Neurotransmitters, Drugs and Brain Function.
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8 Noradrenaline

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INTRODUCTION

Noradrenaline, 3,4-dihydroxyphenylethanolamine (Fig. 8.1), is released from terminals of noradrenergic neurons in the brain, from most postganglionic sympathetic neurons and from chromaffin cells in the adrenal medulla. Its role in the periphery (the so-called 'sympathoadrenal system') has been evident for nearly a century, but its function in the brain is much harder to define. This chapter will describe recent developments in our understanding of the neurochemistry and pharmacology of noradrenergic neurons and adrenoceptors as well as outlining theories to explain how changes in central noradrenergic transmission might influence behaviour.

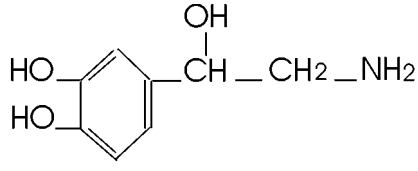


Figure 8.1 The chemical structure of noradrenaline

PATHWAYS WITHIN THE CNS

The cell bodies of central noradrenergic neurons are all clustered within two bilateral groups of nuclei (numbered A1 to A7) in the brainstem (Fig. 8.2). These comprise the locus coeruleus complex and the lateral tegmental nuclei. The locus coeruleus proper (nucleus A6) has attracted most interest because it accounts for approximately 45% of all the noradrenergic neurons in the brain. In the rat, there are only about 1500 noradrenergic cell bodies in the locus coeruleus of each hemisphere but their neurons branch extensively and project throughout the neuraxis. Retrograde tracing has shown that over 50% of neurons within the locus coeruleus innervate both the cortex and the cerebellum, for instance. The majority, if not all, of these fibres are thought to be retained ipsilaterally. The density of innervation varies from brain region to brain region and this is reflected, to some extent, by regional variation in tissue noradrenaline content (Table 8.1).

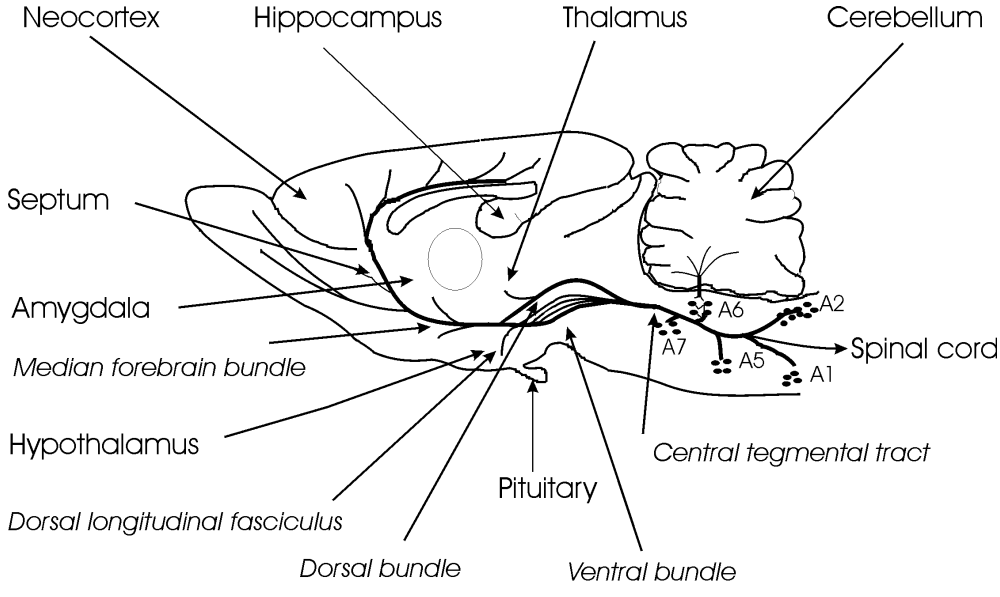


Figure 8.2 The distribution of noradrenergic neurons in the brain. The cell bodies are clustered in nuclei (A1–A7) in the pons/medulla regions of the brainstem and their axons project both rostrally and caudally to most regions of the neuraxis. The major nucleus is the locus coeruleus (A6)

The activity of noradrenergic neurons within the locus coeruleus is governed by two major afferent systems: a GABAergic (inhibitory) input from the nucleus prepositus hyperglossi and an (excitatory) glutamatergic projection from the nucleus paragingantocellularis (Aston-Jones *et al.* 1991). However, dendrites of neurons with cell bodies lying within the locus coeruleus can extend into the area surrounding the nucleus (the pericoerulear region) and could well be influenced by other neurotransmitters and neuromodulators.

Many brain areas are innervated by neurons projecting from both the locus coeruleus and the lateral tegmental system but there are exceptions (Fig. 8.3). The frontal cortex, hippocampus and olfactory bulb seem to be innervated entirely by neurons with cell bodies in the locus coeruleus whereas most hypothalamic nuclei are innervated almost exclusively by neurons projecting from the lateral tegmental system. The paraventricular nucleus (and possibly the suprachiasmatic nucleus, also) is an exception and receives an innervation from both systems.

Table 8.1 The concentration of noradrenaline in different brain regions (μg/g wet weight of tissue)

Cortex	0.1
Hippocampus	0.25
Hypothalamus	0.2
Pons medulla	0.35

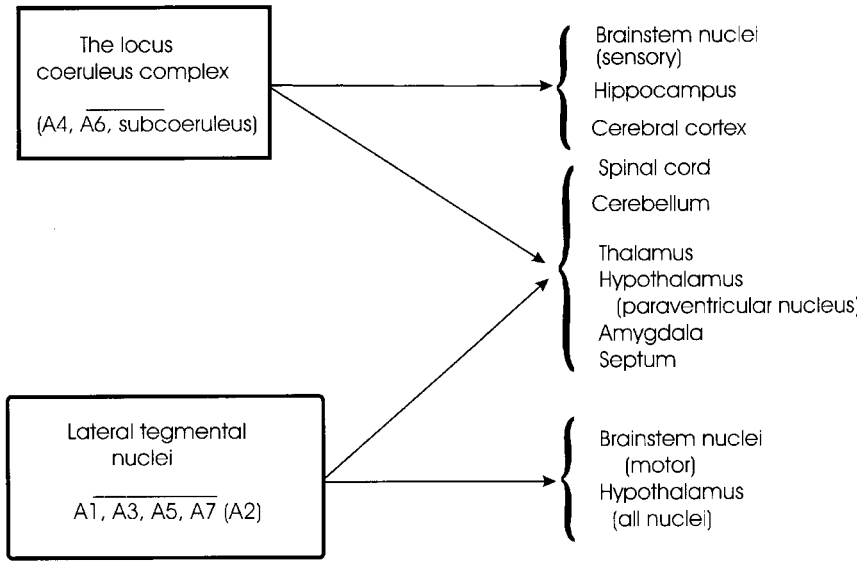


Figure 8.3 Brain areas receiving a prominent noradrenergic innervation. Most brain regions are innervated by neurons projecting from both the locus coeruleus and the lateral tegmental system. However, the frontal cortex, hippocampus and olfactory bulb are innervated exclusively by neurons with cell bodies in the locus coeruleus. With the exception of the paraventricular nucleus (and possibly the suprachiasmatic nucleus) hypothalamic nuclei are innervated by neurons projecting from the lateral tegmental system

The extensive branching and widespread distribution of noradrenergic neurons within the CNS has long been cited as evidence that this is a spatially and functionally diffuse neuronal system. This view was reinforced by an early report that few of these neurons formed specialised synaptic contacts. This fostered the impression that the locus coeruleus represents a 'switch' which, when activated, causes noradrenaline to be discharged from neurons, throughout the brain, in a non-selective manner. However, it is now known that, in the cortex at least, over 90% of the noradrenergic nerve terminals form specialised synaptic contacts with postsynaptic elements (Papadopoulos and Parnavelas 1991). There is also evidence that neurons in different zones of the locus coeruleus are morphologically distinct (at least six different types of noradrenaline-containing cells have been identified) and project to different brain regions or brain systems. In fact, neurons from different noradrenergic nuclei even innervate different types of neuron in the terminal field but, although it is certain that different noradrenergic nuclei have different functions, little is known about their physiological specialisations, largely because of their extensive reciprocal connections. All this evidence (reviewed in Stanford 1995) challenges the view that the central noradrenergic system operates in a non-selective manner.

