Neurotransmitters, Drugs and Brain Function.
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9 5-Hydroxytryptamine

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INTRODUCTION

The idoleamine, 5-hydroxytryptamine (5-HT), like the catecholamines, dopamine and noradrenaline, is found in both the periphery and the brain. In the cardiovascular system, it causes marked vasoconstriction and it is from this action that its alternative name, 'serotonin', derives. This chapter will concentrate on the aspects of 5-HT transmission which have made the greatest advances in recent years, particularly those for which some important and interesting questions remain unanswered. Although this material will obviously focus on 5-HT in the brain, the neurochemical mechanisms that regulate 5-HT transmission, such as its synthesis and inactivation, will apply generally to 5-HT-containing cells in the periphery (e.g. enterochromaffin cells in the gut and neurons of the myenteric plexus). All these processes, together with some well-known drugs that affect them, are summarised in Fig. 9.1.

DISTRIBUTION IN THE CNS

As with the other monoamines, the distribution of 5-HT-releasing neurons in the brain was first characterised in the 1960s using the Falck–Hillarp histochemical technique whereby 5-HT is converted to a compound that is fluorescent under ultra-violet light. This showed that the cell bodies of 5-HT neurons aggregate around the midline of the upper brainstem, forming distinct clusters (or nuclei) (Fig. 9.2). Since then, 5-HT neurons have been found in the noradrenergic locus coeruleus and the area postrema as well. Yet, despite this relatively restricted distribution of cell bodies, their processes project more or less throughout the whole neuraxis. For a detailed review of this topic, see Jacobs and Azmitia (1992) but an outline of key features is given here.

The clusters of 5-HT cell bodies (the so-called Raphé nuclei) were originally perceived as forming nine separate nuclei (designated B1–B9) but current nomenclature has reclassified these to some extent so that, currently the nuclei incorporate cell bodies from more than one of those described originally (Table 9.1). Despite these changes, all these nuclei are still regarded as forming two major groups.

The so-called 'inferior' group (B1–B4) projects mainly to brainstem nuclei, the head nuclei of some cranial nerves and the spinal cord. This means that these neurons are well placed for serving a key role in regulation of motor activity, autonomic function and nociception. In addition, there are numerous interconnections between the different

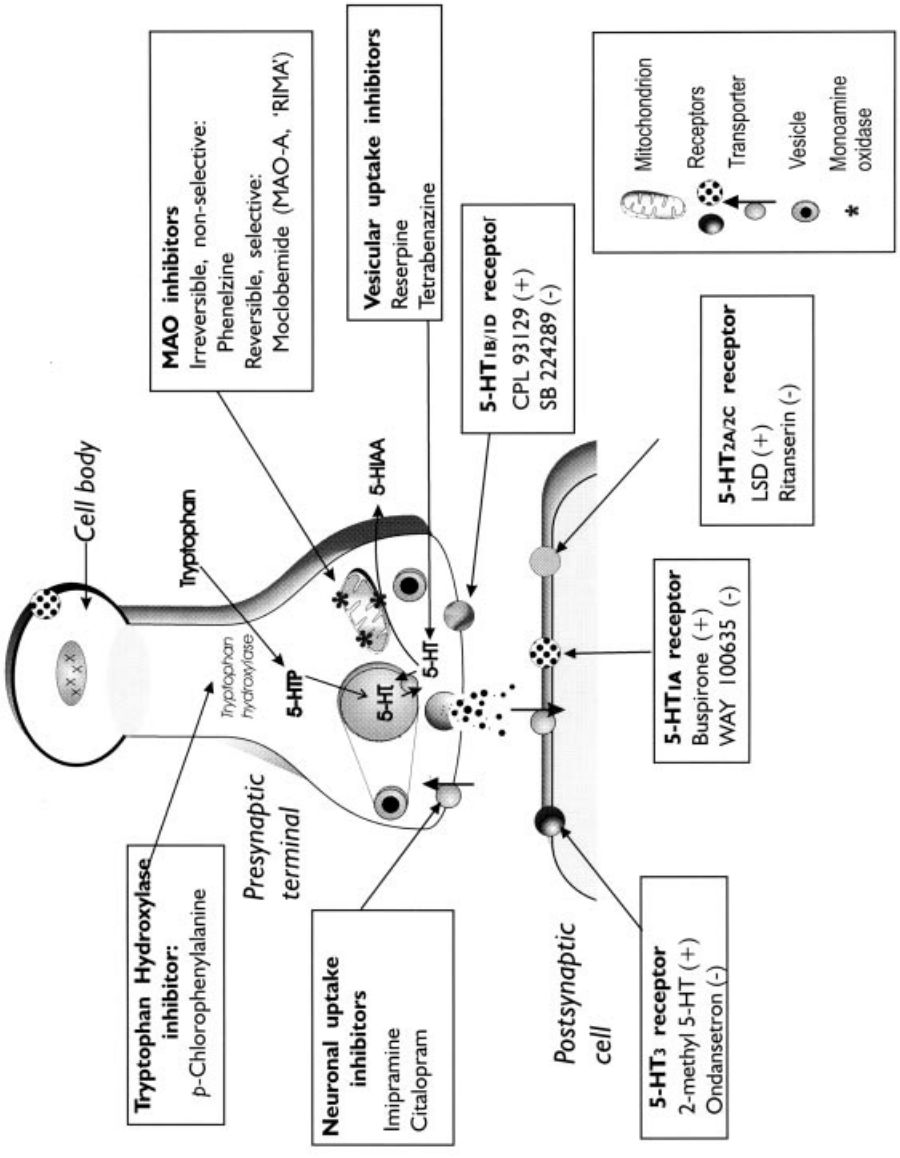

Figure 9.1 The distribution of 5-HT neurons in the brain. The cell bodies are clustered in nuclei (B1–B7) in the pons/upper medullary regions of the brainstem. The rostral cluster ('superior group') project mainly to forebrain areas while the caudal ('inferior') group projects mainly to the medulla and spinal cord. Collectively, these neurons innervate most regions of the central nervous system

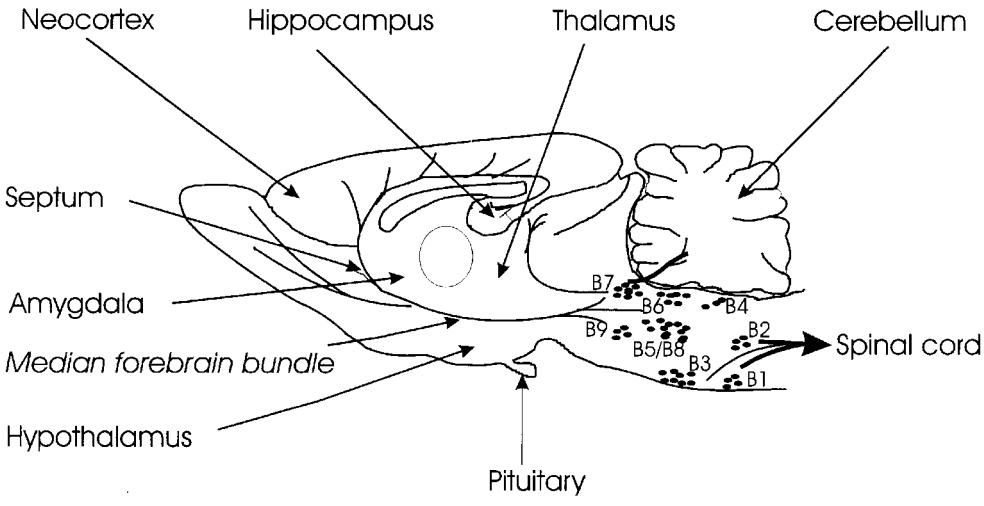

Figure 9.2 The site of action of drugs that modify 5-HT transmission

5-HT nuclei, as well as connections with other monoaminergic nuclei in the brainstem which are also implicated in these physiological functions.

Neurons in the 'superior' group (B5–B9) project rostrally, generally ipsilaterally, in six fibre tracts. The most prominent of these is the median forebrain bundle which contains both myelinated and unmyelinated 5-HT fibres. These mainly innervate limbic and sensory areas of the forebrain. Although extensive branching of the neuronal processes results in a considerable overlap in the terminal axonal fields of the different nuclei, there is evidence for some topographical organisation of the areas to which different nuclei project (Fig. 9.3). For instance, whereas fibres emanating from the dorsal Raphé nucleus (DRN) are the major source of 5-HT terminals in the basal ganglia and cerebellum, neurons in the median Raphé nucleus (MRN) provide the major input to the hippocampus and septum.

