

# Catecholamines: Knowledge and understanding in the 1960s, now, and in the future

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## Abstract

The late 1960s was a heyday for catecholamine research. Technological developments made it feasible to study the regulation of sympathetic neuronal transmission and to map the distribution of noradrenaline and dopamine in the brain. At last, it was possible to explain the mechanism of action of some important drugs that had been used in the clinic for more than a decade (e.g. the first generation of antidepressants) and to contemplate the rational development of new treatments (e.g. *l*-dihydroxyphenylalanine therapy, to compensate for the dopaminergic neuropathy in Parkinson's disease, and  $\beta_1$ -adrenoceptor antagonists as antihypertensives). The fact that drug targeting noradrenergic and/or dopaminergic transmission are still the first-line treatments for many psychiatric disorders (e.g. depression, schizophrenia, and attention deficit hyperactivity disorder) is a testament to the importance of these neurotransmitters and the research that has helped us to understand the regulation of their function. This article celebrates some of the highlights of research at that time, pays tribute to some of the subsequent landmark studies, and appraises the options for where it could go next.

## Keywords

catecholamine; dopamine; drug development; noradrenaline

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## Introduction

Until the late 1960s, most research of catecholamines focused on measuring responses to noradrenaline in peripheral tissues *in vitro* in order to gain a better understanding of the function and regulation of the sympathoadrenal system. The low sensitivity of noradrenaline assays meant that quantitative neurochemical studies were restricted to studying sympathetically innervated tissues of large species or (noradrenaline-storing) chromaffin cells from the adrenal medulla. Like sympathetic neurons, chromaffin cells are activated by preganglionic cholinergic neurons and derive from the same embryonic tissue, and so it was thought that they could serve as a large-scale model for postganglionic sympathetic neurons: a prediction that turned out to be broadly correct.

Three technical developments in the 1960s enabled rapid progress in catecholamine research: refinement of fluorescence histochemistry; development of a sensitive fluorometric assay for catecholamines; and production of radiolabels for biological molecules. Collectively, these technologies made detailed anatomical and neurochemical research on catecholamines in the periphery and, later, the brain, realistic objectives. However, research of dopamine lagged behind that of noradrenaline mainly because it was hard to confirm that this catecholamine was present in neurons that were phenotypically different from those that contained noradrenaline.

In the 1980s, the development of *in vivo* microdialysis, high-performance liquid chromatography (HPLC) coupled with electrochemical detection, and fast cyclic voltammetry (see Anderzhanova and Wotjak, 2013; Young, 1993) made it possible to monitor changes in the concentration of extracellular catecholamines of freely moving animals, following an experimental challenge. These approaches are now complemented by a raft of brain imaging techniques, for example: positron emission tomography (PET; see Finnema et al., 2015; Volkow et al., 2007), single-photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI; see Borsook et al., 2006; DeCharms, 2008). However, unlike microdialysis and voltammetry, neuroimaging techniques suffer the limitation that they provide only 'snapshots' of the functional status of the brain of immobilised subjects.

A detailed and contemporary account of the state-of-the-science of catecholamine research, 50 years ago, is to be found in

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Iversen (1967). A particularly striking point about that book is that only the last chapter, which is relatively short, is devoted to catecholamines in the brain and it offers the tentative conclusion: 'In recent years, the evidence that noradrenaline and dopamine may act as neurotransmitters in the central nervous system has become progressively more convincing'. This comment speaks for itself.

This article highlights some of the 'hot topics' of the late 1960s, some key developments since then and how, in many cases, they directly underpinned therapeutic developments. A testament to the pioneering work by Axelrod's group at the National Institute of Mental Health (NIMH) and Arvid Carlsson (Lund) is that both were awarded Nobel prizes for their different contributions to the field.

## Neurobiology

### Distribution

Noradrenaline ('sympathin') had long been known to effect sympathetic neurotransmission and had been found in the brain in the mid-1940s, but the possibility that it served any specific purpose, other than regulating the cerebral vasculature, was controversial (see Vogt, 1954). Refinement of the formaldehyde condensation histochemical reaction enabled catecholamines to be visualised in freeze-dried tissues and, by the late 1960s, there was a swathe of publications, mapping their distribution in a range of species and organs. This effort revealed that noradrenaline within the brain was confined to an anastomosing network of neurons, with clusters of cell bodies (nuclei) located in the brainstem. This complex network strengthened the view that noradrenaline might have a direct influence on brain function, as Vogt had suggested many years before. The nucleus that has attracted the most interest is the locus coeruleus because, despite containing remarkably few cell bodies (about 1500, bilaterally, in the rat), its neurons project to nearly all regions of the neuraxis and provide nearly half of all noradrenergic nerve terminals in the brain.

Dopamine was known to exist in specialised cells in peripheral tissues and its presence in the brain was reported in the late 1950s, together with its high concentration in the striatum (Bertler and Rosengren, 1959) and nigrostriatal neurons (Anden et al., 1962). However, there was a lingering doubt that neuronal stores of dopamine served merely as a store of substrate for noradrenaline synthesis. Distinguishing between dopamine and noradrenaline using fluorescence histochemistry was, and remains, difficult, but by 1967 it was clear that its distribution in brain neurons differed from that of noradrenaline.

Vogt had also found small quantities of adrenaline in brain extracts in the mid-1950s but, although the Karolinska group found phenylethanolamine *N*-methyltransferase (PNMT)-expressing neurons in the medulla in the early 1970s (see Hökfelt et al., 1974), its status as a neurotransmitter is still uncertain (see Mefford, 1988). In fact, we know remarkably little about its function in the brain, not least because the distribution of cell bodies of PNMT-expressing neurons (in brainstem nuclei, C1–C3) overlaps with noradrenergic nuclei (A1–A3) and they might even act as co-transmitters.