NEUROCHEMISTRY OF NORADRENALINE

The effects of drugs on the synthesis, storage, release and destruction of noradrenaline, summarised in Fig. 8.4, are discussed in the following sections.

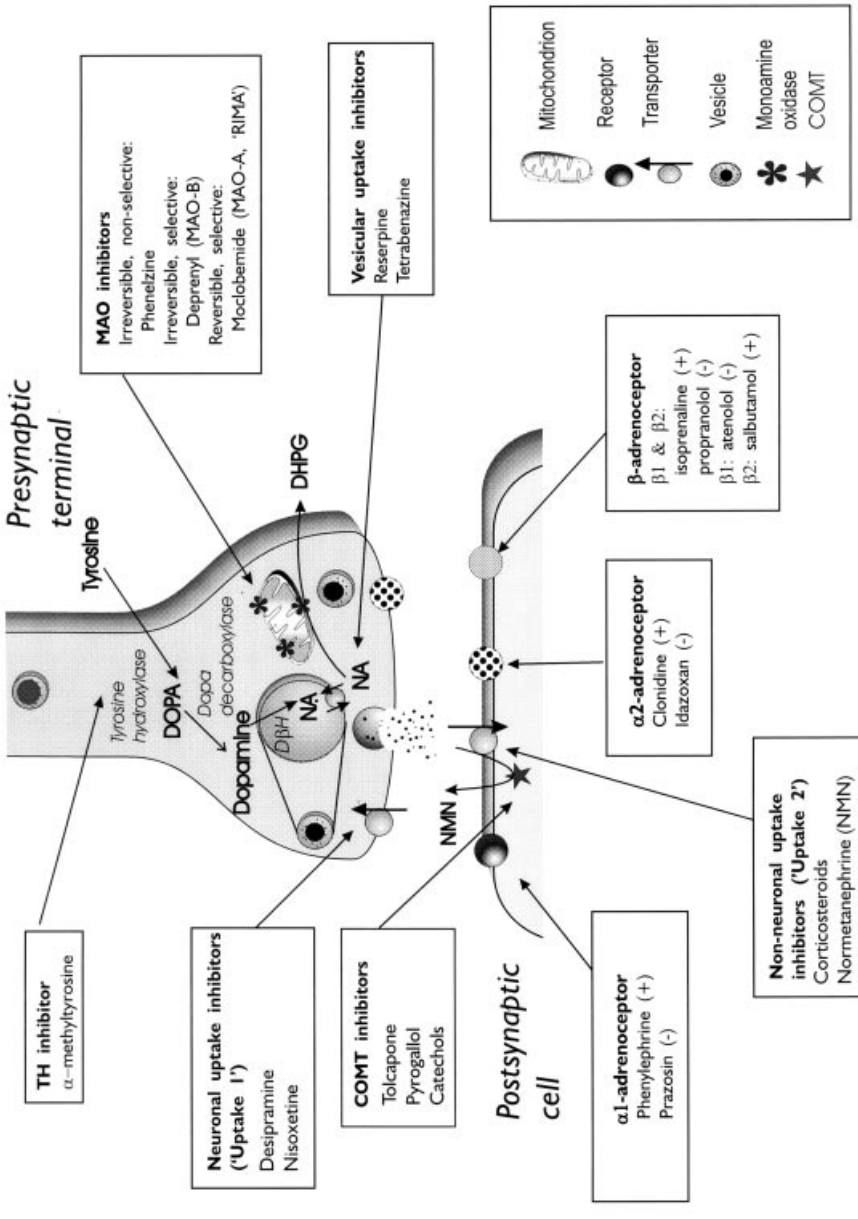


Figure 8.4 The site of action of drugs that modify noradrenergic transmission

SYNTHESIS

The pathway for synthesis of the catecholamines dopamine, noradrenaline and adrenaline, illustrated in Fig. 8.5, was first proposed by Hermann Blaschko in 1939 but was not confirmed until 30 years later. The amino acid *l*-tyrosine is the primary substrate for this pathway and its hydroxylation, by tyrosine hydroxylase (TH), to *l*-dihydroxyphenylalanine (*l*-DOPA) is followed by decarboxylation to form dopamine. These two steps take place in the cytoplasm of catecholamine-releasing neurons. Dopamine is then transported into the storage vesicles where the vesicle-bound enzyme, dopamine-β-hydroxylase (DβH), converts it to noradrenaline (see also Fig. 8.4). It is possible that *l*-phenylalanine can act as an alternative substrate for the pathway, being converted first to *m*-tyrosine and then to *l*-DOPA. TH can bring about both these reactions but the extent to which this happens *in vivo* is uncertain. In all catecholamine-releasing neurons, transmitter synthesis in the terminals greatly exceeds that in the cell bodies or axons and so it can be inferred

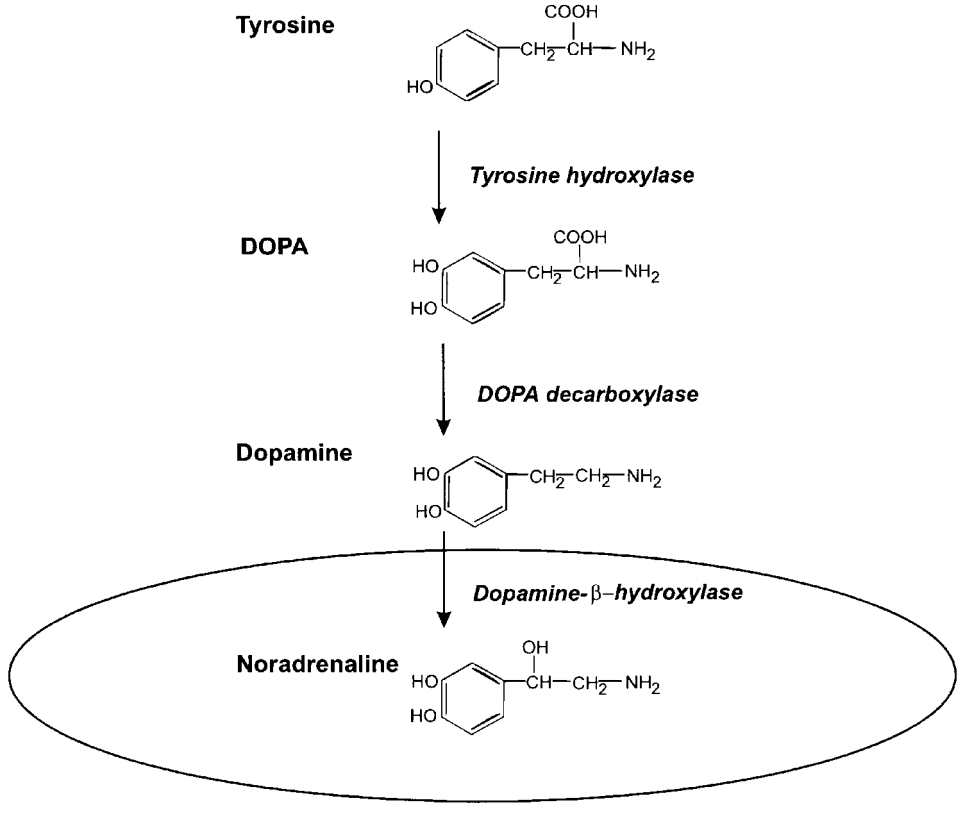


Figure 8.5 The synthetic pathway for noradrenaline. The hydroxylation of the amino acid, tyrosine, which forms dihydroxyphenylalanine (DOPA) is the rate-limiting step. Conversion of dopamine to noradrenaline is effected by the vesicular enzyme, dopamine-β-hydroxylase (DβH) after uptake of dopamine into the vesicles from the cell cytosol

that all the factors (enzymes and storage vesicles) which are vital for this process undergo axoplasmic transport after their assembly in the cell body.

It was recognised as early as the 1960s that conversion of tyrosine to *l*-DOPA was the rate-limiting step in the synthesis of noradrenaline. This emerged from experiments showing that incubation of tissues with high concentrations of tyrosine had no effect on the rate of synthesis of noradrenaline, whereas incubation with high concentrations of *l*-DOPA or dopamine increased it. More evidence came from experiments showing that the rate of conversion of [^{3}H]tyrosine to [^{3}H]DOPA was increased if the sympathetic nerves innervating the test tissue were stimulated, whereas stimulation of nerves innervating tissues incubated in a medium containing [^{3}H]DOPA did not accelerate synthesis of [^{3}H]dopamine or [^{3}H]noradrenaline.