There is also some evidence for morphological differences between DRN and MRN neurons which could impinge on their function. Thus, the terminals of neurons from the DRN are relatively fine, unmyelinated, branch extensively and seem to make no specialised synaptic contacts, suggesting *en passant* release of 5-HT (type I). In contrast,

Table 9.1 The main subdivisions of 5-HT nuclei in the brain

Superior		Inferior	
B6 and B7	Dorsal Raphé nucleus	B1 and B4	Nucleus Raphé pallidus
B5 and B8	Median Raphé nucleus	B2	Nucleus Raphé obscurus
B8	Caudal linear nucleus	B3	Nucleus Raphé magnus
B9	Nucleus prosupralemniscus	B1 and B3	Neurons of the lateral paragigantocellular nucleus and the intermediate reticular nuclei
			Cells in the area postrema

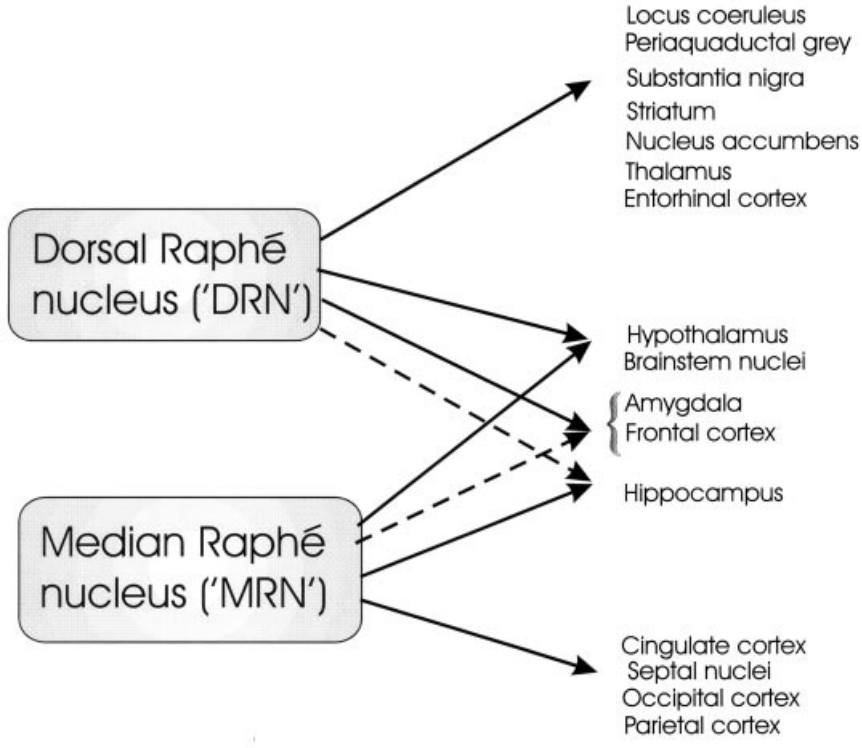

Figure 9.3 Brain regions to which neurons in the dorsal and median Raphé nuclei project. Some areas are innervated by neurons from both nuclei (e.g. hypothalamus) whereas others are innervated predominantly by either the MRN (e.g. the hippocampus) or the DRN (e.g. the amydgala)

those from the MRN are broader, often myelinated, with large varicosities, and they seem to form specialised synaptic contacts, suggesting targeted release of 5-HT (type II). The existence of co-transmitters, especially substance P, thyrotropin releasing hormone (TRH) and enkephalin, gives further options for functional specialisation of different neurons but, as yet, the distribution of these peptides within different nuclei has provided no specific clues as to how this might occur. In any case, species differences in the distribution of co-transmitters is a confounding factor.

In short, although the 5-HT system seems to have a rather non-specific influence on overall brain function, in terms of the brain areas to which these neurons project, there is clearly much to be learned about possible functional and spatial specialisations of neurons projecting from different nuclei.

SYNTHESIS

The first step in the synthesis of 5-HT is hydroxylation of the essential amino acid, tryptophan, by the enzyme tryptophan hydroxylase (Fig. 9.4). This enzyme has several features in common with tyrosine hydroxylase, which converts tyrosine to *l*-DOPA in

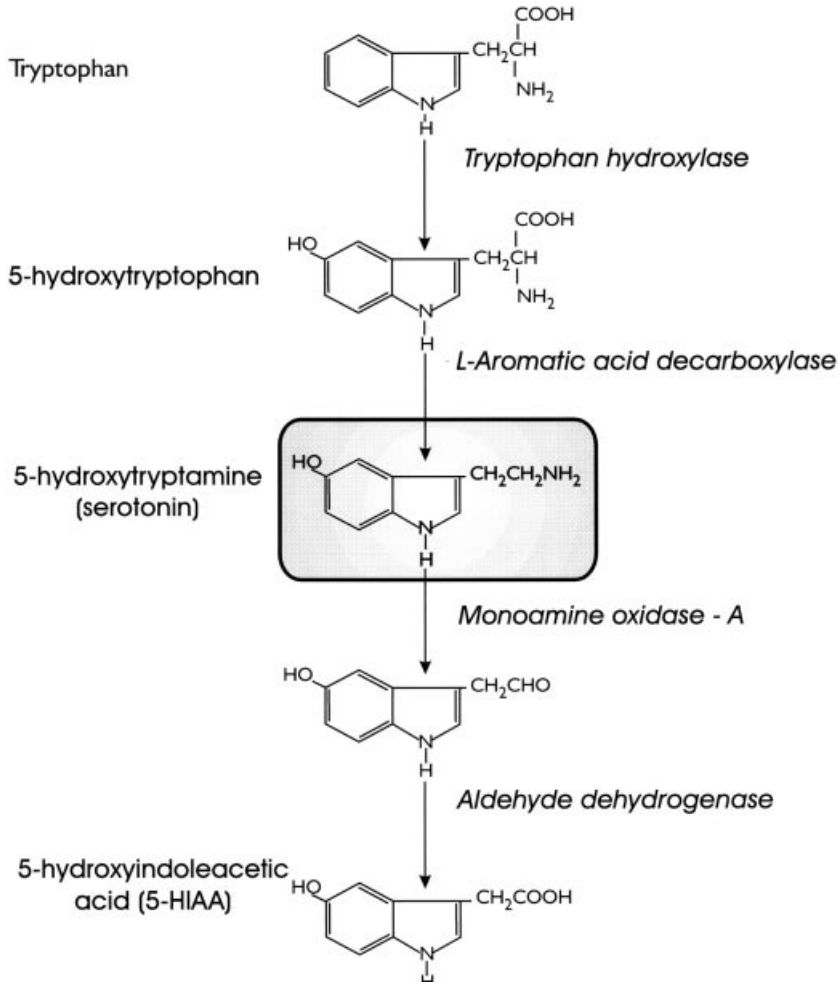

Figure 9.4 The synthesis and metabolism of 5-HT. The primary substrate for the pathway is the essential amino acid, tryptophan and its hydroxylation to 5-hydroxytryptophan is the rate-limiting step in the synthesis of 5-HT. The cytoplasmic enzyme, monoamine oxidase (MAO_A), is ultimately responsible for the catabolism of 5-HT to 5-hydroxyindoleacetic acid

the noradrenaline synthetic pathway. First, it has an absolute requirement for O_2 and the reduced pterin co-factor, tetrahydrobiopterin. Second, hydroxylation of tryptophan, like that of tyrosine, is the rate-limiting step for the whole pathway (reviewed by Boadle-Biber 1993) (see Chapter 8). However, unlike the synthesis of noradrenaline, the availability of the substrate, tryptophan, is a limiting factor in the synthesis of 5-HT. Indeed, the activated form of tryptophan hydroxylase has an extremely high K_m for tryptophan (50 μM), which is much greater than the concentration of tryptophan in the brain (10–30 μM). This means that not only is it unlikely that this enzyme ever becomes saturated with its substrate but also that 5-HT synthesis can be driven by giving extra tryptophan.

This influence of tryptophan availability on the rate of synthesis of 5-HT has some interesting implications. First, it predicts that a dietary deficiency of tryptophan could lead to depletion of the neuronal supply of releasable 5-HT. Indeed, this has been confirmed in humans to the extent that a tryptophan-free diet can cause a resurgence of depression in patients who were otherwise in remission (see Chapter 20). In contrast, a tryptophan-high diet increases synthesis and release of 5-HT. In fact, when given in combination with other drugs that augment 5-HT transmission (e.g. an MAO inhibitor or a 5-HT reuptake inhibitor), tryptophan can cause a life-threatening delirium known as the 'serotonin syndrome' (Gillman 1999).

Transport of tryptophan across the blood–brain barrier and neuronal membranes relies on a specific carrier for large neutral amino acids (LNAAs). Thus, although an increase in the relative concentration of plasma tryptophan, either through dietary intake or its reduced metabolism in a diseased liver, increases its transport into the brain, other LNAAs (such as leucine, isoleucine or valine) can compete for the carrier. This process forms the basis of an intriguing theory linking the intake of carbohydrates in the diet with an individual's mood. It is known that consumption of carbohydrates increases secretion of insulin which, in addition to its well-known glucostatic role, promotes uptake of LNAAs by peripheral tissues. However, it seems that tryptophan is less affected by insulin than the other LNAAs in this respect and so its relative concentration in the plasma increases, thereby increasing its transport into the brain (see Rouch, Nicolaidis and Orosco 1999). The resulting increase in synthesis and release of 5-HT is claimed to enhance mood. Although this scheme is rather controversial, it has been suggested as an explanation for the clinical improvement in some patients, suffering from depression or premenstrual tension, when they eat carbohydrates. It has also been suggested to underlie the carbohydrate-craving experienced by patients suffering from Seasonal Affective Disorder (Wurtman and Wurtman 1995).

