Neurotransmitters, Drugs and Brain Function.
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13 Other Transmitters and Mediators

R. A. WEBSTER

With a Section on Nitric Oxide by A. H. DICKENSON

In the preceding chapters, the synaptic pharmacology of those substances clearly established as NTs in the CNS, i.e. glutamate, GABA, ACh, NA, DA, 5-HT and certain peptides, has been discussed in some detail. There are other substances found in the CNS that could have a minor transmitter role, e.g. ATP, histamine and adrenaline, while still others that cannot claim such a property but clearly modify CNS function in some way, e.g. steroids, prostaglandins and nitric oxide. We will consider each of them in what we hope is appropriate detail.

THE PURINES, ATP AND ADENOSINE

ATP (ADENOSINE TRIPHOSPHATE)

For many years ATP has been clearly established as an important intracellular mediator of neuronal function and the provider of cellular energy. The concept that it may also be a neurotransmitter is more recent. It stems from the finding of Burnstock and his colleagues that it was the mediator of the non-adrenergic, non-cholinergic (so-called NANC) innervation of smooth muscle in the intestine and bladder (see Burnstock *et al.* 1970). Generally ATP is a co-transmitter with a wide range of other NTs and while its role may usually be secondary to them, it actually appears in some sympathetically innervated tissue to mediate the initial contraction of smooth muscle rather than the maintained tone. Structurally it consists of an adenine ring, a ribose element and a triphosphate chain (Fig. 13.1).

ATP certainly fulfils the criteria for a NT. It is mostly synthesised by mitochondrial oxidative phosphorylation using glucose taken up by the nerve terminal. Much of that ATP is, of course, required to help maintain Na^+/K^+ ATPase activity and the resting membrane potential as well as a Ca^{2+}ATPase, protein kinases and the vesicular binding and release of various NTs. But that leaves some for release as a NT. This has been shown in many peripheral tissues and organs with sympathetic and parasympathetic innervation as well as in brain slices, synaptosomes and from *in vivo* studies with microdialysis and the cortical cup. There is also evidence that in sympathetically innervated tissue some extracellular ATP originates from the activated postsynaptic cell. While most of the released ATP comes from vesicles containing other NTs, some

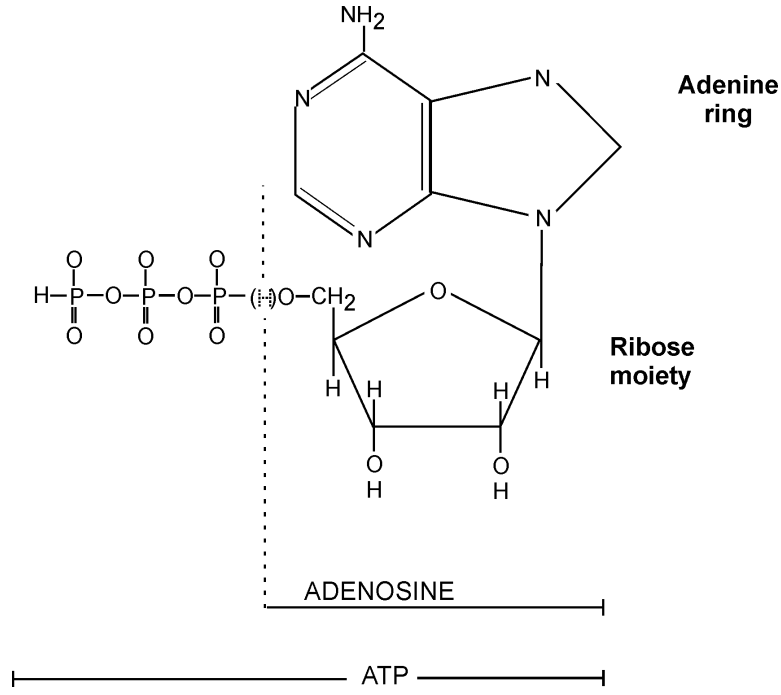

Figure 13.1 Chemical structures of, and relationship between, adenosine and adenosine 5′-triphosphate (ATP). Adenosine contains an adenine ring and ribose component. Phosphorylation of the latter's termial (C_5) hydroxy with three phosphate groups gives ATP

may be stored alone or come directly from the cytoplasm. The extracellular ATP is broken down to adenosine by ecto ATPase.

Unfortunately techniques do not exist for demonstrating purinergic nerves but purinergic receptors have been established. They are divided into two broad groups, P_1 and P_2. The former tend to be located presynaptically, are activated mainly by adenosine and have been reclassified accordingly as A_1 and A_2 (and now A_3). The latter, which respond to ATP, are postsynaptic and as with many other NTs can be divided into two families. Those linked to a fast ionotrophic effect are classified as P_{2x}, with currently six subtypes and those with slow metabotropic effects as P_{2y} with seven subtypes. It is the P_{2x} receptors that mediate the primary transmitter effects of ATP. They have been most studied and while all may be found in the CNS, P_{2x2}, P_{2x4} and P_{2x6} predominate. A schematic representation of a possible ATP (purinergic) synapse is shown in Fig. 13.2.

The role of ATP in the neural control of smooth muscle function is now, as indicated above, well established but its central actions are less clear and have only been studied closely in two areas. In slices of rat medial habenula the synaptic currents, recorded with the whole-cell patch-clamp technique that were evoked by electrical stimulation in the presence of both glutamate and GABA antagonists, were inhibited by the P_{2x} (P_{2x2} preferred) antagonist suramin and by $\alpha\beta$-me-ATP an agonist that desensitises some P_{2x} receptors but not normally the P_{2x2} form. Thus while it is difficult to characterise the precise receptor subunit involved this provides strong evidence for a neurotransmitter

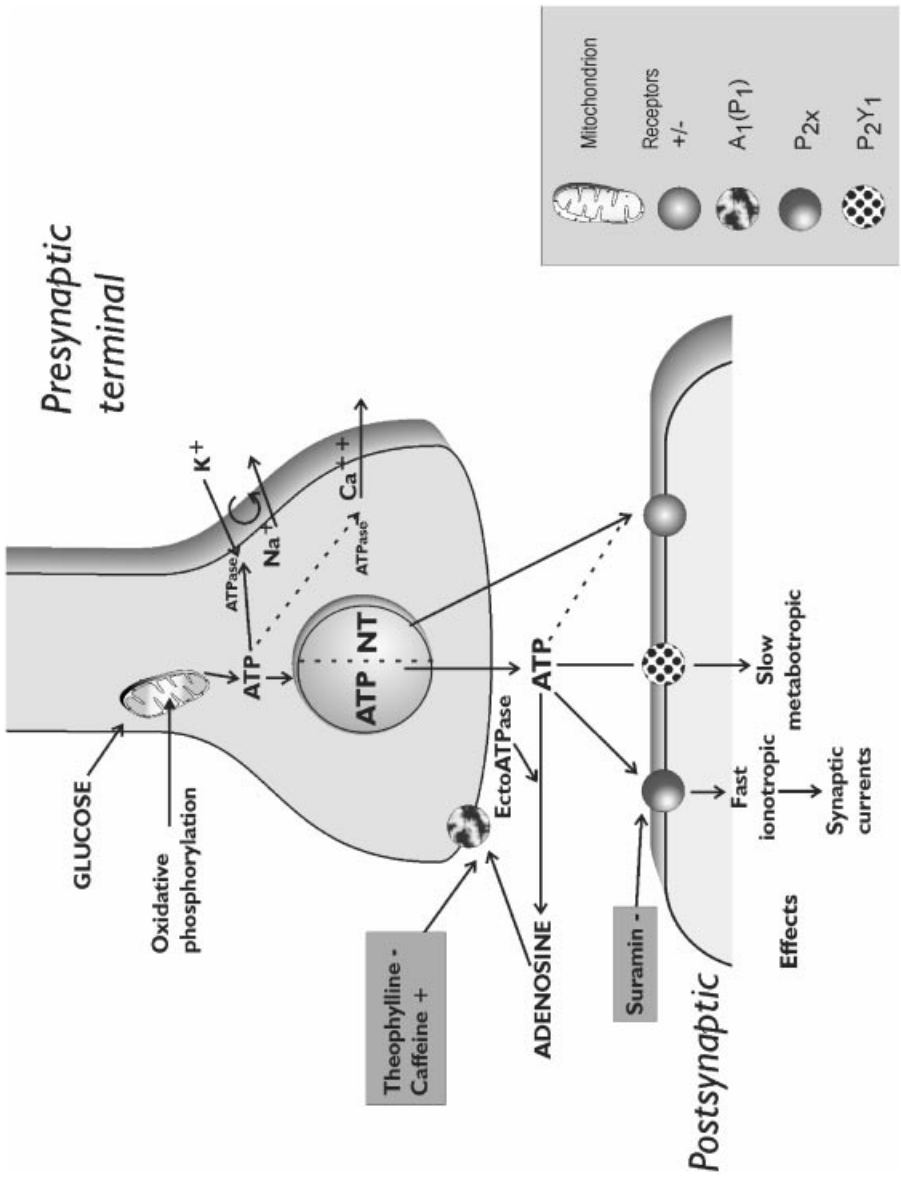

Figure 13.2 Schematic representation of a possible ATP, purinergic, synapse. The effects of ATP, synthesised intraneuronally by mitochondrial oxidative phosphorylation from glucose, on various neuronal ATPases, are shown together with its actions as a conventional neurotransmitter acting at postsynaptic P_2 and presynaptic P_1 receptors