Because the enzymes, DOPA decarboxylase and DβH, have a high affinity for their substrates, neither *l*-DOPA nor dopamine accumulate in noradrenergic nerve terminals under normal conditions. Exceptionally, DβH can become the rate-limiting enzyme, such as during *l*-DOPA treatment of Parkinson's disease which greatly increases the intraneuronal pool of dopamine. It is thought that DβH can also be rate-limiting during periods of intense or prolonged impulse-evoked release of noradrenaline. This is because a high release rate compromises the supply of vesicles that not only store and release noradrenaline but are also the site of its synthesis after uptake of dopamine from the cytosol. Evidence suggests that vesicular uptake of dopamine is reduced after periods of intense neurotransmission; this results in its accumulation in the cytosol until new vesicles are delivered to the terminals.

TH is a mixed-function oxidase with an absolute requirement for reduced pterin co-factor, 6*R*-tetrahydrobiopterin, molecular oxygen and Fe^{2+}. The K_m of the enzyme for its substrate is thought to be well below tissue concentrations of tyrosine and so the enzyme is probably normally about 80% saturated. This makes it unlikely that the supply of tyrosine limits enzyme activity and synthesis of noradrenaline under normal circumstances. However, it was clear from the earliest studies of noradrenergic neurons that the synthesis of noradrenaline was increased during neuronal activity, whether this is induced pharmacologically (e.g. by blockade of presynaptic α_2-adrenoceptors which inhibit release of noradrenaline) or by physiological stimuli (e.g. cold exposure or hypoglycaemia). Such findings suggested that synthesis and release are coupled in some way.

It is now known that regulation of this enzyme involves multiple mechanisms affecting both rapid, transient changes in enzyme activity and long-latency, long-lasting changes in enzyme synthesis involving increased TH gene transcription. The factors controlling the synthesis of noradrenaline have been studied more, and are better understood, than those of most other neurotransmitters and therefore justify detailed consideration.

Regulation of tyrosine hydroxylase activity

Short-term

At first, it was thought that control of TH activity depended on inhibition by its end-product, noradrenaline, which competes with the binding of co-factor. According to this scheme, release of noradrenaline would diminish end-product inhibition of the enzyme and so ensure that synthesis is increased to replenish the stores. When the

neurons are quiescent, the opposite would occur: i.e. intraneuronal accumulation of noradrenaline would automatically blunt synthesis. Much evidence was deemed to support this view. For instance, when metabolic breakdown of cytoplasmic noradrenaline was prevented by treatment with an inhibitor of the enzyme, monoamine oxidase (MAO: see below), the rate of synthesis of [^{3}H]noradrenaline from [^{3}H]tyrosine was markedly reduced (Neff and Costa 1966).

However, as early as the 1970s, it was obvious that end-product inhibition of TH could not be the main factor regulating the rate of noradrenaline synthesis. Clearly, the hydroxylation of tyrosine takes place in the cytoplasm and so it must be cytoplasmic noradrenaline that governs enzyme activity. Yet, it is vesicle-bound transmitter that undergoes impulse-evoked release from the neuron. Also, when neurons are releasing noradrenaline, its reuptake from the synapse is increased and, even though some of this transmitter ends up in the vesicles, or is metabolised by MAO, there should be a transient increase in the concentration of cytoplasmic noradrenaline which would *increase* end-product inhibition of TH.

To overcome these difficulties it was suggested that there was a small 'strategic pool' of cytoplasmic noradrenaline that inhibited the activity of TH. Nevertheless, even this small pool was eventually ruled out as a regulator of TH. This followed *in vitro* experiments investigating the effects of addition of reduced pterin co-factor on the activity of the enzyme derived from the vas deferens. It was predicted that the activity of enzyme derived from control tissues would be increased by the addition of co-factor *in vitro* whereas that enzyme derived from stimulated tissues should not increase because the TH would already have been maximally activated by endogenous co-factor during nerve stimulation. In fact, co-factor increased noradrenaline synthesis in both instances, suggesting that noradrenaline synthesis depended primarily on factors that directly activate TH, rather than on removal of end-product inhibition. The extent to which end-product inhibition of TH contributes to the regulation of its activity under physiologically relevant (e.g. drug-free) conditions remains uncertain.

The first clue to the processes which normally regulate TH activity came from experiments showing that electrical stimulation of sympathetic neurons increased the affinity of this enzyme for its co-factor and reduced its affinity for noradrenaline (for detailed reviews of this topic see Zigmond, Schwarzschild and Rittenhouse 1989; Fillenz 1993; Kaufman 1995; Kumar and Vrana 1996). Several lines of investigation showed that activation of TH was in fact paralleled by its phosphorylation and it was this process that accounted for the changes in the enzyme's kinetics (Table 8.2).

Table 8.2 The effects of phosphorylation of tyrosine hydroxylase on enzyme kinetics (based on Kaufman 1995)

	K_m for co-factor	V_{max}
cAMP-dependent protein kinase (PKA) (pH 6.0)	↑↑	No change
cAMP-dependent protein kinase (PKA) (pH 7.0–7.4)	↓	↑↑
Ca^{2+}-calmodulin dependent protein kinase (CAM-PK II)	No change	↑
Ca^{2+}-phospholipid dependent protein kinase (PKC) (pH 7.0)	↓↓	No change
cGMP-dependent protein kinase (PKG) (pH 6.0)	↓↓	↑
cAMP-independent protein kinase	No change	↑

It is now known that such phosphorylation is activated by several protein kinases, including Ca^{2+}/phospholipid-dependent protein kinase (PKC), which reduces its K_m for co-factor, and cGMP-dependent protein kinase. These factors phosphorylate different sites on the enzyme, although some are shared by different kinases. In rat TH, serine residues, Ser^{8}, Ser^{19}, Ser^{31} and Ser^{40}, have been identified as targets and Ser^{40} seems to be a common target for all the kinases. It is thought that this site produces a conformational change in the enzyme that reduces its affinity for catecholamines. All these regulatory sites reside on the N-terminus of the enzyme, whereas it is the C-terminus that comprises the catalytic site. In addition to all these changes, phosphorylation of the enzyme changes the pH optimum for maximal enzyme activity and so the kinetics of this enzyme depend on the pH of the incubation medium to some extent.

In the periphery, some of the primary triggers for these processes have been identified. Acetylcholine seems to be one such factor because stimulation of preganglionic nerves *in vivo* increases enzyme activity. However, nicotinic and muscarinic receptor antagonists do not completely prevent this increase. The residual activation is attributed to peptides of the secretin–glucagon subgroup, including VIP and secretin; both these peptides activate cAMP synthesis. Purinergic transmitters could also be involved.

Finally, recent findings suggest that, in humans, four different mRNAs for TH are produced from a single gene. The translation products of these mRNAs differ in their amino-acid sequence in the N-terminal domain, rather than the catalytic C-terminus, and are likely to differ in their kinetics and susceptibility to different protein kinases. Regional differences in the distribution of these enzyme isoforms suggest that they might differ functionally, a possibility that is being explored currently.

Long-term

The first reports that TH activity could be altered without changes in its kinetics came from studies of the adrenal medulla of rats in which catecholamine release was stimulated by exposure of rats to a cold environment. The increase in enzyme activity was prevented by protein synthesis inhibitors, suggesting that it was due to an increase in TH gene transcription rather than activation of existing enzyme. Since then, physiological and pharmacological stimuli that increase demand on the transmitter store have consistently been shown to trigger such induction of TH enzyme. Increased TH protein has also been detected in noradrenergic cell bodies of sympathetic ganglia and the locus coeruleus. At all these sites, as in the adrenal medulla, the increase is evident after about 24 h. However, changes in the terminals take several days to appear, presumably because of the time required for axoplasmic transport of the enzyme.

The signal for increased synthesis of TH protein in the adrenal gland certainly depends on an intact cholinergic innervation. Moreover, in the denervated gland, the increase induced by perfusion with exogenous acetylcholine is prevented by nicotinic antagonists. However, nicotinic antagonists do not completely prevent the increase in glands with an intact cholinergic innervation. These findings suggest that activation of nicotinic receptors by ACh is normally only partly responsible for the increase. Other factors now known to regulate TH gene transcription include glucocorticoids and nerve growth factor (NGF). Although details are far from clear, protein kinases (especially PKA), diacyl glycerol and Ca^{2+} are all thought to be crucial intracellular messengers for

increased gene transcription. It should also be borne in mind that enzyme induction is not limited to TH: the same stimuli also increase DβH synthesis but less is known about factors mediating this process.