Not a great deal is known about factors that actually activate tryptophan hydroxylase. In particular, the relative contribution of tryptophan supply versus factors that specifically modify enzyme activity under normal dietary conditions is unknown. However, removal of end-product inhibition of tryptophan hydroxylase has been firmly ruled out. Also, it has been established that this enzyme is activated by electrical stimulation of brain slices, even in the absence of any change in tryptophan concentration, and so other mechanisms are clearly involved.

So far, it has been established from *in vitro* studies that the enzyme undergoes phosphorylation, a process that changes the conformation of the enzyme protein and leads to an increase in its activity. This involves Ca^{2+}/calmodulin-dependent protein kinase II and cAMP-dependent protein kinase which suggests a role for both intracellular Ca^{2+} and enzyme phosphorylation in the activation of tryptophan hydroxylase. Indeed, enzyme purified from brain tissue innervated by rostrally projecting 5-HT neurons, that have been stimulated previously *in vivo*, has a higher activity than that derived from unstimulated tissue but this increase rests on the presence of Ca^{2+} in the incubation medium. Also, when incubated under conditions which are appropriate for phosphorylation, the K_m of tryptophan hydroxylase for its co-factor and substrate is reduced whereas its V_{max} is increased unless the enzyme is purified from neurons that have been stimulated *in vivo*, suggesting that the neuronal depolarisation *in vivo* has already caused phosphorylation of the enzyme. This is supported by evidence that the enzyme activation caused by neuronal depolarisation is blocked by a Ca^{2+}/calmodulin protein kinase inhibitor. However, whereas depolarisation

alone increases enzyme V_{max}, it does not appear to affect the enzyme K_m and so a firm link between neuronal depolarisation and enzyme phosphorylation has yet to be established.

The apparent reliance of enzyme activation on phosphorylation and intracellular Ca^{2+} gives a clue as to how the rate of 5-HT synthesis might be coupled to its impulse-evoked release. Certainly, the impulse-induced increase in intracellular Ca^{2+}, and/or activation of the G protein-coupled receptors that govern synthesis of cAMP, could modify the activity of tryptophan hydroxylase. Indeed, this could explain why activation of either somal 5-HT_{1A} autoreceptors in the Raphé nuclei (which depress the firing rate of 5-HT neurons) or terminal 5-HT_{1B} autoreceptors (which depress 5-HT release) can reduce the production of cAMP and attenuate 5-HT synthesis.

The product of the hydroxylation of tryptophan, 5-hydroxytryptophan, is rapidly decarboxylated to 5-HT by a specific decarboxylase enzyme. This is generally thought to be a soluble enzyme which suggests that 5-HT is synthesised in the cytoplasm, before it is taken up into the storage vesicles. If this is the case, then considerable losses might be incurred from its metabolism by monoamine oxidase before it reaches the storage vesicles. Indeed, this could explain why 5-HT turnover seems to greatly exceed its rate of release.

The high affinity of the decarboxylase enzyme for its substrate (10 μM in the brain) makes it unlikely that this stage could ever become rate-limiting for the pathway as a whole. Nevertheless, the K_m for this enzyme is considerably higher than tissue concentrations of 5-hydroxytryptophan and so, again, supply of this substrate is likely to be a crucial factor.

Finally, as with the noradrenergic system, there is evidence for long-term changes in the rate of synthesis of 5-HT that are triggered by prolonged changes in its rate of release. These can be traced to the rate of gene transcription and the ensuing synthesis of enzyme protein ('enzyme induction'). It has even been shown that mRNA for tryptophan hydroxylase shows a daily rhythm in cultured eye-cups maintained in the dark. Again, not a great deal is known about the underlying control mechanisms but the synthesis of tryptophan hydroxylase, at least, is increased by exposure of 5-HT neurons *in vivo* to the growth factor, brain-derived neurotrophic factor ('BDNF; Siuciak *et al*. 1998). Steroid hormones also seem to modulate tryptophan hydroxylase gene transcription but research in this area is confounded by the variation in this effect across different tissues and different hormones, with both increases and decreases being reported.

STORAGE

As with the other monoamines, 5-HT is found primarily in storage vesicles (30–35 nm diameter) where 'serotonin-binding proteins' (SBPs) have also been identified. These seem to form a macromolecular complex with 5-HT. In fact, three such proteins have now been characterised, but only one of them, 45 kDa SBP, appears to be secreted into the synapse along with 5-HT. Whether they serve any role other than forming an osmotically inert storage matrix for 5-HT is unknown.

In other respects the storage of 5-HT resembles that of noradrenaline with its uptake by vesicles resting on energy-dependent, 'vesicular monoamine transporters' (VMATs) (see Chapter 8). Functional disruption of this transporter, either through competitive inhibition (e.g. by methylenedioxymethamphetamine (MDMA, 'Ecstasy')) or dissipation

of the pH gradient across the vesicle membrane that drives the uptake of 5-HT (e.g. by MDMA, reserpine and *p*-chloroamphetamine) explains why these compounds deplete the vesicular pool of 5-HT.

RELEASE

Impulse-evoked release of 5-HT, like that of noradrenaline, is subject to fine control by a system of autoreceptors, in particular 5-HT_{1A} receptors on the cell bodies of neurons in the Raphé nuclei and 5-$HT_{1B/1D}$ receptors on their terminals. Because these are all $G_{i/o}$ protein-coupled receptors, their activation reduces the synthesis of cAMP so that 5-HT_{1A} agonists (or 5-HT itself) decrease neuronal excitability and the firing of Raphé neurons whereas activation of 5-$HT_{1B/1D}$ receptors seems to disrupt the molecular cascade that links the receptor with transmitter release (see Chapter 4).

There is some evidence that receptors for other neurotransmitters on 5-HT nerve terminals also modify release of 5-HT. These include nicotinic receptors (increase release from striatal synaptosomes), α_{2A}-adrenoceptors (depress cortical release) and H_3-receptors (cortical depression). Because changes in 5-HT release on activation of these receptors is evident in synaptosomal preparations, it is likely that these are true 'heteroceptors'.

Finally, the actions of the so-called '5-HT releasing agent', *d*-fenfluramine, which is well known for its anorectic effects, should be mentioned here. This compound inhibits 5-HT uptake but its metabolite, *d*-norfenfluramine, increases 5-HT release as do high doses of *d*-amphetamine. It is important to realise that this 5-HT release is independent of nerve impulses and the action of such compounds rests on their effects on the 5-HT transporters on the storage vesicles and terminal membrane. Once these drugs have been taken up into 5-HT neurons by the transporter, they cause 5-HT to leak out of its storage vesicles and, ultimately, to be extruded from the neuron by retrotransport (see below and Chapter 4 for further details).

Until recently, *d*-fenfluramine was used to control appetite, in preference to *d*-amphetamine, because it has a lower affinity for the catecholamine transporter and so its uptake into noradrenergic and dopaminergic neurons is much less than that of amphetamine. This is thought to explain why, at anorectic doses, this compound lacks the psychotropic effects and dependence-liability that are real problems with *d*-amphetamine. Unfortunately, despite this therapeutic advantage, this compound has had to be withdrawn from the clinic because of worries that it might cause primary pulmonary hypertension, valvular heart disease and even long-term neuropathy.

INACTIVATION

As with other monoamines, the actions of 5-HT are terminated by its reuptake from the synapse by another member of the family of Na^+/Cl^--dependent transporters. The 5-HT transporter has many features in common with its catecholamine equivalent (described fully in Chapter 8; see Fig. 8.7), including its presumed 12 transmembrane-spanning domains. However, the cloned 5-HT transporter has a K_m for 5-HT of about 450 nM whereas its K_i for both noradrenaline and dopamine is some ten thousand-fold greater (Povlock and Amara 1997) which means that it is relatively selective for uptake

of 5-HT. The uptake process itself requires the inward co-transport of one Na^+ ion and one Cl^- ion while K^+ (or H^+) is carried in the opposite direction. The energy required to maintain the ionic gradients that drive this process is provided by a Na^+/K^+-dependent ATPase.

As might be expected, mRNA for the 5-HT transporter is found in high concentrations in the Raphé nuclei but it is also found in other brain regions. Whether this means that non-5-HT neurons can synthesise this protein is unknown but there is some evidence that it is synthesised in astrocytes, at least. One complication is that there are multiple forms of mRNA for the 5-HT transporter, but there is, as yet, no evidence for transporter subtypes in the CNS. However, it must also be remembered that 5-HT transporters are found in the peripheral tissues, notably platelets, mast cells, the placental brush-border and adrenal chromaffin cells and it is possible that these are not all identical.