### Storage

Refinement of the trihydroxyindole technique greatly increased the sensitivity of spectrofluorometric assay of noradrenaline. For the first time, it was feasible to study the subcellular distribution

of noradrenaline in peripheral neurons and the factors that influence its release. It was even possible to expose a diurnal rhythm for the noradrenaline content of the pineal gland.

By combining evidence from noradrenaline assays with electron microscopy, it was confirmed that noradrenaline was confined within membrane-bound (dense-core) vesicles that are located in the terminal varicosities of sympathetic neurons (Bisby and Fillenz, 1971). Such findings ratified the predicted analogy between postganglionic sympathetic neurons and chromaffin cells of the adrenal medulla. However, a leading question in the 1960s was how does noradrenaline gain access to the vesicles and, given the immense concentration gradient, how it could remain confined there?

The latter question was explained by the discovery of an adenosine triphosphate (ATP)-Mg<sup>2+</sup>-dependent uptake process by neuronal vesicles (Potter and Axelrod, 1963). We now know that this is carried out by one of a family of transporters (VMAT2) whose structure and regulation is well understood (see Blakely and Edwards, 2012). Early on, it was clear that this vesicular uptake was a relatively non-specific process, which became an integral feature of the explanation for the actions of the so-called 'false transmitters' (e.g.  $\alpha$ -methylnoradrenaline and tyramine) and psychostimulants (see below).

### Synthesis

The pathway for the synthesis of noradrenaline was predicted by Blaschko in the 1930s (see Blaschko, 1959). By the mid-1960s, it was confirmed that all the enzymes needed for noradrenaline biosynthesis are expressed by catecholamine-releasing neurons in both the periphery and the brain and are delivered to their terminals by axoplasmic transport. However, by 1967, the details of where in the neurons each step took place were still uncertain. An important finding was that the enzyme, dopamine- $\beta$ -hydroxylase (D $\beta$ H), which converts dopamine to noradrenaline, is bound within the noradrenaline storage vesicles (see De Potter, 1971), confirming that they are the location of the final step in the pathway. This finding also helped resolve a long-term dispute over the process of transmitter release (see below).

In the light of evidence that the tissue content of noradrenaline was remarkably stable, much attention was devoted to finding out how synthesis of this transmitter matched demand for its release. The activity of the enzyme responsible for the first step in the pathway (tyrosine hydroxylase (TH)) was found to be rate limiting, which was a crucial factor in the later development of *L*-dihydroxyphenylalanine (*L*-DOPA) therapy (see below). For many years, end-product inhibition of this enzyme by noradrenaline was thought to be the only mechanism for regulating noradrenaline synthesis (Spector et al., 1967). However, subsequent evidence revealed that end-product inhibition might occur only under exceptional circumstances (e.g. after treatment with an inhibitor of monoamine oxidase (MAO)) and that the activity of TH is normally regulated by a cohort of intracellular protein kinases. These intracellular messengers enable the release of noradrenaline to be coupled with its synthesis through phosphorylation of TH, which reduces its  $K_d$  and/or increases  $V_{max}$ , depending on the kinase. These changes in enzyme activity help ensure that neurons maintain their vesicular transmitter store, despite fluctuations in their firing rate. It is now known that TH also undergoes transcriptional, translational, and epigenetic

regulation, which culminates in long-term adjustments in the production and activity of this enzyme that are essential for maintenance of homeostasis (see Tekin et al., 2014).

### Release

The discovery of D $\beta$ H in storage vesicles also turned out to be crucial for explaining the mechanism of noradrenaline release. An important series of studies showed that, on stimulation of the adrenal medulla or sympathetic nerves, *in vitro* or *in situ*, overflow of noradrenaline from the perfused end organ was paralleled by D $\beta$ H (and other vesicle constituents, such as chromogranins and ATP), but there were no detectable cytoplasmic enzymes (see, e.g., Johnson et al., 1971; Smith and Winkler, 1972). Such evidence reconciled a long-standing debate by confirming that quantal release of neurotransmitter (discovered by electrophysiological studies of the neuromuscular junction) could be explained by vesicular exocytosis, rather than gated (quantal) release of soluble noradrenaline directly from the neuronal cytoplasm. Even so, it is now clear that there can be impulse-independent extrusion of cytoplasmic neurotransmitter on membrane-bound transporters, as is evident with high doses of psychostimulants.

In 1967, impulse-evoked release of noradrenaline was known to be Ca<sup>2+</sup> dependent but the ‘cholinergic link’ hypothesis (see Burn and Rand, 1965) prevailed. This proposed that acetylcholine was expressed in, and released by, sympathetic neurons and that this release increased the concentration of intracellular Ca<sup>2+</sup>, which triggered noradrenaline release. Not even a vestige of this theory remains today, but we now know a great deal about the complex molecular cascade that couples nerve stimulation with vesicular exocytosis of neurotransmitters.

### Metabolism/inactivation

Key elements of the metabolism of noradrenaline by MAO *in vitro* had been worked out in the early 1950s by Blaschko, who was evidently eager to point out its importance in the toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Blaschko, 1989). Iproniazid was identified as an MAO inhibitor in 1952, which later became a core piece of evidence for the catecholamine hypothesis for the cause of depression (Schildkraut, 1965). Although the intracellular location of MAO (in neurons and other types of cell) had already been reported (O’Steen and Callas, 1964), as had Axelrod’s discovery that noradrenaline was also metabolised by catechol-*O*-methyltransferase (COMT; Axelrod and Laroche, 1959), a major conundrum was why inhibitors of MAO did not augment noradrenaline transmission *in vitro*, as was expected at the time, by analogy with the effects of cholinesterase inhibitors at the neuromuscular junction. Whatever, it was clear that the degradation of noradrenaline by MAO was not responsible for the termination of neurotransmission.

