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Review

New perspectives from microdialysis studies in freely-moving, spontaneously hypertensive rats on the pharmacology of drugs for the treatment of ADHD

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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is a common childhood psychiatric condition that is effectively treated by catecholaminergic drugs with a variety of different mechanisms and the SH rat is frequently used as a model of this disorder. *In vivo* microdialysis in freely-moving rats has been employed extensively to provide a better understanding of the pharmacodynamics of drugs at their sites of action. In this review, these three topics are brought together to explore the contribution of *in vivo* microdialysis studies in spontaneously hypertensive (SH) rats to our understanding of the neurochemical deficits in this rat strain and the actions of ADHD drugs on catecholaminergic function in the prefrontocortex (PFC), striatum and nucleus accumbens. What is revealed is that basal efflux of norepinephrine in the PFC is attenuated, whilst striatal and mesolimbic dopaminergic neurotransmission is hyperfunctional; the latter observation fits closely with the hyperactive phenotype of the SH rat. Furthermore, experiments performed with the enantiomers of amphetamine and *threo*-methylphenidate demonstrate that pharmacodynamic effects of drugs reported from experiments in outbred rat strains, e.g. Sprague–Dawleys, do not necessarily translate to the SH rat. When the findings are compared with the clinical efficacy of drugs used in treating ADHD, they indicate that the most efficacious drugs powerfully increase both norepinephrinergic and dopaminergic neurotransmission.

Contents

1. Introduction

In vivo microdialysis in freely-moving rats is now a very well accepted technique that is used to explore neurochemical links to behaviour and to define the pharmacodynamic actions of drugs on central neurotransmitter systems. The catecholamine, dopamine, was the first neurotransmitter to

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be studied by microdialysis and the value of this technique as a tool to determine the effects of drugs not only on dopamine, but also on another catecholamine neurotransmitter, norepinephrine (noradrenaline), has been established through hundreds, if not thousands, of publications in the field. Attention deficit hyperactivity disorder (ADHD) is a behavioural, emotional and cognitive disorder that is effectively treated by a variety of catecholaminergic drugs, initially the stimulants, i.e. *dl*-amphetamine, *d*-amphetamine and *dl-threo*-methylphenidate, to be joined later by drugs like the selective norepinephrine reuptake inhibitor, atomoxetine, and soon by the α_{2A}-adrenoceptor agonist, guanfacine. In spite of the fact that the spontaneously hypertensive (SH) rat has been used extensively to model the behavioural and cognitive deficits in ADHD and to investigate the pharmacological effects of drugs used in the treatment of this disorder, relatively few microdialysis studies have been performed in this rat strain. In this review, we describe how microdialysis experiments in freely-moving rats have contributed to our understanding of the potential disturbances in central catecholaminergic neurotransmission that are present in the brains of SH rats and have compared them with those reported neurochemical deficits that have been observed in subjects with ADHD. Often predictions about the pharmacological actions of drugs used in the treatment of ADHD are based on results either from *in vitro* experiments or from those performed *in vivo* in outbred Wistar or Sprague–Dawley (SD) rats. Another key aspect of this review is a comparison of the actions of various catecholaminergic drugs used to treat ADHD measured by microdialysis experiments in freely-moving SH and outbred, SD rats, which demonstrates that such predictions can be very misleading. In these experiments, the SD rat has not been employed as a control for the SH rat, rather it has been selected as a comparator because the SD is the strain most frequently selected for *in vivo* microdialysis and behavioural experiments. We have described the clinical pharmacology of drugs used to treat ADHD and discuss how the findings from microdialysis experiments in the SH rat help explain the relative efficacies of various catecholaminergic drugs in treating ADHD. Finally, we suggest future directions in the search for new drugs in this therapeutic indication.

2. Attention deficit hyperactivity disorder (ADHD)

ADHD is a complex behavioural, emotional and cognitive disorder that is characterised by its core symptoms of impulsivity, hyperactivity, distractibility, inattentiveness and cognitive impairment. According to the American Psychiatric Association DSM-IV criterion, ADHD is currently subclassified according to symptom clusters, i.e. hyperactive/impulsive, inattentive or combined hyperactive/impulsive-inattentive (American Psychiatric Association, 1994). Willcutt et al. (1999) found that the impulsive/hyperactive form of ADHD, but not the inattentive subtype, was associated with a high rate of co-morbid symptoms of conduct disorder and oppositional defiant disorder. ADHD is a CNS disorder with a childhood onset, and by definition, it includes symptoms that cause impairment before the child reaches the age of 7 years (Tan and Appleton, 2005). In prevalence terms, ADHD is a relatively common disorder with rates generally in the range of 2 to 7% (Taylor et al., 1991), making ADHD one of the most common behavioural and psychological disorders encountered in paediatric medicine. In children, the relative rates of ADHD are about 3 times higher in boys than in girls (Barkley et al., 1990), but in older adolescents this difference disappears (Cohen et al., 1993) and in the young adult population, women predominate with a ratio of 2:1 (Biederman et al., 1994).