role for ATP, although it is not known to what extent blocking P_{2x2} receptors modifies synaptic transmission when the amino acid receptors are functional. Interestingly the currents mediated by P_{2x} receptor activation are smaller and decay much more slowly than those which characterise glutamate's activation of AMPA receptors but are larger and faster than those mediated by its NMDA receptor. Thus in contrast to NMDA currents, those for ATP are less likely to be involved in the temporal integration of synaptic activity (Gibb and Halliday 1996). The P_{2x} receptor is also linked to control of calcium rather than sodium flux and its subunits have two transmembrane domains compared with the four of glutamate's AMPA and ACh's nicotinic receptor.

This above effect of ATP has also been demonstrated on neurons in lamina II of the dorsal horn in transverse slices of rat cord. Here blockade of glutamate GABA and glycine effects left a current, produced by local tissue or dorsal root stimulation which was again sensitive to the P_{2x2} antagonist suramin (Bardoni *et al.* 1997) (Fig. 13.3). Although only 5% of neurons showed this response, the expression of P_{2x} receptors there and the long established release of ATP from the peripheral terminals of dorsal root ganglia neurons and presumably therefore the central ones, have obviously raised interest in ATP being yet another NT involved in the mediation of afferent painful nociceptive stimuli (Chapter 21).

Thus the neurotransmitter role of ATP is well established in the periphary and also in sensory systems but its importance in the CNS remains to be elucidated (see Burnstock 1996). That requires the development of more specific antagonists and methods of mapping its location. The strong linkage of its P_{2x} receptors to calcium currents may also provide a role for ATP in more long-term effects such as plasticity and neuronal development and death.

ADENOSINE

This is not considered to be a neurotransmitter but it may be an important modulator of neuronal activity through its various receptors, A_1, A_2 and A_3. In addition to its ability

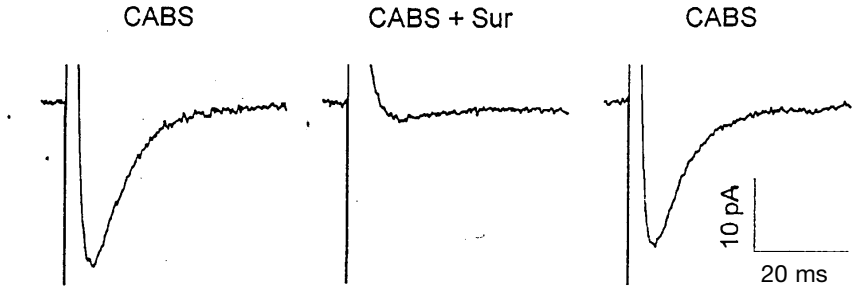

Figure 13.3 Whole-cell patch-clamp recordings of excitatory postsynaptic currents (EPSCs) from dorsal horn neurons of rat (prenatal $P_{2\text{-}13}$) spinal cord slices. The normal evoked EPSC of about 160 pA obtained by focal stimulation of nearby tissue was dramatically reduced by addition of a cocktail (CABS) of CNQX 10 μM, D-APV 50 μM, bicuculline 10 μM and strychnine 5 μM to block glutamate, $GABA_A$ and glycine receptors. The small residual EPSC shown was blocked by the ATP P_2 receptor antagonist suramin and is therefore probably mediated by released ATP. (From Bardoni *et al.* 1997 and reproduced by permission of the *Journal of Neuroscience*)

to reduce the release of a number of NTs it has a strong inhibitory effect on central neurons, enhancing neuronal after-hyperpolarisation through a Ca^{2+}-dependent change in K^+ conductance probably through A_2 receptors. The A_1 receptor appears to be negatively linked to adenylate cyclase through Gi and may mediate the presynaptic inhibition of NT release, with the A_2 acting positively through Gs.

Adenosine comes from the breakdown of ATP. This may occur either extracellularly (Fig. 13.2) or intraneuronally followed by evoked release or transport. Its basal extracellular level is 2 μM but this can increase rapidly when neuronal firing increases and can rise some twentyfold during seizures. The two enzymes responsible for its breakdown are adenosine kinase ($K_m = 2\,\mu M$) and adenosine deaminase ($K_m = 50\,\mu M$). It will be clear that as more adenosine is released during seizures, it will quickly saturate the kinase and its concentration can therefore only be controlled by deaminase. In fact deaminase but not kinase inhibitors are anticonvulsant as is adenosine and its analogues, while its antagonist theophylline is proconvulsant and a central stimulant. Adenosine has even been promoted as an endogenous 'anticonvulsant' (see Dragunow 1986). While that may not be realistic, the antiepileptic benzodiazepine drugs, in addition to their effects on GABA receptors, have been shown to increase the efflux of [$^{-3}$H] adenosine from the rat cortex probably by blocking its uptake and adenosine is often considered to be an endogenous limiter of neuronal activity. Despite this it has also been shown to reduce fast inhibitory postsynaptic potentials (IPSPs) in the rat lateral amygdala probably by presynaptic A_1 inhibitory effect on GABA release (Henbockel and Pap 1999). Adenosine has also been considered to play a role in sleep induction (Chapter 22).

Recently much interest has been shown in the possible neuroproctive effects of adenosine but the responses are complex. Thus A_3 agonists can offer some protection given chronically before ischaemic challenge but given acutely post-challenge they can be neurotoxic (see Jacobsen 1998).

HISTAMINE

The belief that histamine (HT) has a central effect stems from the knowledge that all the classical antihistamines (H_1 receptor antagonists) used to treat allergic reaction, such as hay fever, caused marked sedation if, like mepyramine and promethazine, they can cross the blood–brain barrier, but fail to do so if, like terfenedine and cetirizine, they do not.

The major problem in establishing histamine as a transmitter in the CNS has been the difficulty in demonstrating its actual presence in neurons rather than just in the invading mast cells, in which it is concentrated and from which it is released in the periphery during allergic reactions. The development of immunohistochemical methods for the visualisation of histamine, and its synthesising enzyme histidine decarboxylase, now show there to be definite histaminergic nerves (see Tohyama *et al.* 1991). These are concentrated in the tuberomammillary nucleus in the posterior hypothalamus, not only in the rat but also in humans, and like the other monoaminergic systems (NA and 5-HT) they give off long highly branched axons which ascend in the medial forebrain bundle projecting to the cerebral cortex and hippocampus. Most histamine neurons also contain other transmitters such as GABA, substance P or enkaphalin. The whole brain concentration of histamine is relatively low (50–70 ng/g) but there is much evidence for its central action (see Schwartz *et al.* 1991).

Histamine is synthesised by decarboxylation of histidine, its amino-acid precursor, by the specific enzyme histidine decarboxylase, which like glutaminic acid decarboxylase requires pyridoxal phosphate as co-factor. Histidine is a poor substrate for the L-amino-acid decarboxylase responsible for DA and NA synthesis. The synthesis of histamine in the brain can be increased by the administration of histidine, so its decarboxylase is presumably not saturated normally, but it can be inhibited by α fluoromethylhistidine. No high-affinity neuronal uptake has been demonstrated for histamine although after initial metabolism by histamine *N*-methyl transferase to 3-methylhistamine, it is deaminated by intraneuronal MAO_B to 3-methylimidazole acetic acid (Fig. 13.4). A Ca^{2+}-dependent KCl-induced release of histamine has been demonstrated by microdialysis in the rat hypothalamus (Russell *et al.* 1990) but its overflow in some areas, such as the striatum, is neither increased by KCl nor reduced by tetradotoxin and probably comes from mast cells.