By contrast, cocaine was well known for augmenting responses to the stimulation of sympathetic nerves, but a tentative explanation for this action was that the ensuing vasoconstriction prevented the washout of the released transmitter. This view changed following the production of radiolabeled catecholamines, which confirmed that a process for neuronal uptake of noradrenaline (Whitby et al., 1961) was the mechanism for inactivation of noradrenergic neurotransmission. In the same year, Muscholl (1961) showed that

cocaine inhibited this uptake process. In fact, the link between the potentiation of the noradrenaline response and inhibition of neuronal uptake was so clear that Iversen (1967) even predicted that methylphenidate was a catecholamine uptake inhibitor before that was confirmed experimentally.

Moreover, inhibition of neuronal reuptake was evident for several other groups of compounds, including dibenzazepines (e.g. imipramine; Axelrod et al., 1961), which were already being used as (‘tricyclic’) antidepressants. A landmark study, in which [<sup>3</sup>H]noradrenaline was administered into the lateral ventricle, confirmed that tricyclic antidepressants block the neuronal uptake of noradrenaline in the brain *in vivo* (Glowinski and Axelrod, 1965). It is striking that there was no mention of anaesthesia in that paper, although a later publication from the same group did mention that pentobarbital had been used. There have clearly been major developments in science reporting, as well as catecholamine research, since then.

Several other compounds were found to blunt uptake, also: notably amphetamine and tyramine (Axelrod et al., 1961; Carlsson et al., 1965). However, the field was clearly struggling to explain the difference in the responses to cocaine and amphetamine: particularly why only the latter compound, like tyramine, caused a marked increase in noradrenaline release and acted as a ‘false transmitter’. It was some time before it was realised that cocaine and tricyclics inhibit the neuronal reuptake transporter, whereas *d*-amphetamine and tyramine act as competitive substrates (see Heal et al., 2013, 2014)

### Function

The role of peripheral catecholamines in the stress response was well known, arising from Cannon’s historic work, but a role for noradrenaline in the brain was not taken seriously until 1964 and, even then, only a trickle of papers followed over the next few years. Moreover, those studies concentrated on gross measures of a noradrenaline response (depletion of tissue stores) and depended on the use of intensely stressful stimuli (e.g. electric shocks or prolonged immobilisation) in order to produce a measurable change in the tissue noradrenaline content. Using more sensitive techniques (in vivo microdialysis, voltammetry, and radioligand binding), it has been possible to reveal changes in the synthesis and extracellular concentration of noradrenaline in the brain, together with the downregulation of adrenoceptors, even after naturalistic, non-noxious experimental challenges as mild as handling or exposure to a novel environment (e.g. Dalley and Stanford, 1995; Stanford, 1995; Stanford et al., 1984).

Despite Cannon suggesting, as early as 1911, that changes in the secretion of humoral factors (later identified as catecholamines) affected emotionality, even by the late 1960s there were remarkably few studies of their influence on behaviour. One pioneering example is the disruption of the conditioned emotional response and instrumental conditioning, following the treatment of rats with  $\alpha$ -methyltyrosine, which blocks noradrenaline synthesis and so depletes neuronal stores of noradrenaline (Fuxe and Hanson, 1967). Another was a study of the effects of COMT inhibition on cognition (acquisition/extinction; Merlo and Izquierdo, 1965). However, the limited portfolio of pharmacological tools, and inadequate understanding of the mechanisms of action of those that were available, meant that no meaningful conclusions were feasible at that time.

An important series of studies, 10 years later, reported on the effects of stimulating the locus coeruleus (the major source of noradrenergic nerve terminals in the brain) of non-human primates and concluded that the behavioural response was analogous to anxiety in humans (see Redmond and Huang, 1979). The belief that the excessive release of noradrenaline in the brain can cause anxiety still dominates the field, but has never been confirmed. Nevertheless, it is clear that drugs that blunt noradrenergic transmission (e.g.  $\alpha_2$ -adrenoceptor agonists and  $\beta$ -adrenoceptor antagonists) can relieve anxiety, but that could be through an unrelated process.

We now know that noradrenaline in the brain does not simply contribute to the stress response, but has an important role in the regulation of homeostasis (cardiovascular, metabolic, hormonal), mainly through its actions in the brainstem and hypothalamic nuclei. It also has a well-established role in the modulation of the sleep/waking continuum, such that the activity of neurons in the locus coeruleus increases with the intensity of arousal. However, over recent years, most attention has been directed at its role in cognition, especially attentional performance. For instance, Aston-Jones' work has provided a detailed insight into the role of the locus coeruleus, in focused attention: his Adaptive Gain Theory links tonic versus phasic release of noradrenaline with behavioural flexibility and favourable behavioural adaptation, respectively. Complementing this body of evidence is some sophisticated research *in silico*, which is compatible with the evidence that neurons projecting from the locus coeruleus are especially sensitive to unexpectedly unpredictable environmental stimuli (Marshall et al., 2016).

As regards dopamine, apart from its well-established link with Parkinson's disease (PD; see below) the proposal that all addictive drugs augment dopaminergic transmission in the nucleus accumbens (see Wise, 1987; Wise and Bozarth, 1985) has eclipsed most other research on this neurotransmitter. This concept remains largely unchallenged, subject to the refinement that, as addiction develops, the dopaminergic response is thought to migrate from the ventral striatum (impulsive behaviour) to the dorsal striatum (compulsive behaviour/habit). Nevertheless, there is growing interest in the role of dopamine in mood and cognition, especially regarding its interactions with central noradrenergic transmission, in corticostriatal circuits. This research is the backbone for the evidence that the impairment of dopaminergic function in those forebrain areas could contribute to the cognitive impairment seen in disorders such as schizophrenia, depression, and attention deficit hyperactivity disorder (ADHD; e.g. Chandler et al., 2014).