STORAGE

In common with other classical transmitters, noradrenaline is stored in vesicles that accumulate in the terminal varicosities. This was first shown by experiments that combined sucrose density–gradient centrifugation of tissue homogenates (see Fig. 4.3) with electron microscopy and assay of the noradrenaline content of the different layers of the gradient. These studies confirmed that the noradrenaline-rich layers of the gradient coincided with those layers in which the vesicles were clustered. This suggested that the vesicles were the major storage site for noradrenaline within the nerve terminals. Further studies examined the effects of ligation or cooling the axons of sympathetic neurons for several days. Electron micrographs of the zone around the obstruction showed that the vesicles accumulated on the side nearest the cell body, confirming that they were assembled in the cell body and transported to the terminals by anterograde axoplasmic transport. The life cycle of these vesicles was discussed in more detail in Chapter 4.

The concentration of noradrenaline in the vesicles is thought to be in the region of 0.1–0.2 M and it is estimated that there is a concentration gradient, in the order of 10^4–10^6-fold, driving the transmitter out of the vesicles towards the cytoplasm. The vesicular compartmentalisation of noradrenaline is made possible by its active uptake on vesicular monoamine transporters (VMATs) and its subsequent binding, in an osmotically inert matrix, within the vesicles. One obvious function of these transporters is thus to protect and conserve the releasable vesicular pool of transmitter. However, it is thought that they also protect neurons from potentially toxic effects of an excess of cytoplasmic noradrenaline and also maintain a concentration gradient favouring noradrenaline reuptake from the synapse (see below).

Uptake of noradrenaline into the vesicles depends on an electrochemical gradient driven by an excess of protons inside the vesicle core. This gradient is maintained by an ATP-dependent vesicular H^+-triphosphatase. Uptake of one molecule of noradrenaline into the vesicle by the transporter is balanced by the counter-transport of two H^+ ions (reviewed by Schuldiner 1998). It is thought that either binding or translocation of one H^+ ion increases the affinity of the transporter for noradrenaline and that binding of the second H^+ actually triggers its translocation.

Reserpine irreversibly inhibits the triphosphatase that maintains the proton gradient and so it depletes neurons of their vesicular store of transmitter. This explains why restoration of normal neuronal function rests on delivery of new vesicles from the cell bodies. Some amphetamine derivatives, including methylenedioxymethamphetamine (MDMA), are also substrates for the transporter and, as a result, competitively inhibit noradrenaline uptake. Another way of inhibiting the transporter is by dissipation of the pH gradient across the vesicular membrane: *p*-chloroamphetamine is thought to act in this way.

Much of the early work on these transporters was carried out on the chromaffin granules of the bovine adrenal medulla. These studies revealed the transporter to be a polypeptide of 80 kDa. However, two VMATs have now been characterised and these are the products of different genes. Evidence suggests that both have 12

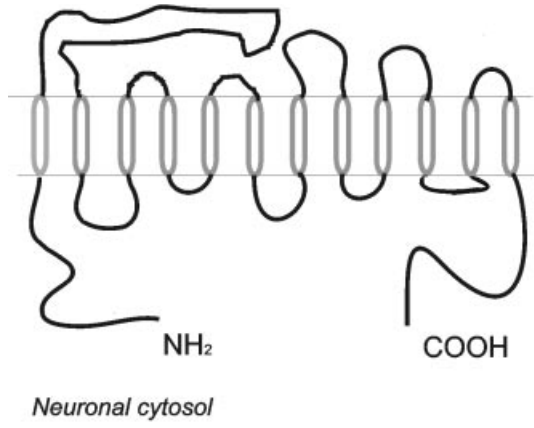


Figure 8.6 Schematic diagram of the proposed structure of the vesicular monoamine transporter. There are 12 transmembrane segments with both the N- and C-termini projecting towards the neuronal cytosol. (Based on Schuldiner 1998)

transmembrane-spanning domains (TMDs) with a large hydrophobic loop, facing the vesicular core, between TMD1 and TMD2. Both the N- and C-termini project towards the neuronal cytosol (Fig. 8.6). There are species differences, but VMAT1 and VMAT2 differ in their distribution. In fact, the expression of these proteins in individual cells might be mutually exclusive. They also differ in their sensitivity to the reversible uptake inhibitor, tetrabenazine, and their affinity for substrates such as amphetamine and histamine. Only VMAT2 binds histamine and tetrabenazine and this protein consistently binds amines with a higher affinity than does VMAT1. In the rat, VMAT1 is found in non-neuronal tissue, including the adrenal medulla, whereas VMAT2 is found in neurons, only. In other species, the distribution is not so distinct, with mRNA for VMAT2 being reported in the adrenal medulla as well as the brain.

RELEASE

Studies of release of noradrenaline from sympathetic neurons provided the first convincing evidence that impulse (Ca^{2+})-dependent release of *any* transmitter depended on vesicular exocytosis. Landmark studies carried out in the 1960s, using the perfused cat spleen preparation, showed that stimulation of the splenic nerve not only led to the detection of noradrenaline in the effluent perfusate but the vesicular enzyme, DβH, was also present. As mentioned above, this enzyme is found only within the noradrenaline storage vesicles and so its appearance along with noradrenaline indicated that both these factors were released from the vesicles. By contrast, there was no sign in the perfusate of any lactate dehydrogenase, an enzyme that is found only in the cell cytosol. The processes by which neuronal excitation increases transmitter release were described in Chapter 4.

While the amount of noradrenaline released from the terminals can be increased by nerve stimulation, it can be increased much more by drugs, like phenoxybenzamine, which block presynaptic α-adrenoceptors. These receptors are normally activated by increased noradrenaline in the synapse and trigger a feedback cascade, mediated by

second messengers, which blunts further release of noradrenaline. These presynaptic autoreceptors play an important part in ensuring that transmitter stores are conserved and preventing excessive stimulation of the postsynaptic cells.

Pharmacological characterisation of this receptor revealed that it was unlike classic α-adrenoceptors found on smooth muscle. In particular, receptors modulating noradrenaline release have a higher affinity for the agonist, clonidine, and the antagonist, yohimbine. This distinctive pharmacology led to the subdivision of α-adrenoceptors into the α_1- and the α_2-subtypes. Although the latter is the subtype responsible for feedback inhibition of noradrenaline release, the majority of α_2-adrenoceptors are actually found postsynaptically in some brain regions. There is still some debate over the identity of the subtype of α_2-adrenoceptors responsible for feedback inhibition of transmitter release. However, most studies agree that the $\alpha_{2A/D}$-subtype has the major role, although the α_{2B}- and α_{2C}-subtypes might contribute to this action. Species differences in the relative contributions of these different receptors are also possible.

It is α_{2A}-adrenoceptors that are found on cell bodies of noradrenergic neurons in the locus coeruleus. These receptors are activated by noradrenaline released from branches ('recurrent collaterals') of noradrenergic neurons projecting from the locus coeruleus and inhibit neuronal firing (Cederbaum and Aghajanian 1976). α_2-Adrenoceptors in the brain thus depress noradrenaline release through two distinct processes: inhibition of the release process following activation of terminal autoreceptors and depression of neuronal firing following activation of receptors on the cell bodies.

The exact process(es) by which α_2-adrenoceptors blunt release of transmitter from the terminals is still controversial but a reduction in the synthesis of the second messenger, cAMP, contributes to this process. α_2-Adrenoceptors are negatively coupled to adenylyl cyclase, through a *Pertussis* toxin-sensitive Gi-like protein, and so their activation will reduce the cAMP production which is vital for several stages of the chemical cascade that culminates in vesicular exocytosis (see Chapter 4). The reduction in cAMP also indirectly reduces Ca^{2+} influx into the terminal and increases K^+ conductance, thereby reducing neuronal excitability (reviewed by Starke 1987). Whichever of these release-controlling processes predominates is uncertain but it is likely that their relative importance depends on the type (or location) of the neuron.