Histamine receptors were first divided into two subclasses H_1 and H_2 by Ash and Schild (1966) on the basis that the then known antihistamines did not inhibit histamine-induced gastric acid secretion. The justification for this subdivision was established some years later when Black (see Black *et al.* 1972) developed drugs, like cimetidine, that affected only the histamine stimulation of gastric acid secretion and had such a dramatic impact on the treatment of peptic ulcers. A recently developed H_2 antagonist zolantidine is the first, however, to show significant brain penetration. A further H_3 receptor has now been established. It is predominantly an autoreceptor on histamine nerves but is also found on the terminals of aminergic, cholinergic and peptide neurons. All three receptors are G-protein-coupled but little is known of the intracellular pathway linked to the H_3 receptor and unlike H_1 and H_2 receptors it still remains to be cloned. Activation of H_1 receptors stimulates IP_3 formation while the H_2 receptor is linked to activation of adenylate cyclase.

Autoradiography and receptor mRNA studies have shown H_1 receptors to be located in most of the brain areas innervated by the ascending histaminergic axons, e.g. cerebral cortex, hippocampus, limbic areas and hypothalamus. Their presence in the cerebellum is not accompanied by appropriate histaminergic innervation. Very few are found in the striatum but this region does show a high density of H_2 receptors. H_2 receptors are also found with H_1 in the cortex, hippocampus and limbic areas, but not in the hypothalamus. Although basically presynaptic the H_3 receptor is also found postsynaptically in the striatum and cerebral cortex (Pollard *et al.* 1993).

Although histamine generally inhibits neuronal firing in the cerebral cortex through H_1 receptors it causes a H_1-mediated excitation in the hypothalamus. It also appears to potentiate NMDA currents although the receptor type has not been established. In the hippocampus it has been shown to block the long-lasting hyperpolarisation (accommodation) that normally follows neuronal firing and is mediated through a Ca^{2+}-activated K^+ conduction.

From time to time it has been suggested that histamine has some role in a number of behaviours and motor activity while the established and marked sedative effect of H_1 receptor antagonists, mentioned at the start of this section, has consistently been considered to indicate a role for histamine in arousal and the sleep–waking cycle (see Chapter 22).

Histamine release in the hypothalamus is higher during the active waking than the quiescent phase of behaviour, whether this is associated with darkness (in rats) or light (rhesus monkey). The firing rate of histamine neurons is also higher during arousal

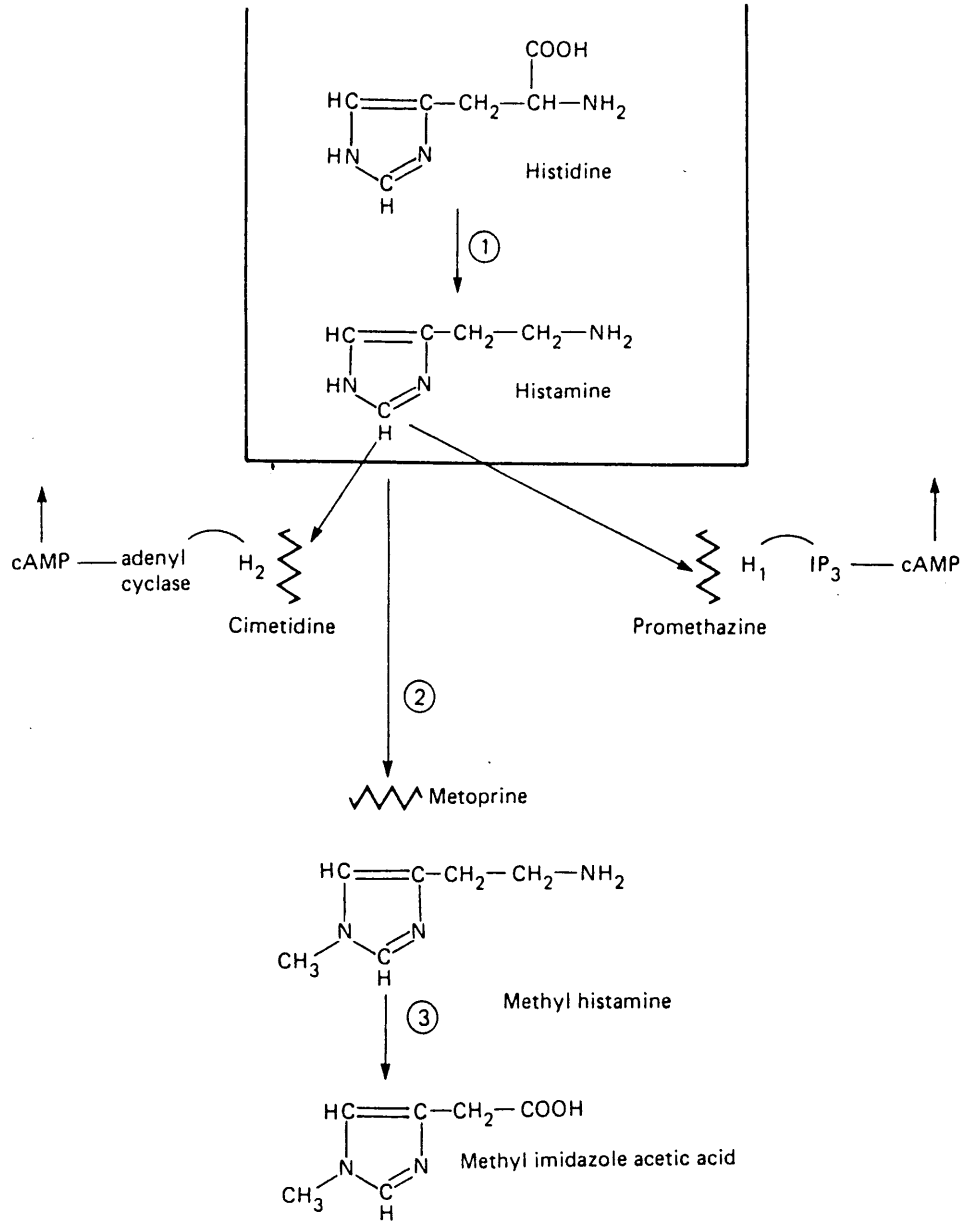

Figure 13.4 Histamine; synthesis, metabolism and receptors. Current knowledge does not justify presentation of a schematic histaminergic synapse. (1) Histidine decarboxylase; (2) histamine-*N*-methyltransferase; (3) mono amine oxidase (MAO_B)

and intraventricular histamine produced EEG arousal in animals. In the cat the H_1 antagonist mepyramine increases the slow-wave sleep pattern while direct injection into the hypothalamus of histamine itself, or an inhibitor of histamine-*N*-methyltransferase to stop histamine breakdown, produces the opposite effect, but it is still sensitive to mepyramine. Such H_1-induced waking effects have not been so clearly established in

humans. In contrast to these excitatory effects elevating brain histamine levels with metoprine, an inhibitor of histamine-*N*-methyltransferase protects rodents against maximal electroshock although the specificity of the effect remains to be established. Agonists and antagonists at the H_3 autoreceptors, which should decrease and increase histamine release, have been shown to augment and reduce slow-wave sleep in rats and cats. (For a review of H_3 neuropharmacology see Leurs *et al*. 1998.)

The fact that the older H_1 antagonists, which reach the brain, are prescribed with the warning that they can affect the patient's ability to drive or operate machinery suggests that they impair pscychomotor performance. There are in fact numerous demonstrations of this using tests which require visual–motor coordination such as vigilance tasks and finger tapping. Since the slowing of such function could result from retarding information processing in the visual cortex it is interesting that the latency of components of the evoked potential, which follows presentation of a changing (reversing) black and white checkerboard pattern, is prolonged significantly in humans by the H_1 antagonist diphenhydramine, which enters the brain, but not by terfenedine which does not (Tharion, McMenemy and Rauch 1994).

There is also some evidence that histamine may be involved in food and water intake and thermoregulation (see Hough and Green 1983).