Inhibitors of 5-HT uptake include the tricyclic antidepressants and the selective serotonin reuptake inhibitors (SSRIs, which are discussed in detail in Chapter 20) as well as compounds like cocaine and *d*-amphetamine. Because cocaine is not transported into the neuron it is thought to bind to a site on the transporter protein. This has a negative allosteric effect on the protein and prevents binding of 5-HT to its domain. It has even been suggested that there could be an endogenous ligand for this site which regulates 5-HT uptake. By contrast, *d*-amphetamine is transported into neurons and so acts as a competitive inhibitor of 5-HT reuptake. Other inhibitors that are transported into the neuron, and which are thought to bind to the same site, include *p*-chloroamphetamine, MDMA and fenfluramine.

Of course, all these transporter inhibitors release 5-HT (see above) but exactly how they do this is uncertain. One suggestion is that, because they can also penetrate the cell membrane directly, they recycle continuously through their active transport into the cell and passive outward diffusion. This is thought gradually to dissipate the ionic gradient that is needed for the transporter to take up 5-HT into the neuron and so culminates in the outward transport of 5-HT into the synapse (Rudnick 1997). This action is compounded by their disruption of the vesicular transporters (VMATs) since, once they gain access to the neuron, they diminish the proton gradient required for the VMATs to function properly, possibly because they are weak bases (see Chapter 8; Fig. 8.6). This leads to leakage of 5-HT into the cytoplasm where its ensuing increased concentration ensures that a large pool is available for its retrotransport into the synapse.

Recent evidence indicates that the 5-HT transporter is subject to post-translational regulatory changes in much the same way as neurotransmitter receptors (Blakeley *et al.* 1998). Protein kinase A and protein kinase C (PKC), at least, are known to be involved in this process. Phosphorylation of the transporter by PKC reduces the V_{max} for 5-HT uptake and leads to sequestration of the transporter into the cell, suggesting that this enzyme has a key role in its intracellular trafficking. Since this phosphorylation is reduced when substrates that are themselves transported across the membrane bind to the transporter (e.g. 5-HT and *d*-amphetamine), it seems that the transport of 5-HT is itself linked with the phosphorylation process. Possibly, this process serves as a homeostatic mechanism which ensures that the supply of functional transporters matches the demand for transmitter uptake. By contrast, ligands that are not transported (e.g. cocaine and the selective serotonin reuptake inhibitors (SSRIs)) prevent the inhibition of phosphorylation by transported ligands. Thus, such inhibitors would reduce 5-HT uptake both by their direct inhibition of the transporter and by disinhibition of its phosphorylation (Ramamoorthy and Blakely 1999).

In platelets, depletion of intracellular Ca^{2+} reduces 5-HT transport and this points to calmodulin as another endogenous regulator and its antagonists do inhibit 5-HT uptake. In contrast, activation of adenosine (A3) receptors seems to upregulate the transporter, possibly through the PKG, NO/cGGP pathway.

Glycosylation sites have also been identified on the transporter and recent findings suggest that the sex steroids, estradiol and testosterone increase transcription of the transporter gene and, in turn, the density of transporters in the DRN but not the MRN (McQueen *et al.* 1999). Although it is not yet clear whether this involves a direct effect on transporter gene expression, this finding does suggest that transporters associated with these two groups of neurons are subject to different control mechanisms.

Many studies have attempted to show changes in 5-HT uptake or the density of transporters either in depressed patients, or after treatment with antidepressants. Most have found a reduction in the density of uptake sites, labelled with the tricyclic reuptake inhibitor, [^{3}H]imipramine, in depression. However, there appears to be no change in the density of uptake sites when these are labelled with the selective serotonin reuptake inhibitor, [3H]paroxetine. Another problem is that, even in studies showing a reduction in transporter density, there are no consistent changes in 5-HT uptake. An intriguing suggestion that could account for this anomaly is that there are 'spare' transporters. More recently, research has been directed towards a search for genetic polymorphisms of the 5-HT transporter gene that might account for disorders including depression, bipolar disorder, anxiety, substance abuse and autism. So far, no certain links with either the expression of, or vulnerability to, any disorder have emerged.

One drug that seems to cause quite marked, long-term changes in 5-HT transporter function is MDMA. Single-photon emission-computed tomography (SPECT) which provides an image of the binding of [^{123}I]2β-carbomethoxy-3β-(4′-iodophenyl)-tropane (β-CIT) to the transporters in the (living) human brain shows that this is greatly reduced (and, in some cases, totally absent) in subjects who claim to use MDMA (Semple *et al.* 1999). Interestingly, fenfluramine, another 5-HT-releasing agent, does not seem to have this effect. It has been suggested that loss of transporters in users of MDMA is due to the death of 5-HT neurons and that this is evidence for its neurotoxic effects. This toxicity is thought to be mediated by the formation of quinones and then free radicals from the metabolites of MDMA, although there are alternative explanations (see Sprague, Everman and Nichols 1998) and some individuals still dispute that this drug is actually neurotoxic in humans. At the very least, there is accumulating evidence for long-term deficits in cognitive and neuroendocrine function in users of MDMA and, of even greater concern, it is not known whether these are reversible.

METABOLISM

5-HT is metabolised primarily by MAO to 5-hydroxyindoleacetic acid (5-HIAA) (Fig. 9.4). *In vitro*, 5-HT is the preferred substrate for the MAO_A, rather than the MAO_B isoenzyme (see Chapter 8) and this appears to be the case *in vivo* since MAO_A, but not MAO_B, knock-out mice have increased concentrations of 5-HT in the brain. Obviously, because of its indole nucleus, 5-HT is not a substrate for the enzyme COMT which metabolises the catechol derivatives, dopamine and noradrenaline. However, other metabolic products of 5-HT are theoretically possible and one, 5-hydroxytryptophol,

which results from the reduction of its intermediate metabolite, 5-hydroxyindoleacetaldehyde, instead of oxidation to 5-HIAA, has been identified in the brain.

The comparatively straightforward link between 5-HT and its primary metabolite, 5-HIAA, encouraged many researchers to use changes in the ratio of tissue concentrations of 5-HIAA and 5-HT as an index of the rate of release of 5-HT *ex vivo*. However, it has been clear for some time that the majority of 5-HT is metabolised in the cytoplasm by MAO before it is released from 5-HT nerve terminals. Consequently, the reliability of the 5-HIAA:5-HT ratio as an index of transmitter release is rather dubious, although it could be used as an acceptable measure of MAO activity. In any case, the development of *in vivo* microdialysis means that changes in the concentration of extracellular 5-HT can now be monitored directly which, under drug-free conditions, provides a far more reliable indication of any changes in the rate of release of 5-HT.

RECEPTORS

Over the last 20 years, the development of receptor-selective ligands, coupled with advances in molecular biology, has resulted in the number of 5-HT receptors increasing from a modest two (identified by Gaddum and Picarelli in 1957) to the 14 recognised to date (Table 9.2). These form seven distinct families which, with the exception of the 5-HT_3 receptor, are all G protein-coupled with seven transmembrane-spanning domains. Apart from 5-ht_{1E}, 5-ht_{1F}, 5-ht_5 and 5-ht_6 subtypes, for which genes have been identified, even though the native receptor protein remains elusive (hence their lower-case nomenclature), all are expressed in the CNS.

All the native 5-HT receptors characterised so far are found postsynaptically, with respect to 5-HT terminals, and some are located presynaptically where they regulate the firing rate of 5-HT neurons and/or release of transmitter from their terminals. There is also evidence that some regulate the release of other transmitters in the terminal field and so could act as 5-HT heteroceptors. For instance, 5-HT_{1B} receptor agonists inhibit K^+-evoked release from synaptosomes preloaded with either [^{3}H]dopamine, [^{3}H]noradrenaline, [^{3}H]prolactin or [^{3}H]glutamate. Apart from regulating neuronal firing and transmitter release, activation of certain 5-HT receptors with selective ligands causes specific behavioural or physiological changes (Table 9.3) but, in some cases, these can vary from species to species. There is also some evidence that 5-HT_{1A} receptors, at least, might influence gene expression and neurogenesis and so they could have far-reaching effects on brain function.

Essential features of the different receptor subtypes are highlighted here and, except where indicated, references to specific points can be found in the definitive review of this subject by Barnes and Sharp (1999).