α_2-Adrenoceptors are not the only receptors to modulate noradrenaline release. In the periphery and CNS (Murugaiah and O'Donnell 1995) activation of presynaptic β-adrenoceptors has the opposite effect: i.e. it augments release of noradrenaline. The increase in cAMP production resulting from activation of these receptors is an obvious explanation for how this might occur. The precise role of these receptors in regulation of noradrenaline release *in vivo* is uncertain because noradrenaline has a relatively low affinity for these receptors. However, one suggestion is that, in the periphery, they are preferentially activated by circulating adrenaline which has a relatively high affinity for these receptors. This activation could enable circulating adrenaline to augment neuronal release of noradrenaline and thereby effect a functional link between these different elements of the sympathoadrenal system. However, the extent to which this actually happens is uncertain as is a physiological role for β-adrenoceptors in regulation of noradrenaline release in the brain.

Noradrenaline release might also be modulated by receptors on noradrenergic nerve terminals that are activated by other neurotransmitters ('heteroceptors'). Unfortunately, most studies of this type of modulation have been carried out in tissue slices and

so it is not possible to rule out the possibility that 'heteroceptors' are in fact part of a polysynaptic loop and that they influence noradrenaline release only indirectly. Nevertheless, there is some evidence from studies of hippocampal synaptosomes that activation of muscarinic, $GABA_B$ or adenosine (A_1) receptors depresses noradrenaline release while activation of $GABA_A$ receptors increases it.

A further possible mechanism, that would enable different types of neurons to modify noradrenaline release, is suggested by recent *in vitro* studies of brain slices. These have revealed that noradrenaline release is increased when the slices are superfused with a solution containing GABA. This release is prevented by an inhibitor of GABA uptake but unaffected by the presence of $GABA_A$ receptor antagonists, such as bicuculline. There is no doubt that this form of release depends on vesicular exocytosis because it is Ca^{2+}-dependent, sensitive to tetrodotoxin and, like impulse-dependent release, it is attenuated by α_2-adrenoceptor agonists (see above). Since uptake of GABA by GABA transporters on noradrenergic nerve terminals ('heterocarriers') involves co-transport of Na^+ ions into the terminal (Fassio *et al.* 1996) it is possible that this uptake increases Na^+ influx enough to depolarise the terminals and trigger exocytotic release of noradrenaline. The extent to which this process occurs under normal physiological conditions *in vivo* remains to be seen.

NEURONAL REUPTAKE OF NORADRENALINE

In common with other monoamines, the actions of released noradrenaline are terminated by its rapid reuptake from the synaptic cleft. This uptake process relies on membrane-bound noradrenaline transporters which are glycoproteins closely related

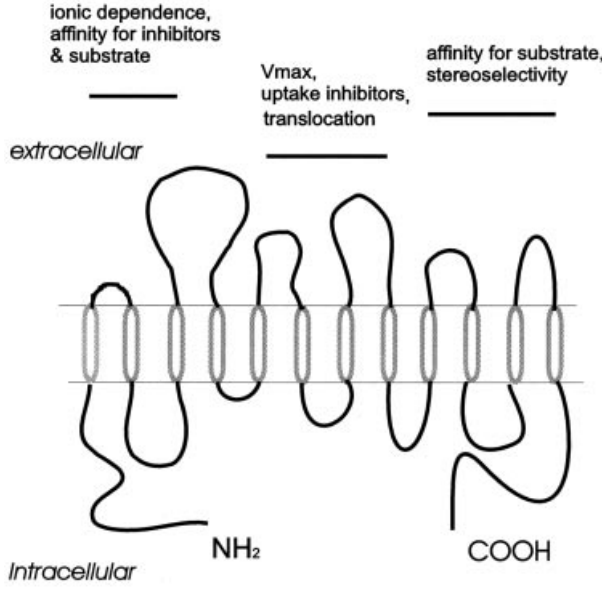


Figure 8.7 Schematic diagram of the proposed structure of the noradrenaline neuronal transporter showing the 12 transmembrane, hydrophobic domains with the N- and C-termini projecting towards the cell cytoplasm. Binding domains for specific ligands are thought to be within regions indicated by the solid bars. (From Stanford 1999, reproduced with permission)

to the transporters for several other transmitters (e.g. GABA and 5-HT). All these transporters have 12 hydrophobic transmembrane domains (TMDs), a large hydrophyllic loop between TM3 and TM4, and intracellular N- and C-termini. The hypothetical structure of the noradrenaline transporter is illustrated in Fig. 8.7. Because co-transport of both Cl^- and Na^+ is required for the uptake of noradrenaline, this is regarded as one of the family of Na^+/Cl^- transporters.

Exactly how this transporter carries noradrenaline across the neuronal membrane is not known but one popular model proposes that it can exist in two interchangeable states. Binding of Na^+ and noradrenaline to a domain on its extracellular surface could trigger a conformation change that results in the sequential opening of outer and inner channel 'gates' on the transporter. This process enables the translocation of noradrenaline from the extracellular space towards the neuronal cytosol.

So far, only one noradrenaline transporter has been cloned. Point-mutation and splicing studies indicate that different zones of the transporter determine its substrate affinity and selectivity, ionic dependence, V_{max}, and the binding site for uptake inhibitors such as desipramine (Povlock and Amara 1997). Because the cloned transporter is a target for the reuptake inhibitor, desipramine, it is thought to reflect the native transporter in the brain and peripheral tissues. However, in the periphery, two native reuptake processes (neuronal uptake, 'uptake$_1$' and extraneuronal uptake, 'uptake$_2$') have been recognised for over 30 years and recently, a third, desipramine-insensitive uptake site has been found in hepatocytes. These are quite distinct uptake mechanisms because they have different substrate affinities and antagonist sensitivities. As yet, few studies have investigated the possibility that more than one uptake process exists in the brain but since two mRNAs for noradrenaline transporters have been isolated from brain tissue (Pacholczyk, Blakely and Amara 1991) there could be more than one transcription factor. Also, the so-called 'extraneuronal transporter' for noradrenaline, responsible for 'uptake 2', has recently been found on glial cells in the brain (Russ *et al.* 1996). At the very least, intracellular messengers could modify substrate affinity of the transporter, by causing its phosphorylation or glycosylation (Bönisch, Hammermann and Brüss 1998), and so markedly affect its function. Whether or not there are different gene products, splice variants, or posttranslational changes, it has been suggested that abnormal distributions of functionally distinctive noradrenaline transporters could underlie some psychiatric and neurological disorders.

METABOLISM

After reuptake into the cytosol, some noradrenaline may be taken up into the storage vesicles by the vesicular transporter and stored in the vesicles for subsequent release (see above). However, it is thought that the majority is broken down within the cytosol of the nerve terminal by monoamine oxidase (MAO; EC1.4.3.4). A second degradative enzyme, catechol-*O*-methyl transferase (COMT; EC2.1.1.6), is found mostly in non-neuronal tissues, such as smooth muscle, endothelial cells or glia. The metabolic pathway for noradrenaline follows a complex sequence of alternatives because the metabolic product of each of these enzymes can act as a substrate for the other (Fig 8.8). This could enable one of these enzymes to compensate for a deficiency in the other to some extent.

MAO is bound to the outer membrane of mitochondria and is responsible for the oxidative deamination of noradrenaline. There are two isoforms of this enzyme, MAO-A