## Landmark developments in pharmacotherapeutics

### *Preclinical pharmacology*

Although examples of most classes of drugs affecting the function of catecholamines that exist today were available in 1967, the portfolio was extremely limited at that time. For instance, it was known that reserpine depleted neuronal catecholamine stores by blocking their intracellular storage (Glowinski et al., 1966). It was also evident that the recovery of the neuronal stores of noradrenaline paralleled the recovery of the vesicular uptake process and the delivery of new vesicles to the nerve terminals.

However, it was not until 1980 that this action of reserpine was explained in terms of its disruption of the intravesicular/cytoplasmic proton gradient (Angelides, 1980). 6-Hydroxydopamine (6OHDA) was also known to cause long-lasting depletion of noradrenaline and dopamine stores and, in the late 1960s, it was discovered to be a neurotoxin for catecholamine-releasing neurons (Bloom et al., 1969; Thoenen and Tranzer, 1968), which has made it an invaluable research tool ever since.

Low selectivity of the vesicular uptake transporter was identified as important for the actions of a range of compounds, such as  $\alpha$ -methylnoradrenaline, tyramine, and amphetamine.  $\alpha$ -Methylnoradrenaline, which reduces blood pressure (and was used clinically for that purpose), was thought to act by replacing noradrenaline in the storage vesicles, but to have a lower efficacy at postsynaptic receptors ('false transmitter'). However, the explanation for the opposite (pressor) effects of *d*-amphetamine and tyramine remained elusive. The high affinity of  $\alpha$ -methylnoradrenaline for postsynaptic  $\alpha_2$ -adrenoceptors was not discovered until 1977 when it was realised that its anti-hypertensive effects arise from a direct effect on the vasomotor centre in the brain. The explanation for the actions of *d*-amphetamine and tyramine had to wait even longer (see Sitte and Freissmuth, 2015).

Beyond doubt, the most important development since 1967 has been the proliferation of catecholaminergic receptors. At that time, there were only two receptors for noradrenaline ( $\alpha$ - and  $\beta$ -adrenoceptors) and one dopamine receptor, for which apomorphine had just been proposed as an agonist. Two subtypes of  $\beta$ -adrenoceptors were defined pharmacologically in 1967 (Lands et al., 1967), but there was no hint of  $\beta_3$ -adrenoceptors or of any subtypes of  $\alpha$ -adrenoceptors. Subdivision of the latter started after the discovery that the activation of  $\alpha$ -adrenoceptors, presumed to lie on noradrenergic nerve terminals, caused feedback inhibition of transmitter release. Initially, these were classified as  $\alpha_2$ -adrenoceptors, to distinguish them from postsynaptic (' $\alpha_1$ -adrenoceptors'). However, it became apparent that these two types of  $\alpha$ -adrenoceptor had different pharmacological profiles and that there are postsynaptic  $\alpha_2$ -adrenoceptors and so the nomenclature was reassigned on the basis of their pharmacology, rather than their presumed location.

As outlined below, the development of selective/preferential ligands for all these receptors has transformed the research of catecholamines and their pharmacology and therapeutics.

### *Clinical developments*

A landmark step in catecholamine therapeutics was the development of the non-selective  $\beta$ -adrenoceptor antagonist, propranolol, as an antihypertensive agent, by James Black (another Nobel laureate; Black et al., 1965), followed by selective  $\beta_1$ -adrenoceptor antagonists to reduce the risk of asthma as an adverse side-effect. However, in the 1960s, the discovery of new drugs was driven by medicinal chemists who were merely synthesising analogues of compounds with proven therapeutic efficacy (e.g. the family of tricyclic antidepressants). Their pre-clinical evaluation usually consisted of pharmacological testing of tissues, *in vitro* or *in situ*, in combination with simple behavioural tests in animals: for example, their prevention of reserpine-induced hypomotility and hypothermia, as a preliminary screen for antidepressants.

**Table 1.** Key drugs to emerge from catecholamine research and development.

Generic name	Trade name <sup>a</sup>	Mechanism of action	Therapeutic applications	Comments	Year of introduction <sup>b</sup>
Propranolol	Inderal	$\beta$ -Adrenoceptor antagonist	Hypertension Cardiac arrhythmias Heart failure	<i>Blockbuster drug</i>	1965
Haloperidol	Haldol	D <sub>2</sub> receptor antagonist (many secondary pharmacological actions)	Schizophrenia Schizoaffective disorder Manic episodes in bipolar disorder	Synthesised by Paul Janssen in 1958 On the World Health Organization's List of Essential Medicines <i>Blockbuster drug</i>	1967
Salbutamol	Ventolin	$\beta_2$ -Adrenoceptor agonist	Asthma Bronchospasm Reversible airways obstruction	Discovered in 1966 by David Jack (Allen and Hanburys) <i>Blockbuster drug</i>	1969
Atenolol	Tenormin	$\beta_1$ -Adrenoceptor antagonist	Hypertension Tachycardia Angina pectoris	Discovered by James Black in 1968 Inventor of rational drug design James Black won the Nobel Prize for Medicine <i>Blockbuster drug</i>	1976
Clonidine	Catapres (hypertension) Dixarit (migraine) Kapvay (USA; ADHD)	$\alpha_1$ -Adrenoceptor agonist	Hypertension Migraine ADHD		1974 (hypertension) 2006 (migraine) 2010 (ADHD)
Sulpiride	Dolmatil	D <sub>2</sub> receptor antagonist	Schizophrenia	First D <sub>2</sub> -selective antipsychotic drug	1983
Prazosin	Hypovase	$\alpha_1$ -Adrenoceptor antagonist	Hypertension Congestive heart failure Raynaud's disease Benign prostatic hyperplasia		1988
Risperidone	Risperdal	D <sub>2</sub> + 5-HT <sub>2A</sub> receptor antagonist (many secondary pharmacological actions)	Schizophrenia Manic episodes in bipolar disorder	First of the 'second generation antipsychotics' <i>Blockbuster drug</i>	1992
Selegiline	Eldepryl	MAOB inhibitor	Adjunctive therapy to L-DOPA in Parkinson's disease		1993
Venlafaxine	Effexor	Noradrenaline + 5-HT reuptake inhibitor	Major depressive disorder Generalised anxiety disorder Social anxiety disorder Panic disorder	First SNRI to be introduced since the tricyclics Venlafaxine actions are predominated by 5-HT reuptake inhibition <i>Blockbuster drug</i>	1994
Reboxetine	Edronax	Noradrenaline-selective reuptake inhibitor	Major depressive disorder	First selective noradrenaline reuptake inhibitor since the tricyclics	1997
Pramipexole	Mirapexin	Dopamine D <sub>2</sub> /D <sub>3</sub> receptor agonist	Parkinson's disease	First agonist of the D <sub>2</sub> receptor family to be clinically approved	1998
Entacapone	Comtess	Catechol-O-methyltransferase inhibitor	Parkinson's disease	First COMT inhibitor to be approved as adjunctive therapy to L-DOPA	1998
Atomoxetine	Strattera	Noradrenaline-selective reuptake inhibitor	ADHD	First selective noradrenaline reuptake inhibitor to be approved for treating ADHD	2002