NEUROSTEROIDS

Neurosteroids differ from nearly all the other transmitters and mediators in that they are lipid-soluble and can easily cross the blood–brain barrier. Thus it is necessary to distinguish those steroids that are produced in the brain from those that find their way there from the circulation after being released from the adrenal cortex or gonads. There are many natural and synthetic steroids that have some effect on neuronal function and can be considered neuroactive but few are actually produced in the brain to act on neurons, i.e. the true neurosteroids.

Steroids which are found in the brain may be grouped as follows. The list is not exhaustive.

(a) Those formed in peripheral glands:

Corticosteroids
- Corticosterone (a glucocorticoid)
- Aldosterone (a minerolocorticoid)

Reproductive steroids
- Oestradiol (an oestrogen)
- Testosterone (an androgen)

All these steroids disappear from the brain in animals after removal of the adrenals or gonads (ovary and testis). This also applies to tetrahydrodeoxycorticosterone for although it is formed by reduction of deoxycorticosterone within the brain, its synthesis depends on that steroid coming from the blood.

(b) Those formed in both the periphery and CNS:

Progesterone (PROG) (a progestogen)

Tetrahydroprogesterone (3α5αThPROG) or allopregnenolone, a reduced metabolite of progesterone

Pregnenolone (PREG) and its reduced (20α dihydropregnenolone) and sulphated (pregnenolone sulphate, PREGS) metabolites

The levels of these steroids are reduced, especially that of progesterone (70%) by adrenalectomy and/or gonadectomy but sufficient remains to indicate some central synthesis.

(c) Those formed within the CNS:

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate, its sulphated derivative

DHEA levels are unaffected by adrenolectomy or gonadectomy

The neurosteroids of most interest are DHEA, PREG, PREGS, PROG and 3α5αThPROG.

The chemical structures and interrelationship of the neurosteroids listed in (b) and (c) above are shown in Fig. 13.5 but the synthetic pathways are not well established. PREG and DHEA are known as 3β-hydroxy-Δ5-steroids and are also found in peripheral glands as intermediaries between cholesterol and active steroids such as PROG and testosterone. It seems that cholesterol may be the starting point of neurosteroid synthesis in the brain. Cytochrome P450scc, the specific hydroxylase needed for cleavage of the cholesterol side-chain to give PREG, has been found widely in white matter and in myelinating oligodendrocytes, but not in neurons. Enzymes are present for the conversion of PREG to PROG and both are reduced in glia and neurons. This does not occur with DHEA and very little is known of either its synthesis or metabolism.

Neurosteroids are widely and fairly evenly distributed in the brain with few noteworthy localisations but the concentration of the conjugated forms (sulphated and reduced) of PREG and DHEA can exceed that of the parent compounds. Values given by Baulieu (1997) are PREG 8.9 ng/g, with 14.2 and 9.2 for its sulphate and hydroxy metabolites, DHEA 0.24 ng/g (1.7 and 0.45) and PROG 2.2 ng/g. By contrast, although the concentration of progesterone rises some twelvefold in plasma and eight times in hippocampus of animals and humans as they pass from the follicular to luteal phase of the ovarian cycle, it increases by 300 in the cortex, suggesting a considerable variation in the ability of different brain regions to concentrate it. In considering the neurosteroids as possible NTs it should be remembered that neither specific steroid neurons nor an evoked neuronal release of steroids have been demonstrated. There are, however, receptors for them in the CNS and no shortage of actions attributed to them.

There is no doubt that steroids have behavioural effects. Given clinically in the therapy of inflammatory conditions, the glucocorticoids are considered to produce euphoria, followed after prolonged or higher dosing by depression and, of course, when we are stressed the corticocosteroids pumped out by the adrenal cortex easily enter the brain and may initiate some of the behavioural response. In women the premenstrual syndrome (irritability, depression and anxiety) is thought to be associated in some way with progesterone since not only does its concentration rise then fall during that time but in post-menopausal women the use of sequential oestrogen and progestogen hormone replacement therapy (HRT) shows that similar mood changes accompany only the addition of progestogen. More specifically, in women with epilepsy while the incidence of seizures decreases when plasma progesterone is high, it increases during the immediate premenstrual period as progesterone levels fall, rather as with withdrawing

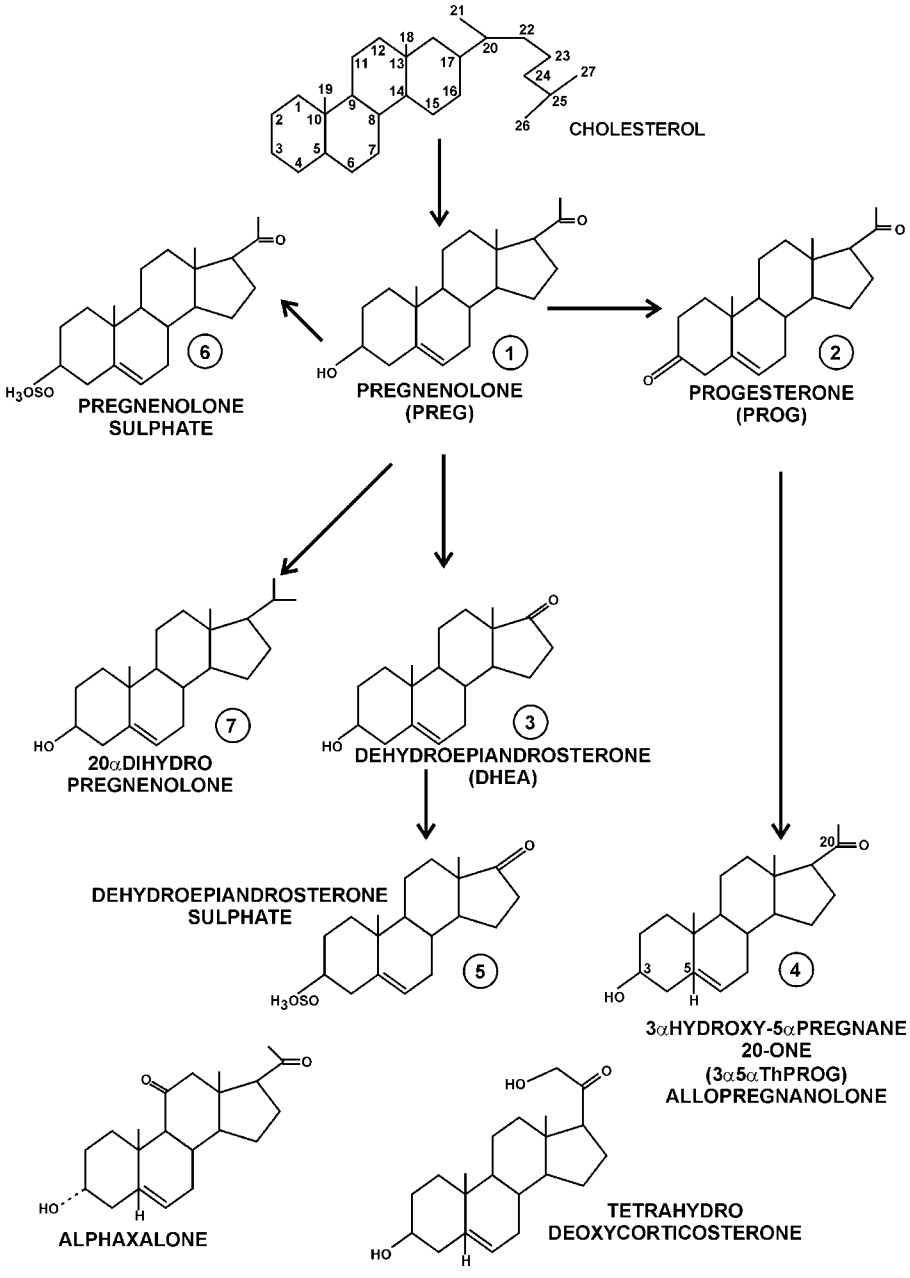

Figure 13.5 Structure and interrelationship of the major neurosteroids. Pregnenolone (PREG) (1) is synthesised from cholesterol and is then either metabolised, reduced to 20α dihydropregnenolone (7) or sulphated (6), or converted by 3β-hydroxysteroid dehydrogenase to progesterone (PROG) (2) or to dehydroepiandrosterone (DHEA) (3). The former (PROG) can then be reduced to allopregnanolone (3α5αThPROG) (4) and DHEA sulphated to dehydroepiandrosterone sulphate (5). It is PREG, DHEA and 3α5αThPROG which appear to be important centrally. The structures of alphaxalone, a steroid anaesthetic and tetrahydrodeoxycorticosterone, which is formed in the brain from deoxycorticosterone of peripheral origin, are also shown

an antiepileptic drug. Of course, it cannot be assumed that plasma levels are reflected in the brain but in rats stressed by insertion in the Morris water maze, so that they have to swim to a safe platform (see Chapter 18), there is still some increase in the concentration of brain PROG and in particular its reduced metabolic (ThPROG), even after adrenalectomy (Purdy *et al.* 1991). The aggressiveness of castrated male mice exposed to lactating females in a cage can also be reduced by DHEA administration.