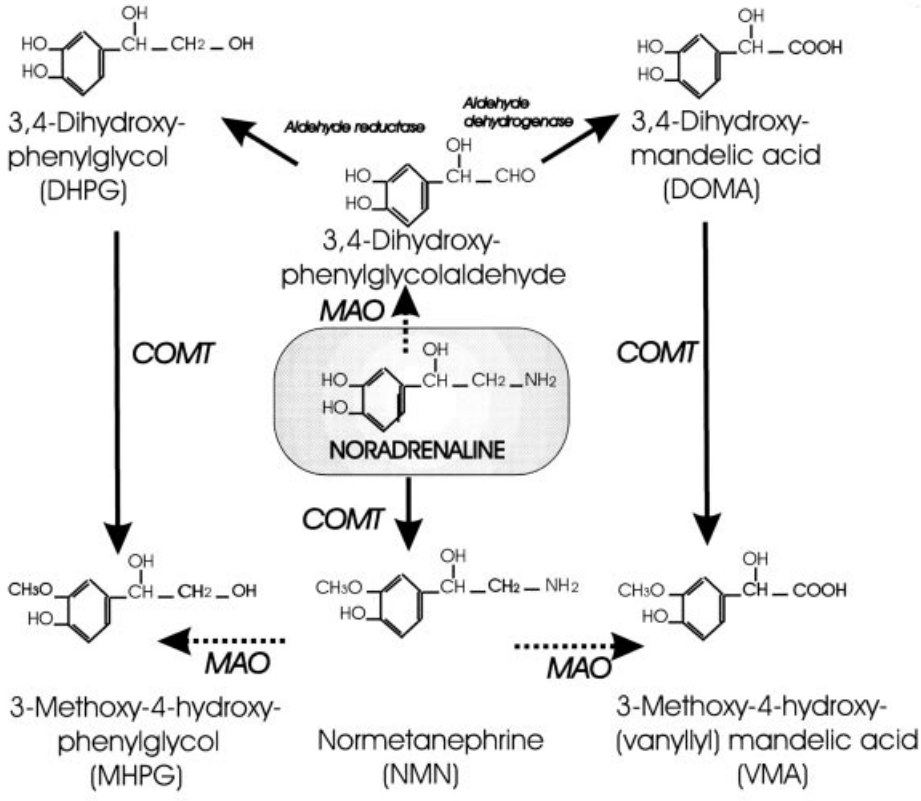


Figure 8.8 The metabolic pathway(s) for noradrenaline. MAO is responsible for the oxidative deamination of noradrenaline derivatives while COMT *O*-methylates noradrenaline. Most intraneuronal metabolism involves MAO while COMT is mainly found extraneuronally. However, both these enzymes can act on each other's products, yielding a complex cocktail of metabolites. The reasons for this complex network of metabolites are not known

and MAO-B, which hybridise to different cDNAs and are encoded by different genes on the X chromosome. MAO-A is the more important *in vivo* because it preferentially metabolises noradrenaline. However, *in vitro*, MAO-B will metabolise noradrenaline at high substrate concentrations. MAO probably also has an important role in development: a genetic deficiency of MAO-A causes some mental retardation and a tendency to bouts of aggression. MAO-B deficiency has no overt effects in the phenotype but a deficiency of both enzymes causes severe mental retardation and behavioural problems (Lenders *et al.* 1996). Of course, some of these abnormalities could be due to disruption of the metabolism of other monoamines, such as tyramine, which are also substrates for MAO.

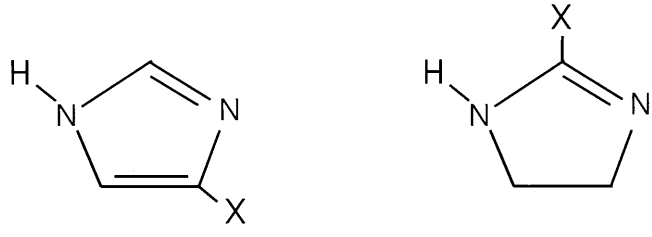
Certainly, such a complex system for metabolism of noradrenaline (which is shared with the other catecholamines) strongly suggests that its function extends beyond that of merely destroying transmitter sequestered from the synapse. However, as yet, little is known about the regulation of this pathway and any influence it might have on noradrenergic transmission. One crucial, additional role for MAO appears to be the

regulation of the intraneuronal stores of noradrenaline. Its predominantly intraneuronal location would suggest that its primary function is to ensure that there is always a low concentration of cytoplasmic noradrenaline. What can happen when the concentration of cytosplasmic noradrenaline is increased is illustrated by amphetamine. This drug causes a rise in the cytoplasmic noradrenaline and results in increased binding of this transmitter to the cytoplasmic side of the transporter which then carries it out of the neuron. Importantly, this form of noradrenaline release ('retrotransport') is independent of neuronal activation or intracellular Ca^{2+}.

By maintaining low concentrations of cytoplasmic noradrenaline, MAO will also regulate the vesicular (releasable) pool of transmitter. When this enzyme is inhibited, the amount of noradrenaline held in the vesicles is greatly increased and there is an increase in transmitter release. It is this action which is thought to underlie the therapeutic effects of an important group of antidepressant drugs, the MAO inhibitors (MAOIs) which are discussed in Chapter 20.

Because of their lack of selectivity and their irreversible inhibition of MAO, the first MAOIs to be developed presented a high risk of adverse interactions with dietary tyramine (see Chapter 20). However, more recently, drugs which are selective for and, more importantly, reversible inhibitors of MAO-A (RIMAs) have been developed (e.g. moclobemide). These drugs are proving to be highly effective antidepressants which avoid the need for a tyramine-free diet.

Further interest in MAO has been aroused as a result of recent research on drugs with an imidazole or imidazoline nucleus (Fig. 8.9). Although many of these compounds are potent and selective α_2-adrenoceptor ligands (e.g. the agonist, clonidine, or the antagonist, idazoxan), not all the binding of these compounds is explained by their high affinity for α_2-adrenoceptors. It is now known that many of these drugs have their own binding sites that are now classified as imidazoline (I-) receptors. One of these, the so-called I_2-receptor, has been found on MAO-B but there is general agreement that the I_2-receptor is not the same as the catalytic site on the MAO enzyme. Instead, it is thought that the I_2-receptor is an allosteric modulator of the catalytic site on MAO which, when activated, reduces enzyme activity. So far, the function of this



Imidazoles

Atipamezole
Cimetidine
Histamine
Medetomidine

Imidazolines

Clonidine
Idazoxan
Monoxidine
p-Aminoclonidine
Phentolamine

Figure 8.9 The chemical structure of imidazole and imidazoline, together with some well-known derivatives

receptor is unknown but it has been suggested that a dysfunction of I_2-receptors could contribute to neurodegenerative disorders such as dementia and Parkinson's disease.

There is also some evidence for subtypes of COMT but this has not yet been exploited pharmacologically. Certainly, the majority of COMT is found as soluble enzyme in the cell cytosol but a small proportion of neuronal enzyme appears to be membrane bound. The functional distinction between these different sources of COMT is unknown. COMT inhibitors also exist (e.g. pyrogallol), mostly as catechol derivatives, but so far, most have proved to be highly toxic. Only recently have drugs been developed which are selective for COMT; one of these agents, tolcapone, is used currently in treatment of Parkinson's disease (see Chapter 15).

NORADRENERGIC RECEPTORS

The division of adrenoceptors into α- and β-types emerged some 50 years ago and was based on the relative potencies of catecholamines in evoking responses in different peripheral tissues. Further subdivision of β-adrenoceptors followed characterisation of their distinctive actions in the heart (β_1), where they enhance the rate and force of myocardial contraction and in the bronchi (β_2), where they cause relaxation of smooth muscle. The binding profile of selective agonists and antagonists was the next criterion for classifying different adrenoceptors and this approach is now complemented by molecular biology. The development of receptor-selective ligands has culminated in the characterisation of three major families of adrenoceptors (α_1, α_2 and β), each with their own subtypes (Fig. 8.10). All these receptors have been cloned and belong to the superfamily of G-protein-coupled receptors predicted to have seven transmembrane domains (Hieble, Bondinell and Ruffolo 1995; Docherty 1998).

The α_1-subgroup is broadly characterised on the basis of their high affinity for binding of the antagonist, prazosin, and low affinity for yohimbine but they seem to be activated to the same extent by catecholamines. There are at least three subtypes which for historical reasons (Hieble, Bondinell and Ruffolo 1995) are now designated α_{1A}, α_{1B} and α_{1D}. α_{1A}-Adrenoceptors are distinguished by their selective antagonists tamsulosim

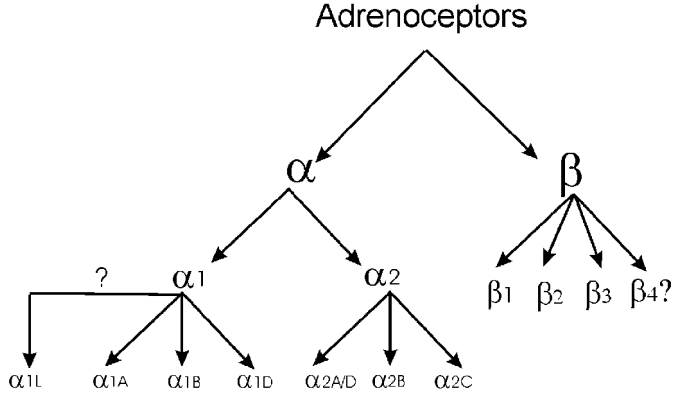


Figure 8.10 Subdivisions of α- and β-adrenoceptor families

and WB4101 but, whereas α_{1B}- and α_{1D}-adrenoceptors have ligands that distinguish them from α_{1A}-adrenoceptors, and from each other, these ligands bind to other transmitter receptors and so they are not really selective. An alternative classification (also based on sensitivity to prazosin) characterised two classes of receptor: α_{1H} and α_{1L} receptors. Whereas those classified as α_{1H} seem to overlap with α_{1A}, α_{1B} and α_{1D} receptors (and are now regarded as the same), there is no known equivalent of the α_{1L} receptor. Although it is still tentatively afforded the status of a separate receptor, it has been suggested that it is an isoform of the α_{1A} subtype (Docherty 1998).