(Continued)

**Table 1.** (Continued)

Generic name	Trade name <sup>a</sup>	Mechanism of action	Therapeutic applications	Comments	Year of introduction <sup>b</sup>
Duloxetine	Cymbalta	Noradrenaline + 5-HT reuptake inhibitor	Major depressive disorder Generalised anxiety disorder Diabetic peripheral neuropathic pain	First SNRI to be introduced since the tricyclics <i>Blockbuster drug</i>	2004
Aripiprazole	Abilify	D <sub>2</sub> receptor partial agonist + 5-HT <sub>2A</sub> receptor antagonist + 5-HT <sub>1A</sub> partial agonist (many secondary pharmacological actions)	Schizophrenia Manic episodes in bipolar disorder	First D <sub>2</sub> partial agonist antipsychotic <i>Blockbuster drug</i>	2004
Guanfacine	Intuniv	α <sub>2</sub> -Adrenoceptor agonist	ADHD	First α <sub>2A</sub> -adrenoceptor agonist to be specifically developed and approved for treating ADHD	2015

ADHD: attention deficit hyperactivity disorder; MAOB: monoamine oxidase B; *L*-DOPA: *L*-dihydroxyphenylalanine; SNRI: serotonin reuptake inhibition; COMT: catechol-*O*-methyltransferase.

<sup>a</sup>Trade names provided are UK and/or European names.

<sup>b</sup>Date of first registration in the United Kingdom and/or Europe.

In the 1970s and 1980s, a large number of highly selective compounds became available, which provided therapeutic benefit in a wide range of cardiovascular, psychiatric, and neurological disorders (see Table 1). However, the subsequent identification of the DNA sequences of the catecholamine receptor family and transporters, when combined with cloning and transfection in stable cell lines and automation, heralded the era of huge chemical libraries and high-throughput screening. This strategy has enabled the identification of lead compounds from several chemical series. Some catecholaminergic molecular targets (e.g. D<sub>1</sub>, D<sub>4</sub>, and, until recently, β<sub>3</sub>-adrenergic receptors) have not yielded new drugs, but have added to the body of knowledge in the field, nonetheless.

### Affective disorders

The discovery, in the 1960s, that the inhibition of neuronal uptake of noradrenaline was the primary target for tricyclic antidepressants turned out to be of immense importance, not least because it seemed to underpin Schildkraut's (1965) proposal that a deficit in catecholamine transmission in the brain could account for depression. It is interesting that any contribution from dopamine was largely neglected until fairly recently.

Although the catecholamine theory of depression (later broadened to include serotonin; e.g. Carlsson et al., 1968) is now regarded as a special case, at best, the disconnect between the typical 6 to 8-week delay in the onset of peak antidepressant efficacy, despite the rapid augmentation of catecholamine transmission by MAO inhibitors and tricyclic antidepressants, was apparent even in the 1960s. It became a major focus of research only in the 1970s when it was realised that this meant that the antidepressant efficacy could not be explained by augmentation of catecholamine transmission, directly, and so must be a neuroadaptive (downstream) response.

A landmark paper in this field reported that the repeated administration of antidepressants caused the downregulation of β-adrenoceptors in the rat cerebral cortex (U'Prichard and Enna, 1979), which were later demonstrated to be of the β<sub>1</sub>-adrenoceptor subtype (Heal et al., 1989). This apparently explained Vetulani and Sulser's (1975) earlier report that antidepressants blunted the

generation of cyclic adenosine monophosphate (cAMP) in the brain. However, the hypothesis that β-adrenoceptor downregulation accounted for the therapeutic efficacy of these drugs did not survive long, mainly because there were some notable exceptions (e.g. selective serotonin reuptake inhibitors (SSRIs)). Conversely, two monoamine reuptake inhibitors that failed to meet their potential as antidepressants did cause β-adrenoceptor downregulation: these were tomoxetine, latterly renamed atomoxetine, and sibutramine, which resurfaced as approved treatments for ADHD and obesity, respectively. It is now accepted that there is either down- or upregulation of many different neurotransmitter receptors, after chronic administration of antidepressants, but a pattern of changes that is common to all these drugs has not emerged and attention has turned to other long-latency adaptive changes instead (e.g. synaptic remodelling, modulation of gene transcription, neurogenesis). Nevertheless, it remains the case that, with the exception of tianeptine, the primary action of all antidepressants is to augment catecholaminergic and/or serotonergic transmission, which leaves a monoamine theory of *antidepressant* largely intact.