These observations, while implicating steroids in brain function and behaviour, cannot be taken as a reliable indicator of their actual effect on neuronal function. Nevertheless, some neurosteroids produce CNS depression with a rapid inhibition of neuronal excitability and one progesterone derivative, alphaxalone (3α-hydroxy-5α pregnane-11, 20 dione, see Fig. 13.5) has been used effectively as an intravenous anaesthetic in humans.

Intracellular steroid receptors, which alter gene expression, exist for corticosteroids, oestrogens and progesterone in the brain, as in the periphery but they cannot account for the relatively rapid depression of CNS function induced by some steroids. This was explained when Harrison and Simmonds (1984) discovered that alphaxalone (the steroid anaesthetic) potentiated the duration of GABA-induced currents at the $GABA_A$ receptor in slices of rat cuneate nucleus just like the barbiturates (Fig. 13.6). Of the

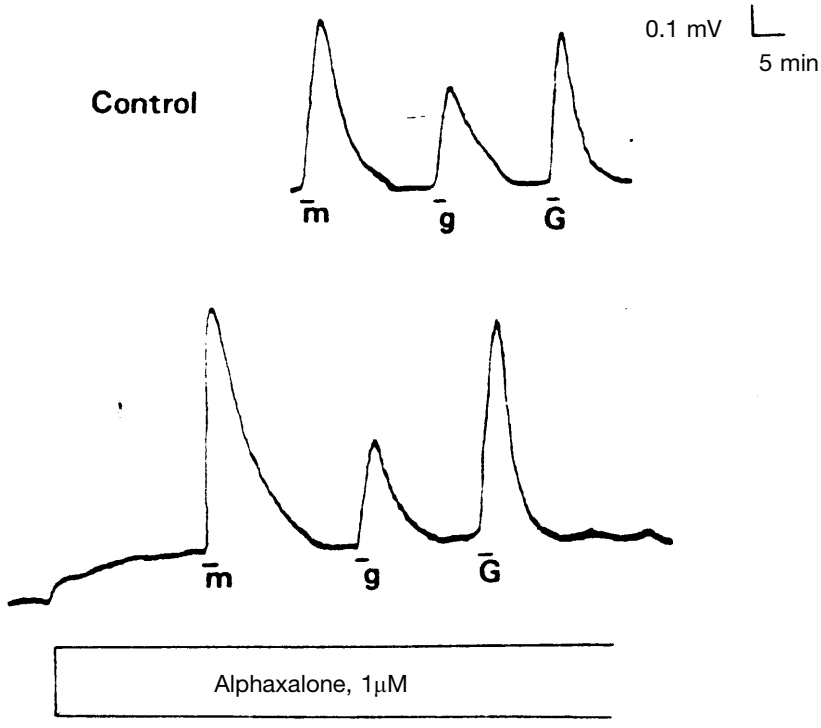

Figure 13.6 Potentiation of GABA action at the $GABA_A$ receptor by the steroid anaesthetic, alphaxalone. Depolarisations recorded extracellularly from dorsal funiculus fibres and terminals in the rat cuneate brain slice after superfusion for 2 min with muscimol 2.5 μM (m), GABA 50 μM (G) and glycine 2 mM (g). In the presence of alphaxalone (1 μM), responses to GABA and the $GABA_A$ agonist muscimol, but not those to glycine, were substantially enhanced. The effect was reversible with responses slowly returning to normal after 3 h. (From Harrison and Simmonds 1984 and reproduced by permission of Elsevier Science)

naturally occurring neurosteroids ThPROG also increases $GABA_A$ receptor currents increasing both the duration and probability of Cl^- channel opening. The sulphated metabolite of PREG is similarly active at low (nM) concentrations. These allosteric effects are still seen after maximal barbiturate potentiation and are not affected by benzodiazepine antagonists suggesting a specific and separate modulating site for the steroids (see Paul and Purdy 1992) although it has not been found. Also while their activity changes with the subunit composition of recombinent $GABA_A$ receptors no specific configuration has been established for their effectiveness but expression of α_2 with $\alpha_1 + B_1$ or $\alpha_2 + B_1$ gives a more responsive receptor than the inclusion of α_3 (Shingai, Sutherland and Barnard 1991).

Not all neurosteroids are inhibitory. The GABA potentiation seen with low concentrations of PREG sulphate changes to antagonism at higher strengths and both this compound and sulphated DHEA, which also inhibits $GABA_A$ receptors, are proconvulsant. There is also evidence that the sulphates of PREG and DHEA potentiate NMDA receptors while glucocorticoids reduce seizure threshold without affecting GABA receptors. Thus even without considering reports of effects on glycine sigma and ACh nicotinic receptors, the electrophysiology of the steroids is complex. In practice, although steriods modulate $GABA_A$ receptors at realistic nM concentrations, unphysiological μM amounts are required at other ligand-gated ion channels (see Rupprecht and Holsboer 1999).

Two unrelated steroid effects warrant some mention. The discovery that PREGS levels are significantly lower in aged than young male rats prompted an interest in its possible role in memory function. In rats, subjected to spatial memory tests in water and Y mazes, impairments in performance were mirrored by reductions in hippocampal PREGS levels (see Baulieu 1997), while aged rats with established memory impairment showed improvement after PREGS administration. Whether this depends on the known ability of PREGS to increase NMDA activity and the accepted role of that receptor in LTP maintenance and memory functions remains to be seen.

In the periphery PROG and PREG may well have an important trophic action since their production in Schwann cells has been shown to result in increased myelin synthesis in regenerating rat sciatic nerve and cultured dorsal root ganglia (see Koenig *et al.* 1995).

With so many different neurosteroids with differing and even opposing neuronal effects, much will depend on their relative concentrations at any time and any evaluation of their function must take this into consideration. Hopefully the synthesis and use of appropriate antagonists will throw more light on the physiological role of steroids in the CNS and facilitate the development and clinical use of new neuroactive steroids (see Gasior e*t al.* 1999).

ADRENALINE

The enzyme *β*-phenylethanolamine-*N*-methyl transferase, which is required to convert noradrenaline (NA) to adrenaline (Ad), is present in the CNS and there is histofluorometric evidence (positive staining with antibodies to that enzyme and to tyrosine hydroxylase and dopamine *β*-hydroxylase as well) for adrenergic cell bodies in two groups (nuclei) alongside NA neurons of the locus coeruleus (LC) but ventral and lateral (C_1) and dorsal and medial (C_2) to it. Projections go to the hypothalamus and in

particular to the dorsal motor nucleus of the vagus as well as to the LC itself. Little is known of the normal function of these relatively minor pathways although stimulation of C_1 causes a release of adrenaline in the hypothalamus and a rise in blood pressure, which is blocked by mixed β-antagonists but not by β_1-antagonists administered icv (Marsden 1987). Such a β_2-mediated response would be better mediated by adrenaline rather than NA, which has little activity at such receptors (see Chapter 7).

TRACE AMINES (TRYPTAMINE, PHENYLETHYLAMINE, TYRAMINE AND OCTOPAMINE)

Decarboxylation, instead of hydroxylation, of the amino-acid precursors of DA and 5-HT results in the formation of amines that are only found in trace amounts in the CNS but have distinct effects when administered into the brain (Fig. 13.7). Since such decarboxylation can be achieved by the non-specific L-aromatic amino acid decarboxylase there is considerable potential for its occurrence, especially if there is a rise in the concentration of the appropriate precursor or some malfunction in their normal hydroxylation through rate-limiting processes. This could shunt tyrosine, tryptophan and phenylalanine through to tyramine, tryptamine and phenylethylamine rather than to the more normally formed dopa, 5-HT and tyroxine (Fig. 13.7). It is this potential for synthesis together with the known central effects of these amines when injected, that preserves an interest in them despite their very low concentrations in whole brain, i.e. phenylethylamine 1.8, *p*-tyramine 2.0, *m*-tyramine 0.3 and tryptamine 0.5 ng/g. Generally concentrations are highest in the striatum (4, 11, 0.3 and 1.5 ng/g respectively) but still very much lower than DA (10 μg/g). Unlike the catecholamines their concentration rises dramatically (50 times) after inhibition of MAO. Turnover can also be increased easily by the provision of extra substrate since decarboxylation is not rate limiting. Distinct anatomical pathways have not been identified since there is no specific enzyme involved in their synthesis that can be used for immunohistochemistry, and they are not sufficiently concentrated for ordinary histofluorescence.