5-HT_{1A}

Although the distribution of these receptors is widespread in the brain, they are found postsynaptically in high concentrations in the hippocampus, septum and amygdala and also on cell bodies of 5-HT neurons in the Raphé nuclei. They are negatively coupled, via $G_{i/o/z}$ proteins, to adenylyl cyclase such that their activation reduces production of cAMP. In turn, this leads to an increase in K^+ conductance and hyperpolarisation of

Table 9.2 The characteristics of 5-HT receptors

Family	Actions	Subtype	Second messenger	Selective/preferential agonist	Selective antagonist	Location
5-HT_1	Hyperpolarisation	5-HT_{1A}	$G_{i/o/z}\rightarrow\downarrow$cAMP	8-OH-DPAT	WAY 100635	Pre- and postsynaptic and heteroceptor
		5-$HT_{1B/D}$	$G_{i/o}\rightarrow\downarrow$cAMP	CP 93129	SB 224289	Pre- and postynaptic and heteroceptor
		5-HT_{1D}	$G_{i/o}\rightarrow\downarrow$cAMP	N/A	BRL 15572	Pre- (?) and postsynaptic and heteroceptor
		5-ht_{1E}	$G_{i/o}$ (?)$\rightarrow\downarrow$cAMP	N/A	N/A	
		5-ht_{1F}	$G_{i/o}$ (?)$\rightarrow\downarrow$cAMP	LY 334370	N/A	
5-HT_2	Depolarisation	5-HT_{2A}	$G_{q/11:}$ activates phospholipase C, $\downarrow gK^+$ and $\uparrow[Ca^{2+}]_{in}$. Also activates PLA2 and arachidonic acid pathway	CP 94253	MDL 100907	Postynaptic and heteroceptor
		5-HT_{2B}	$G_{q/11:}$ activates phospholipase C, $\downarrow gK^+$ and $\uparrow[Ca^{2+}]_{in}$. Also activates PLA2 and arachidonic acid pathway	BW 723C86	SB 204741	
		5-HT_{2C}	$G_{q/11:}$ activates phospholipase C, $\downarrow gK^+$ and $\uparrow[Ca^{2+}]_{in}$. Also activates PLA2 and arachidonic acid pathway	MK 212	SB 242084	Postsynaptic and heteroceptor
5-HT_3	Depolarisation		Ligand-gated Na^+/K^+ channel	2-methyl 5-HT	Ondansetron	Postsynaptic and heteroceptor
5-HT_4			$G_s\rightarrow\uparrow$cAMP	SDZ 216454	GR 113808	Postsynaptic and heteroceptor
5-ht_5	?		G protein-coupled?	N/A	N/A	?
5-ht_6	Depolarisation		$G_s\rightarrow\uparrow$cAMP	N/A	SB 271046	?
5-HT_7	Depolarisation		$G_s\rightarrow\uparrow$cAMP	N/A	SB 258719	?

Table 9.3 Behavioural and physiological responses affected by 5-HT receptors

	5-HT_{1A}	5-HT_{1B}	5-HT_2	5-HT_3	5-HT_4	5-HT_7
Anxiety/panic	✓		✓	(✓?)		
Cognition					(✓?)	
Food intake	✓	✓	✓	✓		
Hallucinations			✓			
Mood	✓		✓			
Nausea/vomiting				✓		
Obsessive behaviour			✓			
Pain		✓	✓	✓		
Psychosis			✓			
Sexual function		✓	✓			
Sleep/circadian rhythms		✓	✓			✓
Thermoregulation	✓	✓	✓			

the host cell resulting in the inhibition of firing of cells in the Raphé nuclei and reduced release of 5-HT from their terminals. The importance of this action, as a possible explanation for the delay in the therapeutic effects of those antidepressants that increase the concentration of extracellular 5-HT, is discussed in Chapter 20.

There is some evidence that pre- and postsynaptic receptors do not respond in exactly the same way to drug challenges and it has even been suggested that they are not identical. For instance, the drug BMY 7378 behaves as an agonist at presynaptic 5-HT_{1A} receptors but has a low intrinsic activity at the postsynaptic site where it acts as an antagonist. However, there is as yet insufficient evidence to claim that there are subtypes of this receptor and, in any case, differences in the receptor reserve at pre- and postsynaptic sites could well explain some of the apparently conflicting findings.

Given that the firing rate, and hence the release of 5-HT, is greater in awake animals than in those that are asleep (see Chapter 22), it is not surprising that the effects of 5-HT_{1A} antagonists on neuronal firing and 5-HT release are more evident in behaving, conscious subjects than in those that are anaesthetised. Indeed, this should be borne in mind when perusing the literature on this subject. However, in terms of their gross physiological effects in awake animals, consistent findings are that 5-HT_{1A} receptor agonists induce hypothermia and increase food intake. They also reduce anxiety and, so far, this is the only action to be exploited clinically. Even so, only one such compound, buspirone, is licensed for use in the clinic and it is still not known whether its anti-anxiety effect is mediated by activation of pre- or postsynaptic 5-HT_{1A} receptors (see Chapter 19).

Another well-known agonist at these receptors is lysergic acid diethylamide (LSD) and, for several years it was thought that this explained its hallucinogenic effects. However, this drug is a non-selective ligand that also binds to 5-$HT_{2A/2C}$ receptors. Although activation of 5-HT_{1A} receptors by LSD seems to have some effects on motor activity, this site can be ruled out as being responsible for its hallucinogenic effects. This is not least because neither buspirone, which is also an agonist of these receptors, nor reserpine, which diminishes 5-HT transmission, have any hallucinogenic actions in humans. In fact, experimental preclinical models strongly indicate that 5-HT_{1A} agonists could be beneficial in treatment of both the positive and negative symptoms of schizophrenia. For instance, they increase the concentration of extracellular dopamine in the frontal cortex but diminish apomorphine-induced stereotypy in rats.

Importantly, they achieve this without inducing extrapyramidal side-effects or increasing prolactin secretion, which are real problems with neuroleptics. These results have been borne out by preliminary clinical trials of buspirone, used in combination with neuroleptics, and several novel 5-HT_{1A} agonists (e.g. BSF 190555) are currently under development for this clinical application (Meltzer 1999).

5-HT_{1B}

These receptors are found postsynaptically, mostly in the basal ganglia, striatum and frontal cortex, but they are also thought to lie on 5-HT nerve terminals where their activation reduces release of 5-HT. However, there seem to be regional differences in the extent to which this population of receptors is tonically activated by extracellular 5-HT and so the literature describing the effects of 5-HT_{1B} antagonists on 5-HT release is somewhat confusing. 5-HT_{1B} receptors are also found in the Raphé nuclei and their antagonism increases release of 5-HT in the DRN. Because of the dearth of 5-HT_{1B} selective ligands, the gross physiological effects that result from activation of these receptors is largely uncharacterised. However, it is possible that they contribute to the regulation of circadian rhythms by blunting 5-HT release in the suprachiasmatic nucleus of the hypothalamus and, in mice in which this receptor has been 'knocked-out', the observed quiescence suggests that their activation increases locomotor activity (see also Chapter 22). Activation of these receptors could also contribute to the anti-migraine effects of sumatriptan, a non-selective 5-$HT_{1B/1D}$ agonist (but see below).

An interesting development in this area is the possibility that there could be an endogenous ligand, for these receptors: 5-HT-moduline. This is a tetrapeptide that is released from neurons and is claimed to be the first allosteric modulator of a G protein-coupled receptor to be identified so far. Functionally, 5-HT-moduline behaves like a 5-HT_{1B} antagonist and so increases terminal release of 5-HT (Massot *et al*. 1998) and it is thought that this could be an important step in maintaining a sustained increase in release of 5-HT during stress.

5-HT_{1D}

Probably the most notable feature of this receptor is the confusion arising from its classification and nomenclature! Soon after characterisation of the 5-HT_{1D} receptor, which was found in certain species (e.g. the human) it was determined that this was in fact a variant of the 5-HT_{1B} receptor which had already been found in other species (e.g. the rat). These receptors were therefore regarded as species variants and came to be described as the 5-$HT_{1B/1D}$ subtype. Since then, another 5-HT_1 receptor subtype has been identified and current nomenclature dictates that this is the (new) 5-HT_{1D} receptor.

So far, little is known about this novel 5-HT_{1D} receptor but, in the rat and human, its mRNA is found, albeit in low concentrations, in the basal ganglia, nucleus accumbens, hippocampus, frontal cortex and Raphé nuclei. It is negatively coupled to adenylyl cyclase and is possibly located presynaptically, on both the 5-HT neuronal cell body and terminals, but this has yet to be confirmed.

Interest in this receptor has been generated by the possibility that its activation accounts for the anti-migraine effects of the non-selective 5-$HT_{1B/1D}$ agonist, sumatriptan. The exact process(es) that account for this action are unresolved but

favoured possibilities include vasoconstriction of cerebral arteries and/or blockade of neurogenic pain and inflammation generated by vascular afferents within the trigeminal nucleus. Because mRNA for the 5-HT_{1B} receptor is not found in this nucleus in humans, activation of the 5-HT_{1D} receptor is thought to be responsible for these effects.

5-ht_{1E} AND 5-ht_{1F}

Although the existence of further 5-HT_1 receptor subtypes with atypical pharmacology was suspected from radioligand binding studies, the 5-ht_{1E} and 5-ht_{1F} receptors have been characterised mainly from the identification of the genes that encode receptors with properties similar to the rest of the 5-HT_1 family. Radioligand binding and the distribution of mRNA for the 5-ht_{1E} receptor suggest that it is found mainly in the striatum but it also seems to be present in the amygdala, frontal cortex and globus pallidus, to some extent. However, the lack of specific ligands has prevented their pharmacological characterisation. As a result of these limitations, they are currently assigned only 'lower-case' status. Recently, the anti-migraine drug, sumatriptan, has been found to bind to 5-ht_{1F} receptors with an affinity similar to that for 5-$HT_{1B/1D}$ receptors and so they might have a role in migraine.