The extension of Schildkraut's theory to include serotonin as a target for antidepressants, together with the notorious 'cheese reaction' of the MAO inhibitors and the cardiotoxicity of the tricyclics, justified focusing on developing SSRIs as a preferred first-line therapy. However, the realisation that the cardiotoxicity of tricyclics was attributed to their anticholinergic effects in combination with their inhibition of noradrenaline uptake cleared the way for the development of selective noradrenaline reuptake inhibitors, which led to reboxetine being licensed in Europe in 1997 (Table 1). This was followed by drugs that combined serotonin and noradrenaline reuptake inhibitors (SNRIs), but venlafaxine was the sole example in the formulary for many years.

Subsequently, the dopamine-mediated euphoriant and mood-elevating properties of psychostimulant drugs, like cocaine and *d*-amphetamine, prompted the development of triple monoamine reuptake inhibitors as an approach to enhancing antidepressant efficacy and/or reducing the therapeutic lag. Although several such compounds have been evaluated in clinical trials, none has proved to be an effective antidepressant. This leads to the

uncomfortable conclusion that significant dopamine reuptake inhibition possibly hinders, rather than enhances, the antidepressant efficacy. Now that almost all combinations of reuptake inhibition have been explored in clinical trials, it is difficult to envisage a new catecholaminergic compound that will meet the much-needed step change in antidepressant treatment.

### *Schizophrenia and other psychoses*

Antipsychotic drugs (formerly known as neuroleptics or major tranquilisers) have been used to treat schizophrenia since the 1950s, when almost nothing was known about their mechanism of action. Experiments in animals, showing that antipsychotics prevented apomorphine-induced emesis and amphetamine-induced toxicity suggested that a dopaminergic mechanism could be involved (see Snyder, 1973). Keabian et al. (1972) published the first report that dopamine receptors were positively coupled to adenylyl cyclase and suggested that the inhibition of these receptors was a property shared by all antipsychotic drugs. This finding triggered intensive research, which confirmed and extended the finding, but also uncovered a discrepancy, namely, that haloperidol, one of the most potent antipsychotic drugs, was suspiciously weak at inhibiting dopamine-sensitive adenylyl cyclase. Creese et al. (1976) then published a seminal paper reporting that the dopamine receptor binding affinity predicted the clinical efficacy of antipsychotic drugs, including the anomalous haloperidol. On the basis of this evidence, it was inferred that dopamine receptor antagonism was the mechanism of action shared by all antipsychotics, which remains the case to this day. Creese et al. (1979) later resolved the haloperidol paradox with the discovery that there were two dopamine receptors, labelled with [<sup>3</sup>H]dopamine and [<sup>3</sup>H]haloperidol, respectively. These were classified as type 1 and type 2 dopaminergic receptors (DRD1 and DRD2), with only the former being positively coupled to adenylyl cyclase and the latter being the target for antipsychotic agents. A major development since then has been the discovery of two *families* of dopamine receptors, with the DRD1 family subdivided into the D<sub>1</sub> and D<sub>5</sub> subtypes and the DRD2 family comprising the D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> subtypes. However, none of the compounds that have been developed as selective antagonists for any of these subtypes has turned out to be an effective antipsychotic (infamously, D<sub>4</sub> receptor antagonism).

A problem for pharmacotherapeutics was that, although haloperidol was extremely efficacious among the established antipsychotics, it had the highest propensity to produce extrapyramidal (Parkinsonian) side-effects and tardive dyskinesia in patients: the former problem apparently deriving directly from its antagonism of DRD2. This opened the door for the development of another dibenzazepine derivative, clozapine. This drug became the 'gold standard' because its extrapyramidal ('Parkinsonian') side-effects were less common and less pronounced than with its predecessors, which led to it being described as an 'atypical' antipsychotic. A significant finding was that, despite being a less selective and less efficacious DRD2 antagonist than its predecessors, it was more effective in treating cases of schizophrenia that did not respond to other antipsychotics. This prompted a wave of 'second generation (atypical) antipsychotics' (SGAs), led by risperidone (licensed in 1992), with others following hot on its heels. However, their low binding affinity for DRD2 indicated that this was not the only target to account for their therapeutic

actions. Instead, its antagonism of 5-HT<sub>2A</sub> receptors became a focus of interest, mainly because all 5-HT<sub>2A</sub> agonists are hallucinogenic. This led Meltzer et al. (1989) to propose that the key pharmacological characteristic of clozapine was the ratio of its affinity for DRD2/5-HT<sub>2</sub> as an antagonist, with its affinity for 5-HT<sub>2A</sub> receptors being several orders of magnitude higher than that for DRD2. This has been a major driver in drug development, ever since, and the field has moved away from selective DRD2 antagonism to drugs that have a broad spectrum of actions that all contribute to the alleviation of schizophrenia.

The most recent innovation in this field has been the development of aripiprazole. This compound is a DRD2 partial agonist, which explains its low potential to induce extrapyramidal side-effects (EPSs) and its low propensity to produce hyperprolactinaemia, both of which are caused by DRD2 antagonism. However, this action is combined with 5-HT<sub>2A</sub> antagonism and 5-HT<sub>1A</sub> partial agonism: the latter is thought to lead to an increase in dopamine release in the prefrontal cortex (PFC), which is thought to explain the beneficial effects of aripiprazole on the negative symptoms of schizophrenia. Such an increase could also explain why aripiprazole can have beneficial effects on the cognitive impairment that is a major feature of schizophrenia, but is typically not improved by the first or second generation of antipsychotics.