TRYPTAMINE

Although tryptamine can be detected in brain there has been much debate over whether it exists separately from 5-HT or merely co-exists with it. Specific high-affinity binding sites have been demonstrated for tryptamine in rat cortex. These appear to be different from 5-HT sites but until appropriate antagonists are found it remains possible that they form a subset of the ever-increasing number of 5-HT receptors (see Chapter 9). The behavioural response in rats to tryptophan plus a MAO inhibitor (Grahame-Smith 1971) is accompanied by an elevation of brain tryptamine as well as 5-HT and is less marked if the synthesis of tryptamine is reduced by a decarboxylase inhibitor that does not have a significant effect on 5-HT levels. In fact after a MAOI, tryptamine produces a behavioural response in rats similar to that of tryptophan apart from the absence of certain features like tremor and wet-dog shake. The complexity of the situation is illustrated by studies of the effect of intra-hypothalamic injections of 5-HT and tryptamine on rat body temperature (Cox, Lee and Martin 1981). In these it was shown that 5-HT decreased temperature while tryptamine actually increased it but it was not possible to block one effect preferentially with a whole range of antagonists, despite

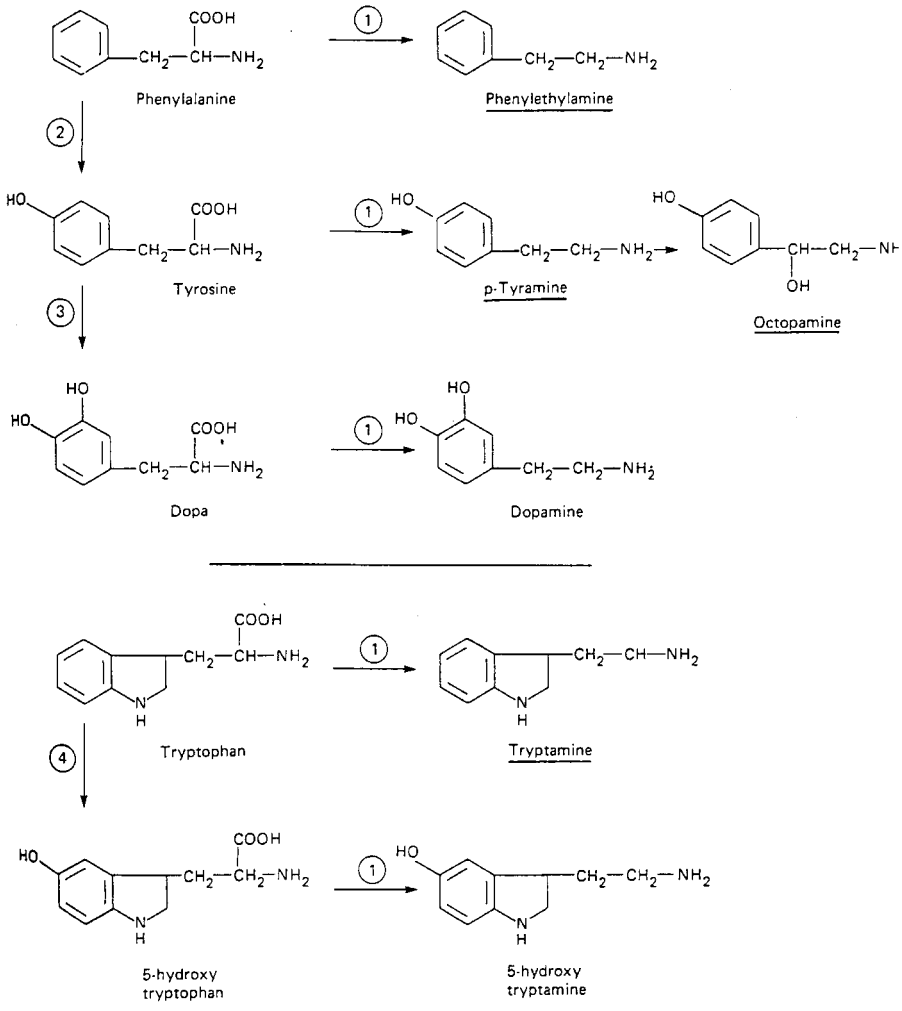

Figure 13.7 Synthesis and structure of the trace amines phenylethylamine, *p*-tyramine and tryptamine. These are all formed by decarboxylation rather than hydroxylation of the precursors of the established monoamine neurotransmitters, dopamine and 5-HT. (1) Decarboxylation by aromatic L-amino acid decarboxylase; (2) phenylaline hydroxylase; (3) tyrosine hydroxylase; (4) tryptophan hydroxylase

some differences in effectiveness. Also although it was the tryptamine and not the 5-HT response, which was abolished after destruction of 5-HT neurons with 5,7-dihydroxytryptamine and implies that tryptamine was releasing 5-HT, it was found that raphé (5-HT) neuron stimulation produces hyperthermia, like tryptamine, rather than hypothermia, like 5-HT.

These opposing effects of tryptamine and 5-HT are also seen when they are applied directly to cortical neurons by iontopheresis. Tryptamine is predominantly depressant while 5-HT is mainly excitatory. Surprisingly, the 5-HT antagonist metergoline is more effective against tryptamine and the depressant effects. When the medial Raphé nucleus

is stimulated this produces inhibition of cortical neurons followed by excitation but it is the inhibition (tryptamine-like) that is blocked by metergoline. In keeping with this finding is the observation that depletion of 5-HT with pCPA reduced only the excitatory (5-HT) response while 5,7-dihydroxytryptamine, which destroys the neurons, abolished both effects (see Jones 1983). The inference from these studies and those on temperature is that some neurons in the raphé nucleus release something other than 5-HT. This might be tryptamine but if it is not, then its effects are presumably modified by tryptamine.

Possibly there is a subclass of 5-HT receptors that would be preferentially activated by tryptamine if its endogenous concentrations were ever adequate. Indeed the term 'tryptamine receptor' as first used by Gaddum to describe the effects of all indole amines may be one to which we should return.

PHENYLETHYLAMINE

The relationship of phenylethylamine to dopamine is not unlike that of tryptamine to 5-HT. Present in low concentrations in the brain there is some evidence for distinct binding sites but not for specific neurons. When injected icv it causes stereotyped behaviour similar to, but more marked than, that seen with amphetamine. These effects are blocked by neuroleptics (DA antagonists) and since phenylethylamine does not bind directly to DA receptors it is assumed to release DA, like amphetamine. This is substantiated by the fact that in rats with unilateral 6-OHDA lesions of the SN its systemic administration causes ipsilateral rotation like amphetamine (see Chapter 6). Phenylethylamine certainly increases the overflow of [^{3}H]-DA from striatal synaptosomes and slices and of endogenous DA *in vivo*, but part of this may be due to block of DA uptake. In any case such effects only occur with concentrations of 5×10^{-5} M, which are not likely to be encountered *in vivo* and it is not Ca^{2+}-dependent. Peripherally phenylethylamine causes contractions of the rat fundus just like amphetamine, tryptamine and 5-HT. These are reduced by some 5-HT antagonists, like methysergide, but not by DA antagonists. Thus some of its central effects may be mediated through a tryptamine receptor. Needless to say, the DA and amphetamine-like activity and structure of phenylethylamine, together with the facility for its synthesis in the CNS, has led to unproven suggestions of its involvement in schizophrenia. In fact there is some evidence for increased excretion of its metabolite (phenyl acetic acid) in the subgroup of paranoid schizophrenics.