5-HT_{2A}

These receptors are mainly found in the cortex and basal ganglia. They are coupled to phospholipase A and have an excitatory effect on the host cell as a result of the ensuing reduction in K^+ conductance. The many well-known agonist ligands for this receptor include DOI (1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride), DOM (2,5-dimethoxy-4-methylamphetamine) and LSD. In rodents, these compounds cause a characteristic 'head twitch' and increase motor activity and, in humans, they are all hallucinogens. Preclinical studies reinforce the view that 5-HT_{2A} (and possibly 5-HT_{2C}) receptor activation underlies the hallucinogenic effects of these compounds (Krebs-Thomson, Paulus and Geyer 1998). This is consistent with recent evidence that all the atypical neuroleptics, such as clozapine, risperidone and olanzepine, act as antagonists at this receptor, an action that could well contribute to their therapeutic effects in schizophrenia.

In addition to their psychotropic effects, activation of 5-HT_{2A} receptors induces hyperthermia, which could explain this dangerous action of MDMA. Finally, an unusual feature of these receptors is that they are downregulated by prolonged exposure to antagonists, as well as agonists. The reason for this is uncertain but it could suggest that drugs which hitherto have been regarded as antagonists are, in fact, inverse agonists.

5-HT_{2B}

The use of labelled antibodies suggests their presence in the amygdala, septum, hypothalamus and cerebellum. However, little is known about these receptors, mainly because of the shortage, until recently, of selective ligands, their low density and the limited distribution of their mRNA in the brain.

5-HT_{2C}

Again, conventions for nomenclature have somewhat confused the status of this receptor which was first known as a 5-HT_{1C} receptor. However, as information accrued from cloning studies, pharmacological characterisation, and discovery of its second messenger system, it became evident that this receptor shared the characteristics of the 5-HT_2 receptor family, rather than those of a 5-HT_1 receptor. The switch in classification from a 5-HT_{1C} to a 5-HT_{2C} receptor explains the gap in the 5-HT_1 receptor family.

The 5-HT_{2C} receptor was first found in the choroid plexus, where it is thought to regulate the formation of CSF, but it has since been found in cortical and limbic areas as well as the basal ganglia. In the choroid plexus, at least, its actions seem to be mediated by activation of phospholipase C with a resulting depolarisation of the host cell. Like the 5-HT_{2A} subtype, 5-HT_{2C} receptors are downregulated by prolonged exposure to antagonists (inverse agonists?) as well as agonists. The discovery that 5-HT_{2C} receptor mRNA is subject to posttranslational changes suggests that there could be several different isoforms of this receptor and it cannot be assumed that they are functionally the same.

As far as can be certain, given the lack of selective ligands, their activation elsewhere in the brain is thought to culminate in reduced locomotor activity and hyperthermia. However, interest in these receptors as possible therapeutic targets is fostered by evidence that their agonists, such as *m*CPP, appear to be profoundly anxiogenic (see Chapter 19) and reduce food intake (see below).

5-HT_3

These receptors are quite different from any other monoamine receptor in that they are not coupled to G proteins. Instead, they comprise a pentameric complex of subunits that incorporates an ion channel. This is selective for the cations Na^+ and K^+ which, when opened, leads to depolarisation of the host cell. 5-HT_3 receptors are found at high concentrations in the brainstem and area postrema. However, they are also found elsewhere in lower concentrations, notably in the cortex, amygdala and hippocampus, where they are thought to be associated mainly with GABAergic neurons.

Interestingly, their function is modified by many agents that allosterically modify $GABA_A$ receptor function (e.g. barbiturates and steroids). A further parallel with the $GABA_A$ receptor is that there could well be differences in subunit composition of 5-HT_3 receptors such that different heteromeric complexes form receptors which are functionally distinct.

These receptors are best known for their stimulation of dopamine release. Indeed, it is attenuation of dopamine release in the area postrema by the 5-HT_3 receptor antagonist, ondansetron, that is thought to explain its anti-emetic effects. However, they are also thought to influence release of other neurotransmitters including GABA, acetylcholine and noradrenaline; they are even thought to increase somatodendritic release of 5-HT in the Raphé nuclei. So far, despite vigorous attempts to find other clinical applications for ondansetron, none has proved convincing.

5-HT_4

This receptor is positively coupled to adenylyl cyclase which results in closure of K^+ channels, culminating in an increase in excitability of the host cell and a delay in

repolarisation. Its density is high in the basal ganglia and the nucleus accumbens but studies of the pharmacology of this receptor may well be complicated by the discovery that it has several 'splice' variants (four in humans). So far, the literature on its behavioural effects is somewhat inconsistent but agonists of this receptor are being explored as possible cognitive enhancers.

5-ht_5 AND 5-ht_6

The existence of these receptors was predicted by cDNA sequence analysis and their protein products have been studied in cell expression systems. 5-ht_5 receptor mRNA is found in the cortex, hippocampus, olfactory bulbs and cerebellum but the native receptor has still not been characterised and so it has been assigned only 'lower-case' nomenclature. However, studies using antibodies generated against these receptors have shown that they are present on glial cells and investigations of cloned receptors suggest that they are negatively coupled to $G_{i/o}$ proteins and reduce activation of adenylyl cyclase

In contrast, the 5-ht_6 receptor is positively coupled to G_s proteins and increases adenylyl cyclase activity. Again, the native 5-ht_6 receptor has not been characterised but *in situ* hybridisation suggests that its mRNA is present in the amygdala, nucleus accumbens, striatum, cortex and olfactory tubercle. Many antipsychotic agents and some antidepressant drugs show high-affinity binding to this receptor where they act as antagonists but it remains to be seen whether this contributes to their therapeutic profile. The recent development of selective antagonists for 5-ht_6 receptors could help to answer this question but, so far, the most promising findings are that their antagonists increase seizure threshold and could turn out to be beneficial in the treatment of epilepsy.

5-HT_7

Again, these receptors are positively coupled to adenylyl cyclase through a G_s protein. However, at least three splice variants are expressed in human tissue and the impact of these different isoforms on the function of these receptors is not known. Radioligand binding and the distribution of 5-HT_7 receptor mRNA suggest that the density of these receptors is high in the thalamus, hippocampus and hypothalamus where (in the suprachiasmatic nucleus) they are thought to synchronise circadian rhythms with the light cycle (see Chapter 22).

WHY ARE THERE SO MANY RECEPTORS?

It is obvious that strenuous efforts have been invested in the research of 5-HT receptors and, in particular, in the development of receptor-selective agonists and antagonists. All this has been done in the hope that it might be possible to control a specific 'switch' in the brain that governs a particular aspect of 5-HT function and which would be beneficial therapeutically. A further ambition is that, by avoiding activation of other 5-HT receptors, the risk of any unwanted side-effects would be eliminated. Of course, it is equally possible that reduction in non-specific receptor interactions could actually unmask some side-effects.

This approach has worked to some extent in that the 5-HT_{1A} agonist, buspirone, is an acknowledged anxiolytic drug that lacks some of the problems associated with benzodiazepines. Also, the benefits of the 5-HT_3 antagonist, ondansetron, in relieving nausea, with minimal side-effects, are undisputed. However, in other respects, this approach to drug development has been disappointing. This is probably because all the 5-HT receptors have a wide and overlapping distribution in the brain and it would be naive to suppose that any physiological response relies exclusively on the activation of any single 5-HT receptor.

What the overall physiological consequences of either an increase or decrease in 5-HT transmission in any brain region might be is beyond the scope of this chapter. However, it is certain that the diverse cocktail of 5-HT receptors in every brain region gives scope for flexibility and refinement in the 5-HT response that would not be possible if there were only the two receptors identified by Gaddum. This flexibility applies not only to the qualitative features of the response but also its duration. Another dimension of sophistication is added by the different affinities of 5-HT for each of its receptors and differences in their rates of desensitisation. An interesting discussion of how all these variables could affect overall 5-HT transmission in the brain can be found in Uphouse (1997).

WHAT DOES 5-HT DO IN THE BRAIN?

The final challenge is to define the function of 5-HT in the brain. This is not easy because the actions of drugs that target specific receptors leads us to believe that 5-HT helps to regulate: mood, anxiety, sleep, body temperature, appetite, sexual behaviour, movement, intestinal motility, cardiovascular function (central and peripheral) and nociception, at least. While a detailed explanation of the physiology of each of these functions is not possible here, and many are covered in appropriate chapters of this book, two topics are of particular interest. One is the general role of 5-HT during the waking state: this is discussed below because, in the light of recent discoveries, we might have to modify the currently accepted view. A second is the role of 5-HT in feeding, a subject to which this chapter has referred to some extent already. This will be covered here because the regulation of body weight is becoming an increasingly important research area, reflecting the growing concern about the serious health problems linked with obesity.