All α_1-adrenoceptors are coupled to the $G_{q/11}$ family of G-proteins and possibly other G-proteins as well. When activated, they increase the concentration of intracellular Ca^{2+} through the phospholipase C/diacyl glycerol/IP_3 pathways (Ruffolo and Hieble 1994) but other routes have been suggested too. These include: direct coupling to Ca^{2+} (dihydropyridine sensitive and insensitive) channels, phospholipase D, phospholipase A2, arachidonic acid release and protein kinase C. Their activation of mitogen-activated protein (MAP) kinase suggests that they also have a role in cell proliferation. All three subtypes are found throughout the brain but their relative densities differ from one region to another. A detailed review of the classification of α_1-adrenoceptors is to be found in Zhong and Minneman (1999).

α_2-Adrenoceptors all have a high affinity for yohimbine (although there are species differences) and are negatively coupled to a *Pertussis* toxin-sensitive $G_{i/o}$-protein in some tissues whereas, in others, they appear to be insensitive to this toxin. Their activation inhibits target cell activity, resulting from reduced cAMP production, an increase in K^+ current and a reduced Ca^{2+} current. However, stimulatory effects of α_2-adrenoceptors have also been reported, although the underlying mechanisms are unclear. Paradoxically, the different receptor subtypes are characterised by their affinity for prazosin: the α_{2A}-subtype (found in human platelets) has a low affinity for this ligand, while the α_{2B}-subtype (isolated from neonatal rat lung) has a higher affinity. The α_{2C}-adrenoceptor, first isolated from opossum kidney (OK) cells, is distinguished by its characteristic relative affinities for yohimbine and prazosin. There is also functional evidence for an α_{2D}-adrenoceptor. This has not been granted the status of a separate subtype, partly because it has not been possible to produce a distinctive receptor clone, and it is now regarded as the rodent homologue of the human α_{2A}-subtype. (Bylund *et al*. 1994).

It is the $\alpha_{2A/D}$-adrenoceptor that predominates in the locus coeruleus and this subtype seems to be responsible for reducing neuronal excitability and transmitter release. Strangely, immunocytochemical studies suggest that most α_{2C}-receptors are intracellular. The explanation for this finding and its functional implications are as yet unknown but it could reflect differences in intracellular trafficking of different receptor subtypes.

Contrasting with the α_2-adrenoceptors, β-adrenoceptors activate cAMP synthesis and are coupled to G_s-proteins. The β_1 and β_2 subtypes were distinguished in the 1960s but the β_3-adrenoceptor has been characterised only recently, largely on the basis of its low affinity for the antagonist, propranolol. Unlike β_1- and β_2-adrenoceptors, this subtype is not found in the brain but probably has an important role in lipolysis by mobilising triglyceride stores and promoting thermogenesis (Giacobino 1995).

In the brain, autoradiography has shown that β_1- and β_2-adrenoceptors have quite distinct distributions. Thus, approximately 60% of the β-adrenoceptors in the neocortex are of the β_1-subtype while, in the cerebellum, it is the β_2-subtype that

predominates. As yet, the functional implications of this uneven distribution are unclear and await the development of more subtype selective agents. However, unlike the α-adrenoceptor families, the affinity of catecholamines for β-adrenoceptors differs markedly: noradrenaline is a relatively weak agonist at the β_2-subtype whereas it is more potent than adrenaline at β_3-receptors.

Electrophysiological studies of the β-adrenoceptor have produced complex findings. In cardiac tissue, their activation leads to an increase in Ca^{2+} conductance and so they are regarded as excitatory receptors. β-Adrenoceptor activation in cortical pyramidal cells causes an increase in excitability mediated by a reduction of a Ca^{2+}-activated K^+ current. A different response is evoked in thalamic relay neurons where these receptors cause depolarisation and an increase in input conductance by resetting a hyperpolarisation-induced cation current. In the dentate gyrus their activation causes an increase in voltage-dependent Ca^{2+} currents through opening of Ca^{2+} channels.

Because of these disparate findings, it is difficult to assign particular electrophysiological changes to each of the adrenoceptors let alone to noradrenaline, more generally. A shortage of selective ligands aggravates this problem. Another difficulty concerns the uncertain location of the receptors responsible for initiating any changes. In tissue slices, the target receptors could be located on interneurons, rather than mediating direct axo-somatic interactions, for instance. The net effect of receptor activation could also depend on the underlying tonic activity of the target cell as well as the influence of other neurotransmitters that converge on the same G-protein.

Despite these obstacles, it has been suggested that the overall effect of interactions between noradrenaline and its receptors could be to increase the excitability and responsiveness of the target cells. This could make an important contribution to the governance of arousal and selective attention (McCormick, Pape and Williamson 1991). Another, similar suggestion is that noradrenergic transmission increases the signal-to-noise ratio of cell responses to incoming stimuli: i.e. it reduces the basal activity of target cells but increases their response to excitatory inputs. This is the so-called 'Kety hypothesis' (reviewed by Harley 1987).

WHAT IS THE FUNCTION OF NORADRENALINE IN THE BRAIN?

Because central noradrenergic pathways are so diffuse, and the synaptic effects of noradrenaline have a comparatively slow time-course, these neurons could have a wide range of functions, depending on the brain region being targeted and the neurobiological status of the individual. In general terms, however, it is agreed that noradrenergic neurons influence arousal. This encompasses not only the sleep/waking cycle (see Chapter 22) but also more specific activities, such as selective attention and vigilance (Aston-Jones *et al.* 1994). Indeed, depression and anxiety, both of which are relieved by drugs that modify noradrenergic transmission, can be regarded as arousal disorders. Yet, despite nearly 40 years of research, it is still uncertain whether an increase in noradrenergic transmission contributes to unpleasant emotional responses to environmental stimuli (e.g. fear and anxiety) or whether its main role is to ameliorate the emotional impact of such stimuli (i.e. contributes to 'coping').

Many electrophysiological studies have shown that single-unit activity of noradrenergic neurons in the locus coeruleus is increased by sensory stimuli. Effective stimuli range from those causing physical discomfort (e.g. footshock) and interoceptive

cues (e.g. hypoglycaemia) to certain arousing environmental stimuli (e.g. the approach of the experimenter). On the basis of this evidence, it has been suggested that central noradrenergic neurons could form part of an 'alarm system'. This would be consistent with the attenuation of the neuronal response on repeated presentation of the test stimulus, the presumption being that this change underlies behavioural habituation.

The precise features of environmental stimuli that provoke increased noradrenergic transmission are unclear but recent experiments using *in vivo* microdialysis suggest that neither 'novelty' nor the 'aversiveness' of the stimulus alone is responsible (McQuade and Stanford 2000). Electrophysiological studies suggest that it could be the 'salience' (i.e. its significance or relevance to the individual), or change in salience, of a stimulus that is the key factor and that increased noradrenergic transmission in the brain mediates changes in selective attention.

