 selectivity, no means of separation of the three receptors. However, the consequences of receptor activation are clear in that the GalR1 and 3 receptors are inhibitory and the GalR2 excitatory, although some mixed effects have been reported with the latter. The 1 and 3 receptors open potassium channels whereas the GalR2 receptor mobilises internal calcium, possibly via IP3 mechanisms. The distribution of the 1 and 2 receptors differs, with the former being enriched in hippocampus, spinal cord and peripheral nerves whereas GalR2 has a wider distribution. Galanin co-exists with enkephalin, NPY and substance P, modulates ACh release and is found in GABA-containing neurons in the spinal cord.

Most is known about galanin in the spinal cord and the normal almost undetectable levels of the peptide increase after nerve damage with gene induction occurring. After inflammation there is upregulation of GalR2. In normal animals spinal application of galanin has mixed effects on both spinal neurons and peripheral nerve activity and these are likely to reflect GalR1 and 2 receptors located together. Selective agents for the two receptors are needed.

CONCLUSIONS

It is clear that the peptides present as an interesting group of diverse substances. Their discovery stimulated great expectations but most of these remain to be resolved. As indicated above it seems likely that their low concentrations in the brain and their very

slow turnover limits their potential for mediating high levels of activity while their slow time-course of action and apparent need to co-locate with so-called classical transmitters may preclude them from primary activity. Nonetheless they are there and, perhaps more importantly, so are their receptors. It is to be hoped that the synthesis of appropriate agonists and antagonists will make it possible to study the actions of the peptides and possibly develop appropriate therapy, even if this turns out to be a secondary line of attack.

The localisation of a particular peptide to a particular brain area and possibly associated with a particular transmitter (e.g. CCK with dopamine in mesolimbic pathways) has often prompted a prediction of function (e.g. CCK may have a role in schizophrenia). Animal studies in which the peptide has been injected into the appropriate brain area or tested on slices taken from the brain area have sometimes been taken to confirm such hypotheses. These approaches have lined up the peptides for a whole range of potential roles, some of which are listed in Table 12.4. Whether these predictions are realities will depend on the availability of chemical agents and their evaluation, not only in animals but also in humans.

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