THE ROLE OF 5-HT IN THE WAKING STATE

One puzzle concerning 5-HT transmission in the brain, and a defining feature of 5-HT neurons in the DRN and MRN, is their slow, rhythmic firing rate of 1–2 spikes/s. In fact, this 'clock-like' discharge is even maintained *in vitro*. Certainly, electrophysiological studies *in vivo* have shown that neurons in the DRN do respond to environmental stimuli but, unlike noradrenergic neurons, they do not seem to have a role in homeostasis or the response to aversive stimuli. This is deduced from findings that the single-unit response of neurons in the DRN is not affected by a range of aversive stimuli such as environmental heat or systemic pyrogens; drug-induced changes in systemic blood pressure or glucoregulatory challenge. They are even apparently unaffected by a variety of painful stimuli. In all these cases, the activity of these neurons during the

stimulus is no greater than that expressed during active waking (Jacobs and Azmitia 1992). In fact, it is generally thought that the only consistent change in response of these neurons is that reflecting changes in the sleep–waking cycle such that these neurons are maximally active during waking but can become totally quiescent during rapid eye movement (REM) sleep (see Chapter 22).

As a result of such findings, it has been suggested that 5-HT neurons in the brain are concerned merely with regulation of motor responses. Specifically, that serotonergic transmission serves to coordinate target cell responses by adjusting their excitability to match the animal's general level of arousal. In so doing, they could be responsible for gating motor output and coordinating homeostatic and sensory function (Jacobs and Azmitia 1992; Jacobs and Fornal 1999). This would be consistent with evidence that increases in the firing rate of neurons in the DRN precede an increase in arousal (see Chapter 22). This could mean that the frequency of discharge codes the state of arousal and primes target cells for forthcoming changes in the motor response to sensory inputs. It has even been claimed that 5-HT neurons projecting to the primary visual cortex are involved more in the interpretation of movement in the visual field than its qualitative features.

Evidence deemed to support this theory comes from the discovery of a population of neurons in the DRN that, unlike the majority, do not show any increase on waking and some may even reduce their firing during orientation to environmental stimuli. However, they do increase their activity during vegetative motor behaviours involving oral–buccal movements (chewing, grooming). Some are even active during anticipation of food, suggesting that they are capable of developing responses to conditioned environmental cues.

To some extent, this proposal is supported by microdialysis studies of changes in 5-HT efflux in the terminal fields of 5-HT neurons. For instance, increased 5-HT efflux in the striatum, induced by immobilisation of rats, occurs only during the period of increased motor activity that follows the animals' release (Takahashi *et al.* 1998). A single swim stress also fails to increase 5-HT efflux in the medial prefrontal cortex of rats.

However, evidence that challenges the 'motor output' theory is that a second bout of swim stress does increase 5-HT efflux (Petty *et al.* 1997). Indeed, it would be interesting to know whether this reflects any long-term influence of 5-HT-moduline on regulation of 5-HT release (see above). Also, 5-HT efflux is increased in the Raphé nuclei and their terminal fields after handling of rats (Adell, Casanovas and Artigas 1997), hypoglycaemic shock (Vahabzadeh, Boutelle and Fillenz 1995) and even during a conditioned fear response in which animals actually freeze (i.e. when their motor response is suppressed; Yoshioka *et al.* 1995). Another finding that is difficult to reconcile with 5-HT simply governing motor responses is that its efflux in the brain is increased by exposure of rats to inescapable, uncontrollable stress during which animals develop 'learned helplessness' (see Chapter 20; Petty *et al.* 1994) and yet the striking feature of this behaviour is that there is a deficit in animals' motor activity. Finally, there is compelling evidence that 5-HT transmission in the amygdala affects emotional, rather than merely motor, components of anxiety (see Chapter 19).

Overall, it remains to be seen whether or not changes in the release of 5-HT in the terminal field parallel changes in the firing rate of neurons in the Raphé nuclei. Certainly the network of hetero- and presynaptic receptors, described above, could make it feasible to adjust 5-HT release in the terminal field despite the 'clock-like' firing

rate of these neurons. However, this question might be resolved by the recent discovery of a subpopulation of 5-HT neurons in the Raphé nuclei that is affected by aversive stimuli. Thus, in brain slices, taken from rats that had previously experienced stress *in vivo*, the 'stress hormone', corticotropin-releasing factor (CRF), increased the neuronal firing rate of neurons in the region of the caudal DRN/rostral MRN. It is proposed that these (presumed) 5-HT neurons form a distinct mesocorticolimbic group that, unlike neurons in other zones of the DRN and MRN, contribute to the stress response *in vivo* (Lowry *et al*. 2000). If so, this could explain the neuronal origin of the increase in 5-HT efflux in forebrain areas during stress and could suggest that certain 5-HT neurons have an important role in the stress response rather than merely governing motor activity.

THE ROLE OF 5-HT IN GOVERNING FOOD INTAKE

Obviously, regulation of food intake depends on many neurotransmitters and hormones but this final section will outline the role played by central 5-HT transmission in this process. It had been the belief for some time that increased 5-HT transmission in the brain reduces food intake (Blundell 1977) and this certainly explains the satiety in rats that follows infusion of 5-HT into the paraventricular nucleus (PVN) of the hypothalamus. However, recent studies using microdialysis have found that 5-HT efflux in the lateral hypothalamus is itself increased by food intake, suggesting the existence of a feedback control system. In fact, because the increase in 5-HT efflux is greater in genetically obese rats than in their lean counterparts, it has been proposed that there is a deficiency in the 5-HT inhibition of food intake in obesity.

The increase in food intake induced by 5-HT_{1A} receptor agonists is entirely consistent with the view that increased 5-HT transmission in the brain reduces food intake (hypophagia) since the activation of 5-HT_{1A} autoreceptors in the Raphé nuclei would ensure a reduction in 5-HT release from the nerve terminals. Of course, this explanation does not tackle the question of what role, if any, is served by postsynaptic 5-HT_{1A} receptors and, in fact, recent studies suggest that links between 5-HT_{1A} receptor activation and food intake are far more complex than originally proposed. For instance, in microdialysis studies of the lateral hypothalamus, 5-HT efflux was reduced, as expected, by systemic administration of the 5-HT_{1A} agonist, 8-OH-DPAT, in freely feeding, but not food-deprived, rats. The finding that 8-OH-DPAT reduces food intake in fasted pigs but increases it in satiated animals is even more difficult to explain. Clearly, more research is needed before these apparently incongruous findings can be reconciled.

So far, the role(s) of other 5-HT receptors in feeding seem to be more consistent and it is generally agreed that activation of 5-HT_{1B} or 5-$HT_{2A/2C}$ receptors reduces food intake. It has even been reported that stress-induced hypophagia is ameliorated by 5-HT_{2A} antagonists and that this indicates a link between abnormal 5-HT_{2A}-receptor mediated transmission in the PVN and anorexia nervosa (see Samanin and Grignaschi 1996).

A link between 5-HT transmission and hypophagia is further reinforced by the pharmacology of drugs that reduce food intake ('anorectic' agents), such as *d*-fenfluramine or sibutramine. Both these compounds have actions that should lead to increased synaptic concentrations of 5-HT in the brain, albeit through different mechanisms (see below), but whether or not this actually explains the anorectic actions of *d*-fenfluramine is controversial.

Certainly, infusion of *d*-fenfluramine into the region of the PVN reduces food intake by increasing satiety. Moreover, microdialysis studies have shown that systemic administration of this drug causes a rapid but short-lived increase in the extracellular concentration of 5-HT in this brain region. This increased efflux is due to the effects of *d*-fenfluramine on the vesicular transporter that leads to impulse-independent extrusion of 5-HT into the synapse by the terminal membrane transporter. Although this action fits well with the hypophagic effects of 5-HT, the increased 5-HT release is possibly not responsible for the effects of *d*-fenfluramine. This doubt has emerged from studies such as those showing that neither a neurotoxic lesion of 5-HT neurons in the brain, nor depletion of neuronal transmitter stores by administration of the synthesis inhibitor, *p*CPA, prevents the reduction in food intake caused by *d*-fenfluramine (see Curzon, Gibson and Oluyomi 1997). Instead, a direct effect of this compound and/or its metabolite, norfenfluramine, on 5-HT receptors has been proposed.

Another drug that reduces food intake through advancing satiety is the non-selective 5-HT/noradrenaline reuptake inhibitor, sibutramine. However sibutramine also increases thermogenesis by increasing metabolism in brown adipose tissue (BAT). This action, which contributes to loss of body weight, is thought to be mediated by an increase in activation of sympathetic afferents to BAT by central 5-HT neurons because, like sibutramine's effect on satiety, this action is prevented by administration of a peripheral ganglion blocking agent.

An important distinction between the effects of sibutramine and *d*-fenfluramine is highlighted by microdialysis studies (Heal *et al*. 1998). These show that the rate of increase in 5-HT efflux in the region of the PVN, after administration of sibutramine, is slow, progressive and long-lasting. This is because it relies on the accumulation of extracellular 5-HT following the inhibition of its reuptake after impulse-dependent release. This time-course contrasts with the rapid and transient increase in 5-HT efflux which results from the fenfluramine type of impulse-independent release from nerve terminals. In fact, this rapid increase in 5-HT release is thought to underlie the serious adverse side-effects of *d*-fenfluramine that have led to its withdrawal from the clinic.