Even if this turns out to be the case, it is likely that noradrenergic neurons in different brain regions make different contributions to this process. This complication is suggested by the results of a recent microdialysis study in which release of noradrenaline in response to the sound of a buzzer alone was provoked after repeated

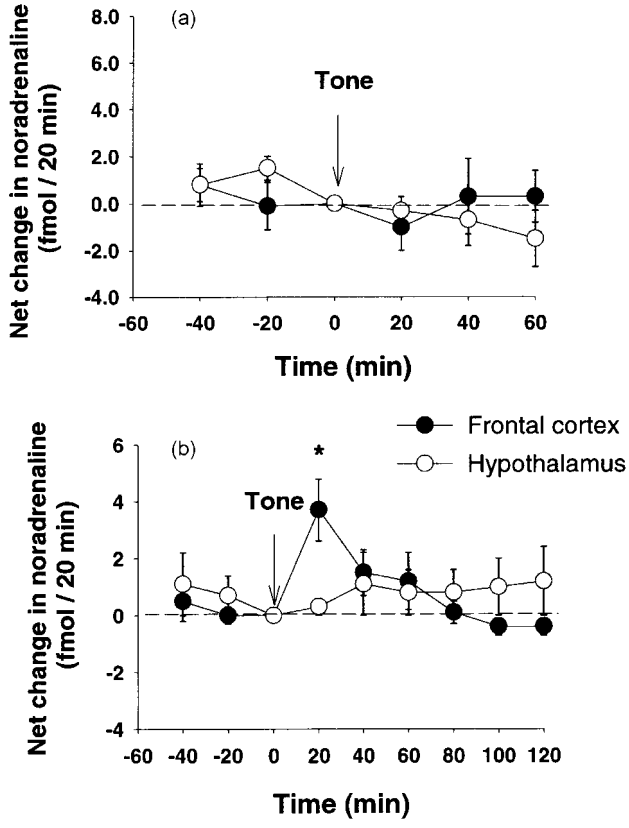


Figure 8.11 Noradrenaline efflux, measured by microdialysis, in the rat frontal cortex and hypothalamus. (a) Repeated exposure to a tone, alone, has no effect on noradrenaline efflux in either brain region. (b) After repeated pairing of the tone with transfer of the rat to a brightly lit (aversive) arena, the sound of the tone alone triggers a significant (*: $P < 0.05$, cf. last basal sample) increase in noradrenaline efflux in the frontal cortex, but not the hypothalamus. (Based on a figure from McQuade and Stanford 2000)

pairing of this normally neutral stimulus with transfer of the rats to a brightly-lit novel arena. This adaptive change occurred in the frontal cortex but not the hypothalamus suggesting that only noradrenergic neurons innervating the former brain region (i.e. those arising from the locus coeruleus) show adaptive changes in response to a change in the salience of an environmental stimulus (McQuade and Stanford 2000) (Fig. 8.11).

Another concept is that noradrenergic transmission influences the emotional impact of a given stimulus, i.e. individuals' ability to 'cope'. One obvious possibility is that inadequate noradrenergic transmission explains depression, whereas moderate activity provokes attentive interest that is vital for appropriate cognitive function, and excessive noradrenergic activation culminates in anxiety or agitation. Evidence supporting this single axis for central noradrenergic function/dysfunction is discussed in Chapters 19 and 20.

It is equally possible that the role and consequences of central noradrenergic transmission depend on the type or severity of the stimulus and individual differences in the neurobiological coding of behaviour. This would mean that the optimal behavioural response to a given environmental stimulus requires a specific increase in noradrenergic transmission. The optimal response could be determined genetically or by the individuals' previous experience of that stimulus, or both. Deviation of the response, in either direction (i.e. either under- or overactivity), would then result in a deficit in 'coping' (Fig. 8.12(a)). However, it is also possible to envisage disruption of this neurochemical coding of behaviour in the ways illustrated in Figs 8.12(b) and 8.12(c). If there is a shift of the curve to either the right or the left, then the noradrenergic response that would be optimal in normal subjects now produces a suboptimal coping response. In the case of a shift to the left, a reduction in noradrenergic transmission would be required to restore optimal coping whereas for a shift to the right, an increase would be required.

This hypothetical scheme means that there are two possible sources of mismatch that could account for an abnormal behavioural response to a given stimulus and result in an 'inability to cope'. One is that the underlying coding is correct but it is the noradrenergic response evoked by the stimulus that is inappropriate. A second is that the amplitude of the noradrenergic response to arousing stimuli is normal but the underlying coding is not.

Several findings support this model. For instance, an early report suggested that there is a positive correlation between the density of (postsynaptic) β-adrenoceptors in rat cortex and behavioural resistance to a mild environmental stress (novelty and frustration) but a negative correlation between these parameters when the stress is intensified (Stanford and Salmon 1992). More recently, it has been proposed that the phasic response of neurons in the locus coeruleus (which governs 'attentiveness') depends on their tonic activity (which determines arousal). Evidence suggests that the relationship between these two parameters is described by a bell-shaped curve and so an optimal phasic response is manifest only at intermediate levels of tonic activity (Rajkowski *et al.* 1998).

Obviously, it is extremely unlikely that noradrenergic transmission is the sole factor to determine the behavioural response to even simple environmental stimuli. Indeed, a bell-shaped dose–response curve immediately suggests the intervention of one or more additional factors (neurotransmitters?). Such interactions with other neurotransmitters could well define the relationship between noradrenergic transmission and the coding of the coping response.

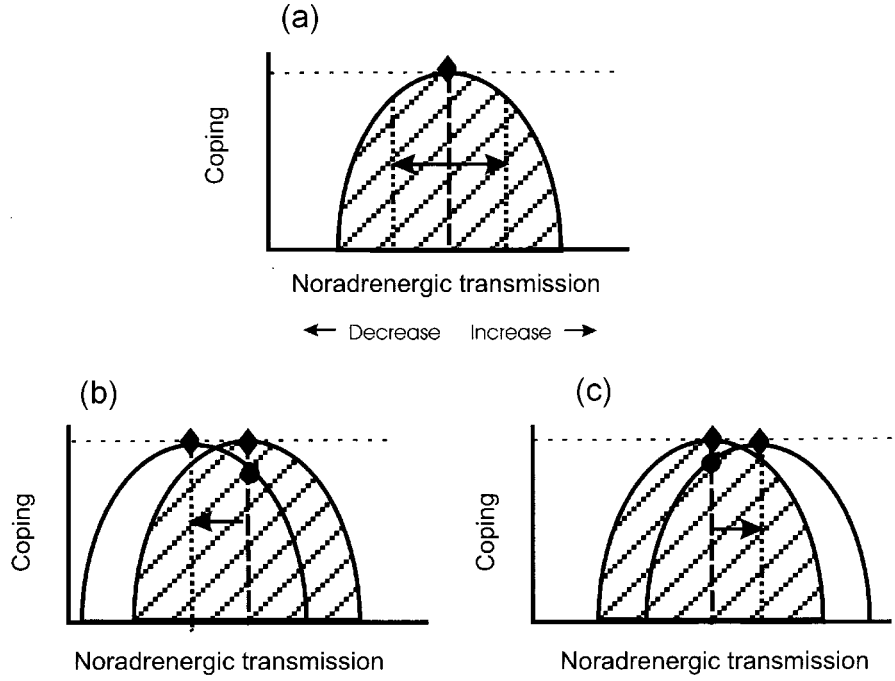


Figure 8.12 Schematic diagram showing the hypothetical relationship between noradrenergic transmission and an individual's ability to 'cope' with aversive environmental stimuli. (a) Optimal coping is attained when the brain rallies a specific noradrenergic response which is determined either genetically and/or by previous experience of the stimulus. Either a reduction or an increase in noradrenergic transmission produces a functional mismatch and diminishes coping. (b) The hatched area depicts the normal relationship between changes in noradrenergic transmission and coping with aversive stimuli (as illustrated in (a)). In these normal subjects, optimal coping is attained when the noradrenergic response to a specific stimulus corresponds to that marked (◆). If there is a leftward shift of the curve that describes the neurochemical coding of coping, then the (predetermined) noradrenergic response that would be optimal in normal individuals now produces suboptimal coping (●). One remedy for such a dysfunction is to reduce noradrenergic transmission so as to restore optimal coping. Similarly, in the case of a rightward shift of the coping curve (c), a predetermined noradrenergic response to a specific stimulus, that would be optimal in normal individuals, will again produce suboptimal coping (●). This time, the remedy is to increase noradrenergic transmission. In both (b) and (c) an alternative way to restore optimal coping would be to reverse the shift in the noradrenergic transmission/coping curve. This could explain the changes in mood that occur after chronic administration of drugs that cause long-latency changes in neurochemical factors that influence noradrenergic transmission (see Chapters 19 and 20)

SUMMARY

Much remains to be learned about the neurochemical regulation of noradrenergic transmission and even more research is required before we can define the role(s) of this neurotransmitter in the brain. Nevertheless, it is evident that these neurons are a crucial component of the network of monoamine influences on the limbic system and that they

are capable of both short- and long-term adaptive changes that will influence emotion, motivation, cognition and many other aspects of behaviour.

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