TYRAMINE

p-Tyramine is produced by decarboxylation of tyrosine and is present in the CNS in higher (threefold) concentrations than *m*-tyramine, the hydroxylated derivative of phenylethylamine. In the periphery *p*-tyramine is easily hydroxylated to octopamine, which has some direct effects on α_1 adrenoceptors, unlike tyramine which functions by releasing NA. When tested on central neurons tyramine always produces the same effects as NA but they are slower and less marked, implying an indirect action. By contrast octopamine often produces the opposite effect to NA and it is probable that octopamine may have a functional role in the invertebrate CNS where it is found in higher concentrations (5 μg/g) than in the mammalian brain (0.5 ng/g). Neither tyramine nor octopamine have distinct behavioural effects, unlike phenylethylamine,

and little is known of their central effects, although depressed patients have been shown to excrete less conjugated tyramine than normal subjects after challenge with tyrosine.

PROSTAGLANDINS

The main problem with any study of prostaglandins (PGs) is that although brain concentrations can exceed 0.1 μg/g, they appear to be formed on demand, rather than preformed and stored and they have very short half-lives (seconds). Also specific effective antagonists remain to be developed and PGs are widely and evenly distributed, unlike many NTs. Thus any analysis of their central effects rests heavily on either studying PG release, or their effects when applied directly (icv injection). Certainly the brain has the enzymatic ability to synthesise both prostaglandins (cycloxygenase) and leukotrienes (lypoxygenase) from arachidonic acid (AA) (see Fig. 13.8) and a number of central functions have been proposed for them (see Piomelli 1994).

When injected into the brain (often in rather large concentrations) PGE_2 but not PGF_2 is a depressant and causes sedation and catatonia. PGEs can be found in superfusates of cat cortex and their concentration is increased by direct electrical stimulation as well as by afferent nerve activation. In fact, when given intraventricularly PGE_1 and PGE_2 antagonise convulsions induced by leptazol and electroshock but whether PGs have any

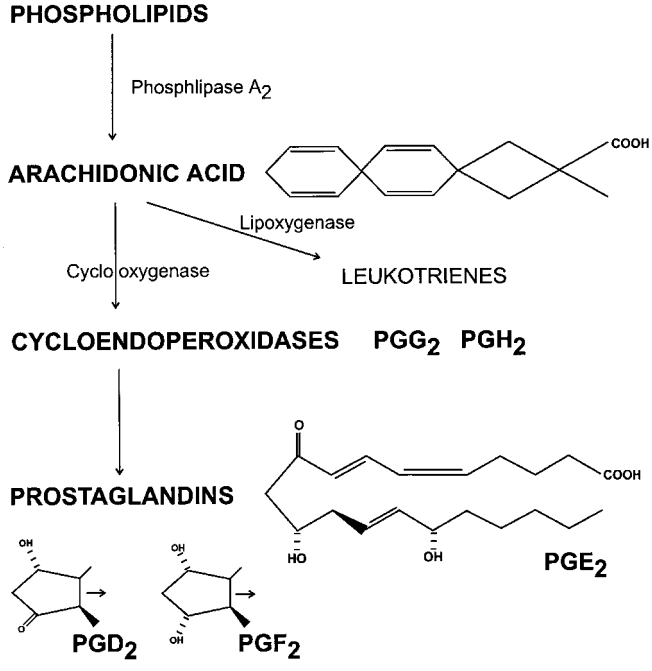

Figure 13.8 Products of arachidonic acid metabolism. The action of cyclooxygenase produces the cyclic endoperoxides PGG_2 and PGH_2 and the prostaglandins PGE_2, $PGF_{2\alpha}$ and PGD_2: lypoxygenase activity gives rise to leukotrienes. The classification D,E,F,G,H depends on the number and position of the hydroxy groups. The subscript (2) describes the number of double bonds. It is the (2) derivatives that appear to be active pharmacologically. ◀ Bonds which lie in front of the plane of the cyclopentane ring. ≡ Bonds which lie behind the plane of the ring.

role in initiating or controlling convulsive activity is uncertain. The levels of a number of PGs, especially PGD_2 and PGE_2, are reported to be significantly lowered in spontaneously convulsing gerbils and in these animals the levels of brain lypoxygenase derivatives have also been found to increase after the onset of seizures (Simmet, Seregia and Hertting 1987), although such changes could result from, rather than cause, the convulsions.

PGD_2 and PGE_2 receptors are concentrated in the preoptic region of the basal forebrain which is known to be imporant in sleep production and when injected into that area PGD_2 does induce sleep. In fact PGD_2 synthesis increases in rat cortex in the light period of a dark/light cycle when rats normally sleep and when infused into the third ventricle it induces a seemingly natural sleep at very low ($-\mu M$) concentrations.

One area of particular interest, in view of the anti-pyretic effects of cyclo-oxygenase inhibitors like aspirin, is the possible role of PGs in the control of body temperature. Thus icv injections of PGE_1 and PGE_2 elevate body temperature and PGE levels increase in CSF following pyrogen-induced fever. Unfortunately this release does not occur near the anterior hypothalamus, which is considered to control body temperature, and iontophoretically applied PGE_2 does not affect the firing of hypothalamic neurons. Also lesions of the anterior hypothalamus abolish PGE- but not pyrogen-induced fever. The situation remains to be resolved (see Wolfe 1982).

Interest in the PGs has recently reverted to their precursor arachidonic acid (AA), which seems to be able to act intracellulary as a second messenger, and also extracellularly. In this latter mode it may play a part in LTP. It is known that AA produces a long-lasting enhancement of synaptic transmission in the hippocampus that resembles LTP and in fact activation of NMDA receptors leads to the release of AA by phospholipase A_2 (see Dumuis *et al.* 1988) and inhibition of this enzyme prevents the induction of LTP. AA has also been shown to block the uptake of glutamate (see Williams and Bliss 1989) which would potentiate its effects on NMDA receptors. This would not only prolong LTP but also cause neurotoxicity.

NITRIC OXIDE

INTRODUCTION

The results of a number of studies demonstrate that the gas nitric oxide (NO) plays a functional role in the central nervous system. This all originated with the discovery that the so-called endothelium-derived relaxing factor (EDRF), found in blood vessels, and thought to be a peptide, was in fact NO. The potential roles of this freely diffusible gas have subsequently been extended to many other tissues and organs but we will concentrate on the possible neuronal roles of what is obviously a novel mediator. There are also suggestions that the closely related carbon monoxide may also have a function in the central nervous system.

Many brain and spinal cord neurons have the capacity to produce NO and experimental evidence indicates a role for this gas in neuronal transmission in animals. A major issue is that the effects of a gas are not limited to the release site and interpretation of the apparent neuronal actions of NO is complicated by the fact that some of the observed effects may be via changes in local blood flow.

Being a gas, NO can diffuse freely once produced, and so is not constrained by the usual mechanisms of release and uptake that confine most transmitters to the synapse. Likewise, the fact that it is not stored means that the criteria of presence and storage are

not met by this highly labile and freely diffusible molecule. Finally, its ability to cross lipid barriers means that it is a transcellular mediator rather than a molecule that acts on a surface receptor close to its release site. Thus while it cannot be considered as a neurotransmitter, NO can still have important actions in the central nervous system.

SYNTHESIS

NO is the product of the oxidation of one of the guanidino nitrogens of the amino acid, L-arginine by the enzyme nitric oxide synthase (NOS). L-arginine is then hydroxylated and a second oxygen atom is incorporated to produce NO and citrulline (see Fig. 13.9). The production of NO occurs in many tissues. There are three isoforms of the enzyme, endothelial (eNOS), inducible (iNOS) and neuronal (nNOS). Whereas nNOS and eNOS are regulated by calcium, iNOS is not. This control stems from the production of a calcium–calmodulin complex that then binds NOS and switches on the production of NO from arginine. Then, as internal calcium levels drop, the production of NO also ceases. In many parts of the central nervous system, NMDA receptors are expressed on neurons with the capacity to produce nitric oxide. Thus, calcium influx through the NMDA receptor channel appears to trigger production of nitric oxide by activation of nNOS. To complicate matters, nNOS is also found outside the CNS in epithelial cells and skeletal muscle. Nitric oxide seems to play a much greater role in neuronal