Although the above findings confirm that 5-HT efflux is increased by anorectic drugs, the question remains as to how this leads to a reduction in food intake and, in particular, what 5-HT receptors are involved? Unfortunately, despite the development of selective 5-HT receptor ligands, this question remains unanswered. Nevertheless, it seems that *d*-fenfluramine and sibutramine increase satiety in different ways because $5\text{-HT}_{2A/2C}$ receptor antagonists, such as ritanserin, do not block this effect of *d*-fenfluramine but they do inhibit that of sibutramine. In fact, it is still not at all certain which 5-HT receptor(s) mediate the effects of *d*-fenfluramine. Some studies suggest that 5-HT_{1B} receptors are responsible but evidence for this is inconsistent. Another possibility is that it is in fact the metabolite of *d*-fenfluramine, norfenfluramine, which increases satiety through its potent activation of 5-HT_{2C} receptors. Although this remains unconfirmed, 5-HT_{2C} knock-out mutant mice are less sensitive to the effects of this drug on satiety than are their wild-type counterparts.

A final, important distinction between sibutramine and *d*-fenfluramine is that the actions of the former, but not the latter, rest on its modification of both 5-HT and noradrenergic transmission. Thus, the reduction in food intake by sibutramine is partially blocked by α_1- or β_1-adrenoceptor antagonists as well as $5\text{-HT}_{2A/2C}$ or $5\text{-HT}_{2B/2C}$ antagonists. In fact, there appears to be a synergistic interaction between these two transmitter systems. This is illustrated by a study of the effects of the selective serotonin

reuptake inhibitor, fluoxetine, and the selective noradrenaline uptake inhibitor, nisoxetine on food intake and BAT thermogenesis. Using doses of these drugs that were ineffective on either measure when given alone, they did increase both satiety (Jackson *et al*. 1997) and BAT thermogenesis when given in combination. The 'hard-wiring' of this 5-HT/noradrenaline interaction is currently being researched.

Clearly, this is a complex physiological system, involving multiple feedback and feedforward pathways that interacts with other 'food-factors' (e.g. NPY, leptin, orexins, corticosteroids and other monoamines) and it will take a great deal of research to unravel all these networks. However, the progress that has been made so far underlines the crucial role played by 5-HT in this process and reinforces the view that drugs targeting specific 5-HT receptors could turn out to be effective anti-obesity agents that lack the adverse effects of 5-HT releasing agents.

REFERENCES

Adell, A, Casanovas, JM and Artigas, F (1997) Comparative study in the rat of the actions of different types of stress in the release of 5-HT in the raphe nuclei and forebrain areas. *Neuropharmacology* **36**: 735–741.

Barnes, NM and Sharp, T (1999) A review of central 5-HT receptors and their function. *Neuropharmacology* **38**: 1083–1152.

Blakely, RD, Ramamoorthy, S, Schroeter, S, Qian, Y, Apparsundaram, S, Galli, A and DeFelice, LJ (1998) Regulated phosphorylation and trafficking of antidepressant-sensitive serotonin transporter proteins. *Biol. Psychiatry* **44**: 169–178.

Blundell, JE (1977) Is there a role for serotonin (5-hydroxytryptamine) in feeding? *Int. J Obesity* **1**: 15–42.

Boadle-Biber, MC (1993) Regulation of serotonin synthesis. *Prog. Biophys. Molec. Biol.* **60**: 1–15.

Curzon, G, Gibson, EL and Oluyomi, AO (1997) Appetite suppression by commonly used drugs depends on 5-HT receptors but not on 5-HT availability. *Trends Pharmacol. Sci.* **18**: 21–25.

Gaddum, JH and Picarelli, ZP (1957) Two kinds of tryptamine receptor. *Brit. J. Pharmacol.* **12**: 323–328. (Reproduced in *Brit. J. Pharmacol.* **120** (Suppl): 134–139.)

Gillman, PK (1999) The serotonin syndrome and its treatment. *J. Psychopharmacol.* **13**: 100–109.

Heal, DJ, Cheetham, SC, Prow, MR, Martin, KF and Buckett, WR (1998) A comparison of the effects on central 5-HT function of sibutramine hydrochloride and other weight-modifying drugs. *Brit. J. Pharmacol.* **125**: 301–308.

Jackson, HC, Needham, AM, Hutchins, LJ, Mazurkiewicz, SE and Heal, DJ (1997) Comparison of the effects of sibutramine and other monoamine reuptake inhibitors on food intake in the rat. *Brit. J. Pharmacol.* **121**: 1758–1762.

Jacobs, BL and Azmitia, EC (1992) Structure and function of the brain serotonin system. *Physiol. Rev.* **72**: 165–229.

Jacobs, BL and Fornal, CA (1999) Activity of serotonergic neurons in behaving animals. *Neuropsychopharmacology* **21**: 9S–15S.

Krebs-Thomson, K, Paulus, MP and Geyer, MA (1998) Effects of hallucinogens on locomotor and investigatory activity and patterns: influence of 5-HT_{2A} and 5-HT_{2C} receptors. *Neuropsychopharmacology* **18**: 339–351.

Leibowitz, SF, Alexander, JT (1998) Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biol. Psychiatry* **44**: 851–864.

Lowry, CA, Odda, JE, Lightman, SL and Ingram, CD (2000) Corticotropin-releasing factor (CRF) increases the *in vitro* firing rates of serotonergic neurones in the rat dorsal raphé nucleus: evidence for selective activation of a topographically organised mesolimbocortical system. *J. Psychopharmacol.* **14** (Suppl 2): A72.

Massot, O, Rousselle, JC, Grimaldi, B, Cloez-Tayarani, I, Fillion, MP, Plantefol, M, Bonnin, A, Prudhomme, N and Fillion, G (1998) Molecular, cellular and physiological characteristics of

5-HT-moduline, a novel endogenous modulator of 5-HT1B receptor subtype. *Ann. NY Acad. Sci.* **861**: 174–182.

McQueen, JK, Wilson, H, Sumner, BEH and Fink, G (1999) Serotonin transporter (SERT) mRNA and binding site densities in male rat brain affected by sex steroids. *Molec. Brain Res.* **63**: 241–247.

Meltzer, HY (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* **21** (Suppl 2): 106S–115S.

Petty, F, Kramer, G, Wilson, L and Jordan, S (1994) *In vivo* serotonin release and learned helplessness. *Psychiatry Res.* **52**: 285–293.

Petty, F, Jordan, S, Kramer, GL, Zukas, PK and Wu, J (1997) Benzodiazepine prevention of swim-stress induced sensitization of cortical biogenic amines: an *in vivo* microdialysis study. *Neurochem. Res.* **22**: 1101–1104.

Povlock, SL and Amara, SG (1997) The structure and function of norepinephrine, dopamine and serotonin transporters. In: *Neurotransmitter Transporters*. Humana Press, New York, pp 1–28.

Ramamoorthy, S and Blakely, RD (1999) Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. *Science* **285**: 763–766.

Rouch, C, Nicolaidis, S and Orosco, M (1999) Determination, using microdialysis, of hypothalamic serotonin variations in response to different macronutrients. *Physiol. Behav.* **65**: 653–657.

Rudnick, G (1997) Mechanism of biogenic amine neurotransmitter transporters. In: *Neurotransmitter Transporters*. Humana Press, New York, pp 1–28, 73–100.

Samanin, R and Grignaschi, G (1996) Role of 5-hydroxytryptamine receptor subtypes in satiety and animal models of eating disorders. In *Drug Receptor Subtypes and Ingestive Behaviour* (Eds Cooper, SJ and Clifton, PG), Academic Press, London, pp. 39–58.

Semple, DM, Ebmeier KP, Glabus MF, O'Carroll, RE and Johnstone, EC (1999) Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. *Br. J. Psychiatry* **175**: 63–69.

Siuciak, JA, Clark, MS, Rind, HB, Whittemore, SR and Russo, AF (1998) BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain. *J. Neurosci. Res.* **52**: 149–158.

Sprague, JE, Everman, SL and Nichols, DE (1998) An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. *Neurotoxicology* **19**: 427–442.

Stock, MJ (1997) Sibutramine: a review of the pharmacology of a novel anti-obesity agent. *Int. J. Obesity* **21** (Suppl 1): S25–S29.

Takahashi, H, Takada, Y, Nagai, N, Urano, T and Takada, A (1998) Extracellular serotonin in the striatum is increased after immobilisation stress only in the nighttime. *Behav. Brain Res.* **91**: 185–201.

Uphouse, L (1997) Multiple serotonin receptors: too many, not enough, or just the right number? *Neurosci. Biobehav. Rev.* **21**: 679–698.

Vahabzadeh, A. Boutelle, MG and Fillenz, M (1995) Effects of changes in rat brain glucose on serotonergic and noradrenergic neurons. *Eur. J. Neurosci.* **7**: 175–179.

Wurtman, RJ and Wurtman, JJ (1995) Brain serotonin, carbohydrate-craving, obesity and depression. *Obesity Res.* **3**: 477S–480S.

Yoshioka, M, Matsumoto, M, Togashi, H and Saito, H (1995) Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. *Pharmacol. Biochem. Behav.* **51**: 515–519.