Looking to the future, DRD2 partial agonism or antagonism (perhaps via a different mix across the D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> subtypes) is likely to remain a core component of catecholaminergic mechanisms in antipsychotic efficacy, but finding ways of treating the cognitive impairment in schizophrenia will undoubtedly be a major target for future research. The challenge will be to identify a combination of pharmacological mechanisms that unlock clozapine-like efficacy without producing the unacceptable adverse effects (metabolic, cardiac, and motor) that are prominent with this class of compounds. Aripiprazole could provide clues in this respect because this drug carries a relatively low risk of a harmful increase in body weight, compared with its predecessors.

### *ADHD*

ADHD is a common childhood-onset psychiatric disorder that has been treated with the psychostimulants, amphetamine and methylphenidate, since the mid-20th century. With the response rates of ~70%, these two drugs are regarded as highly effective, but both are subject to regulatory restrictions because they pose significant risks of diversion, misuse and abuse.

There is good evidence to suggest that a neuroanatomical locus for ADHD is the PFC. This brain region is unusual because differences in the topographical locations of noradrenaline and dopamine transporters, together with the higher affinity of the former for dopamine, leads to neuronally released dopamine being predominantly sequestered by the noradrenaline reuptake transporter, into noradrenergic neurons, rather than into dopaminergic nerve terminals via the dopamine reuptake transporter. As a consequence, selective noradrenaline reuptake inhibitors will augment both dopaminergic and noradrenergic neurotransmission in the PFC and so possibly mimic the actions of methylphenidate and *d*-amphetamine. Consistent with this proposal, the search for ADHD drugs that are non-stimulant and pose no risks for recreational abuse led to the development of the selective noradrenaline reuptake inhibitor, atomoxetine (formerly known as tomoxetine), which was approved for this clinical indication in 2002. Because

atomoxetine is not a dopamine transporter (DAT) inhibitor, it does not augment dopamine transmission in the ventral striatum (including the nucleus accumbens), an action that mediates the potential for misuse and abuse of the psychostimulants.

Based on her research showing that  $\alpha_2$ -adrenoceptor agonists potentiated cognitive function in aged primates, Arnsten postulated that  $\alpha_{2A}$ -adrenoceptor agonists could be an effective treatment for ADHD. Backed by evidence from a small clinical trial (Hunt et al., 1995), guanfacine was approved to treat ADHD in 2015, but like all  $\alpha_{2A}$ -adrenoceptor agonists, this drug has the disadvantage of causing a profound reduction in blood pressure. Moreover, a mechanism focused exclusively on enhancing neurotransmission mediated by postsynaptic  $\alpha_{2A}$ -adrenoceptors excludes the contribution from the concomitant activation of dopamine receptors in the PFC that is offered by the stimulants and atomoxetine.

The most interesting direction for targeted modulation of catecholamine transmission in the search for new ADHD drugs is the development of potent noradrenaline + dopamine reuptake inhibitors that carry a low risk for recreational abuse. Modafinil has such a pharmacological profile and was shown to be effective in treating ADHD, but its development was halted following a report of an adverse side-effect in a child (a rash that resembled Stevens–Johnson syndrome). Nonetheless, modafinil is used off licence to treat adult ADHD. Dasotraline and centanafadine are two less stimulant dopamine/noradrenaline reuptake inhibitors that are in late-stage clinical development. Future research in this area will depend on whether they deliver efficacy and an onset of action that matches the efficacy of the ‘gold standard’ stimulants, without the associated misuse liability. A very recent setback has been the issue of a non-approvable letter by the Food and Drug Administration (FDA) for dasotraline in the treatment of ADHD based on safety concerns. Further development work is in progress to address the FDA’s concerns. Even if these drugs ultimately fulfil that promise, they probably represent the upper limit of what can be achieved in the treatment of ADHD through targeted modulation of catecholamine transmission.

## PD

PD is a progressive neurodegenerative disorder with a physical manifestation that progresses inexorably from early problems with fine movement, mild tremor, and bradykinesia to almost total motor incapacity. Hornykiewicz (2004) made the important discovery of a neuropathy of dopamine-containing neurons in the nigrostriatal tract of Parkinson’s patients in 1959. And 2 years later, in collaboration with Birkmeyer, he demonstrated that the motor deficits in PD patients were dramatically alleviated by intravenous administration of *l*-DOPA, which is the immediate precursor in the synthesis of dopamine but, unlike dopamine, crosses the blood–brain barrier.

Scepticism about dopaminergic denervation being a causal factor of motor impairment in PD and the potential benefits of *l*-DOPA therapy abounded for many years. However, the discovery by Cotzias et al. (1967) that high-dose, oral *l*-DOPA therapy was also effective provided a simple and effective route for treatment that still serves as a first-line treatment and transforms the lives of PD sufferers.

However, the fundamental problem in PD is that it is a progressive neurodegenerative disorder and all catecholaminergic drugs provide only symptomatic relief. Also, although the initial benefits

of *l*-DOPA therapy can be spectacular, it gradually loses its effect over time with dyskinesias appearing when the drug is first taken followed by motor ‘freezing’ later in the day (the ‘on/off’ effect). Strategies to potentiate the *l*-DOPA therapy include preventing its catabolism in the brain by monoamine oxidase B (MAOB) using inhibitors, such as selegiline (1993), or COMT inhibitors (e.g. entacapone; 1998). There were also suggestions that the MAOB inhibitors slow disease progression by preventing the generation of oxidative free radicals arising from dopamine catabolism, but this is controversial. However, there is some concern over the pressor effects of the prototype, selegiline, and its metabolism to the dopaminergic neurotoxin, methamphetamine.