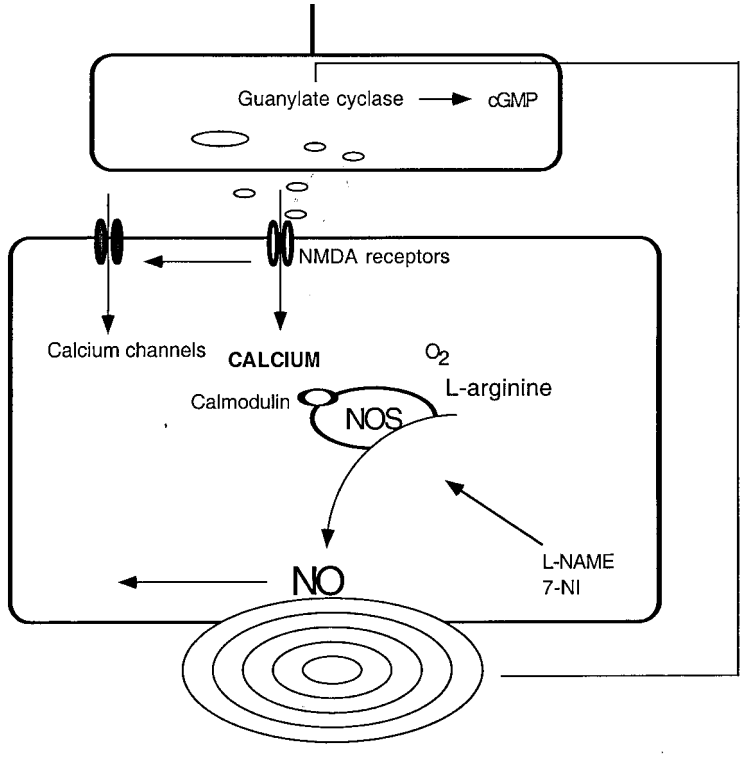

Figure 13.9 The production and actions of nitric oxide (NO). The influx of calcium through either calcium channels or NMDA receptors triggers NOS to convert L-arginine to NO. L-NAME and 7-NI inhibit this process. NO, once produced, can diffuse in a sphere and then can activate guanylate cyclase

transmission following excessive stimulation/pathology in some regions of the brain and so although the enzyme is constitutive it can clearly be unregulated.

CELLULAR ACTIONS

The main action of NO is on the enzyme-soluble guanylate cyclase. NO activates this enzyme by binding to the heme moiety and so there is an increased conversion of GTP to cGMP. This reduces intracellular calcium and this action, and also partly through activation of cGMP-dependent protein kinases, relaxes smooth muscle. The same mechanism of action occurs in neurons but NO can also inhibit other enzymes with a heme group such as cyclooxygenase and lipoxygenase. How these effects translate into what appear to be mostly excitatory effects in the CNS is unclear. Thus agents that increase NO production cause increases in neuronal excitability and vice versa. The results of a number of studies manipulating the levels of the gas show that NO plays a role as a neuronal communicator. There is, however, the problem of a lack of selective agents that modulate the production and actions of NO.

PHARMACOLOGY — INHIBITORS

Application of L-arginine and nitrates and nitrites (that donate NO) has been used to drive the system but, as always, blocking the effects of a potential mediator provides the best approach. There have been reports of a large number of putative inhibitors of NOS but there are two agents that have been widely used, L-N^G nitroarginine (L-NAME) together with the closely related L-N^G monomethylarginine (L-NMAA). These agents block NOS at the arginine site, acting as false substrates, and have no selectivity for any of the three forms of the enzyme. Thus, any study of the physiological role of NO in neurons based on the use of these compounds will be carried out in animals where the vascular effects of NO are also blocked leading to severe hypertension. This may well lead to problems of interpretation and even local application of these compounds directly within the CNS will change local blood flow.

However, more recently, a functionally selective inhibitor of nNOS has been described — 7-nitroindazole (7-NI). It is puzzling that *in vitro* this compound has no selectivity for nNOS over eNOS but after systemic administration, fails to change blood pressure yet alters neuronal responses that are thought to result from production of NO. A suggested resolution of this action is that 7-NI is metabolised in the periphery but not the CNS, so that once it has crossed the blood–brain barrier, it can only act on nNOS.

FUNCTION — EXCITOTOXICITY

The proposal that NO or its reactant products mediate toxicity in the brain remains controversial in part because of the use of non-selective agents such as those listed above that block NO formation in neuronal, glial, and vascular compartments. Nevertheless, a major area of research has been into the potential role of NO in neuronal excitotoxicity. Functional deficits following cerebral ischaemia are consistently reduced by blockers of NOS and in mutant mice deficient in NOS activity, infarct volumes were significantly smaller one to three days after cerebral artery occlusion, and the neurological deficits were less than those in normal mice. Changes in blood flow or vascular anatomy did not account for these differences. By contrast, infarct size in the mutant became larger

after eNOS inhibition by L-NAME administration. Hence, after middle cerebral artery occlusion neuronal NO production appears to exacerbate acute ischemic injury, whereas vascular NO protects. The data emphasise the importance of developing selective inhibitors of the neuronal isoform.

NOCICEPTION

Behavioural studies are generally unable to find a role for spinal NO in nociceptive reflexes in normal animals, whereas NO inhibitors are highly effective in blocking these same reflexes following the induction of peripheral inflammation or neuropathy. Although a complication is that NO may also play a role in peripheral vascular events during inflammation, these results do suggest that the gas is produced only under certain conditions.

The nNOS inhibitor 7-NI causes a greater inhibition of the wind-up and hyperexcitability of dorsal horn neurons (see Chapter 21) than the immediate response due to direct afferent C-fibre stimulation in normal animals. The preferential inhibition of the NMDA receptor-mediated neuronal hyperexcitability and wind-up of the neurons by 7-NI conforms to the idea that the NO generated in the spinal cord during the transmission of nociceptive information is a consequence of NMDA receptor activation. This also agrees well with a number of other observations, including electrophysiological studies in which block of NO production reduces the excitatory effects of NMDA on neurons and behavioural studies where block of NO production reduced the behavioural effects of NMDA. It seems that following the development of peripheral inflammation and consequent hyperalgesia the NMDA receptor is able to participate in spinal nociceptive reflexes providing a mechanism whereby NO is generated. Thus NOS inhibitors do block nociceptive reflexes in behavioural studies in animals with peripheral inflammation. However, once NO is generated in the spinal cord, the mechanism by which it produces its effects, such as the role played by NO in the wind-up process, has yet to be confirmed. Although NO can act in the neuron in which it is produced to increase levels of cGMP, NO can also diffuse to other neurons to produce its effects. It has been shown that activation of NMDA receptors in the cord can produce an NO-mediated release of glutamate, some of which may represent release from primary afferent terminals following the retrograde diffusion of NO. Nitric oxide can also evoke the release of CGRP and substance P from the dorsal horn of the spinal cord. An NO-evoked release of glutamate, CGRP and substance P may operate as a positive feedback system to further generate wind-up and centrally mediated hyperalgesia. Thus, the development of clinically useful neuronal NOS inhibitors could provide a novel approach to indirectly controlling NMDA receptor-mediated transmission. As with agents directly acting at the NMDA receptor–channel complex, side-effects may preclude their use.

LONG-TERM POTENTIATION

The idea of retrograde messengers such as NO has also been advanced with regard to hippocampal LTP (Chapter 20). There is a marked lack of consensus on whether NO plays a role in LTP and much discussion on why different groups find different results. The importance of the need for a diffusible messenger in the initiation of long-term changes comes from the fact that LTP is induced by activation of postsynaptic NMDA receptors yet maintained by presynaptic changes. Thus, there is a requirement for a mediator to be generated by NMDA receptor activation and then diffuse back to the

presynaptic terminals. NO could fulfil this role. Unfortunately, some studies have shown that NOS inhibition blocks LTP whereas others have failed to show this.

SUMMARY AND PERSPECTIVES

NO differs from the more conventional NTs like the amino acids and monoamines in that it is not released from nerve terminals by arriving action potentials. Thus unlike them it is not a primary messenger. It could be regarded as a second messenger except that its effects appear to be mediated by the production of cGMP, itself an established second messenger. In that sense, NO is more like a G-protein. The fact that its synthesis and release from neurons, and so its actions, are dependent on and stimulated by Ca^{2+} influx, often after NMDA receptor activation, inevitably links NO to more extreme excitatory effects such as LTP, excitotoxicity, pain and possibly also epilepsy. Whether blocking its synthesis will be a more effective therapeutic approach than the use of NMDA receptor antagonists is problematic in that even if really specific NOS inhibitors are developed these effects will potentially be at least as widespread as block of NMDA receptors. Where NO inhibition may have the advantage is that it should only operate under conditions of NMDA action that are above normal and so may only affect adverse but not normal neuronal function. This should only occur in those brain areas and pathways showing that extreme level of activity. Time will tell . . .

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