The use of dopamine receptor full agonists, such as bromocriptine, for treating PD was first tried in the 1970s. However, their propensity to overstimulate supersensitive dopamine receptors, followed thereafter by receptor tachyphylaxis, resembled the problems experienced with *l*-DOPA. For this reason, research has focused on developing partial agonists of the DRD2 family, with varying mixes of efficacy at the  $D_2$  and  $D_3$  subtypes. This approach culminated in the introduction of pramipexole for the treatment of PD in 1998.

As regards tools for research of PD, a landmark study by Ungerstedt and Arbuthnott (1970) demonstrated that unilateral injections of the neurotoxin, 6-hydroxydopamine, into the dopaminergic nigrostriatal tract of rats evoked rotational behaviour when they were challenged with dopaminergic drugs. The direction of rotation depends on whether the drug activates the receptor directly (i.e. an agonist) or indirectly (i.e. a dopamine-releasing agent) because postsynaptic dopamine receptors on the lesioned side of the brain develop denervation supersensitivity. This simple behavioural test continues to provide a versatile tool for investigating the neurobiology of PD and a screen for the evaluation of putative treatments.

A breakthrough in understanding the aetiology of PD came from a tragic accident involving the intake of a toxic byproduct from the illicit synthesis of desmethylprodine, which resulted in a full-blown PD-like syndrome. The toxic byproduct was 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which after catabolism by MAOB generates the mitochondrial toxin, 1-methyl-4-phenylpyridinium (MPP+). MPTP does not induce a permanent Parkinsonian syndrome in rodents; this outcome occurs only when the compound is administered to primates. MPTP-treated non-human primates have generated a wealth of new insights into the neurochemistry and neuroanatomy of PD and remain an excellent model for evaluating putative new treatments for PD. The discovery of MPTP means that much future research in PD is likely to focus on preventing mitochondrial dysfunction, which is now believed to drive the neurodegenerative process.

## Abuse of drugs that target catecholamines

The psychotropic effects and the dependence liability of the prototypical stimulants, *d*-amphetamine (a  $\beta$ -phenylethylamine), and the tropane alkaloid, cocaine, were well known in the late 1960s (see Heal et al., 2013, 2014). It was not until the 1980s that it was discovered that their augmentation of dopaminergic transmission accounted for both these properties, whereas their effects on noradrenergic transmission mainly increased arousal. Another major class is the dopaminergic/serotonergic compounds,



for example, 3,4-methylenedioxymethamphetamine (MDMA), mephedrone, and a raft of the so-called ‘legal highs’. These have emerged from increasingly sophisticated attempts to create illicit drugs with pharmacological properties that are similar to *d*-amphetamine cocaine, or MDMA, but are sufficiently structurally different from these controlled drugs to evade legal restrictions. The synthetic cathinones are a particularly fertile area for illicit drug design and Iversen et al. (2014) estimated that almost 200 new stimulants had appeared in the United Kingdom since 2010 with several more emerging every week. How to deal with this epidemic is a societal and legal problem and is likely to remain so for the foreseeable future. As this confirms, there is a dark side to innovations in catecholaminergic pharmacology.

A related problem is the unmet need for drugs to treat individuals with substance misuse disorders linked to psychostimulants. Attempts to apply substitution therapy (analogous to that used to treat opiate dependence) have been singularly unsuccessful. Drugs that act as (non-stimulant) inhibitors of dopamine reuptake (e.g. bupropion and vanoxerine (GBR12909)) or noradrenaline reuptake (e.g. atomoxetine), through to psychostimulants that are thought to have comparatively low abuse potential (e.g. methylphenidate and lisdexamfetamine), have all failed in clinical trials. That said, catecholaminergic drugs can be useful treatments for other substance misuse. For instance, the selective dopamine reuptake inhibitor, bupropion, is used to aid smoking cessation, and the  $\alpha_2$ -adrenoceptor agonist, lofexidine, for treating the acute opiate withdrawal syndrome.

### Future directions in catecholamine research

The pace of catecholamine research over the last 50 years, which has transformed many fields of neuroscience, seems to have stalled somewhat. However, a much-needed step change in our knowledge and understanding of these neurotransmitter systems could well arise from the findings that are emerging from three related fields, all of which are gathering pace. One is the functional interactions between catecholamines and their co-transmitters, especially peptides and amino acids (see Stanford, 2014, for example). Another is the regulation of heterodimerisation between catecholamine receptors with those for other neurotransmitters/neuromodulators and a better understanding of how this affects the function of downstream targets (including gene transcription). A third is how catecholamine transmission influences, and is influenced by, glial cells. These are all rich seams for research that could well reinvigorate the pharmacology of catecholamines.

Regarding therapeutics, some of the early successes, particularly the introduction of  $\beta_1$ -adrenoceptor antagonists (as antihypertensives) and  $\beta_2$ -adrenoceptor agonists (as antiasthmatics) pushed drug development towards ever higher receptor selectivity. However, this approach did not always produce the anticipated improvements in efficacy or reductions in side-effects. In fact, for many therapeutic indications, experience has revealed that multiple pharmacological actions (‘enriched pharmacology’ or ‘dirty drugs’ depending on the perspective) offer the optimum balance of efficacy and safety. With most of the molecular targets associated with catecholamine transmission having been explored, both in isolation and in combination with additive pharmacological mechanisms, pharmaceutical research and development has turned full

circle to identifying potential therapeutic applications for novel compounds by pharmacological phenotyping using ‘black box’ behavioural protocols. In this situation, in vitro pharmacological profiling of molecules to identify its mechanism(s) of action by high-throughput screening techniques is conducted retrospectively. With this approach, there is no preconception about what pharmacology is necessary for therapeutic efficacy. It is too early to predict whether this approach will be successful.

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