3. Neurochemical and neuroanatomical basis of ADHD

Studies of the neuropharmacology, genetics and neuropsychology of ADHD indicate that the neurobiological cause of ADHD probably lies, at least to a major degree, with dysregulation of brain catecholaminergic systems in the prefrontocortex (PFC) and its connections to striatal areas (Durston, 2003; Arnsten and Dudley, 2005; Russell et al., 2005). It has been proposed that the inattentive subtype of ADHD may arise due to a dysfunction of dopamine functioning in the inhibitory control of the frontal cortex and the hyperactive/impulsive subtype due to impairment of functioning in subcortical structures (Lahey et al., 1994; Solanto, 2002; Johansen et al., 2002). ADHD sufferers have been suggested to have low brain norepinephrinergic neurotransmitter activity (Oades, 1987; Halperin et al., 1997), particularly in relation to that of dopamine. Levels of the dopamine transporter have also been reported to be increased in ADHD sufferers (Dougherty et al., 1999).

Neuroimaging techniques are increasingly being applied to the study of ADHD and such studies have shown anatomical alterations of dopamine-enriched brain areas, e.g. globus pallidus and frontal cortex in children with this condition (Castellanos, 2001). These investigations have indicated smaller and less active striatal neural networks and reduced dopamine metabolism in the cortex in patients with ADHD (Sieg et al., 1995; Castellanos et al., 1996; Ernst et al., 1998). In addition to prefrontocortical and striatal areas, abnormality of function of the basal ganglia, cerebellum, and parietal cortex has also been implicated in the causation of ADHD. These regions are part of unique circuits that project both to and from the PFC (Casey et al., 2007).

4. Pharmacotherapy of ADHD

From a pharmacological perspective, drugs for the treatment of ADHD fit into a very restricted classification, i.e. they selectively potentiate norepinephrinergic or dopaminergic neurotransmission in the brain or they enhance the function of both catecholamines simultaneously. The norepinephrinergic drugs, based on their *in vitro* pharmacology at least, are the selective norepinephrine reuptake inhibitor, atomoxetine (Bolden-Watson and Richelson, 1993) and the α_2-adrenoceptor agonist, guanfacine. Those drugs that simultaneously enhance both norepinephrinergic and dopaminergic neurotransmission include the monoamine releasers/reuptake inhibitors consisting of *dl*-amphetamine and its isomers, *dl-threo*-methylphenidate and its *d*-isomer. Although not an approved medication for the management of ADHD, bupropion has nonetheless been shown to have some efficacy in the treatment of this disorder (Wilens et al., 2001, 2005). Based on its *in vitro* pharmacology, bupropion is a weak, moderately selective dopamine reuptake inhibitor (Richelson and Pfenning, 1984; Hyttel, 1982). Stimulants like amphetamine or methylphenidate are the most widely prescribed drugs for the treatment for ADHD with clinical studies reporting that ~70% of patients will have a positive response to them (Spencer et al., 1996). The non-stimulant monoamine reuptake inhibitors, atomoxetine and bupropion, deliver 50–60% response rates (Spencer et al., 1998; Wilens et al., 2001).

It was Bradley (1937), who first reported that the behaviour of children suffering from what we now call ADHD, was dramatically improved when they were given *dl*-amphetamine. This paradoxical calming effect of a psychostimulant in a psychological and cognitive disorder that is characterised by hyperactivity and sometimes aggression revolutionised the clinical management of ADHD and the stimulants remain the mainstay of ADHD treatment to this day (see Table 1). Although *d*-amphetamine was introduced into the market as a single enantiomer product in the 1940s, systematic clinical trials to determine the relative efficacy of amphetamine's isomers in ADHD were not performed until the 1970s. In these trials, *d*-amphetamine was found to be more efficacious in alleviating the symptoms of ADHD than *l*-amphetamine and to have a more rapid onset of clinical effect (Arnold et al., 1972, 1973), but this was not confirmed in a later trial by Arnold et al. (1976), whilst Gross (1976) reported that *d*-amphetamine was more efficacious in the treatment of ADHD than racemic amphetamine. A more recent introduction is “mixed salts” amphetamine, which is a formulated amphetamine product containing a 3:1 mixture of *d*- and *l*-isomers of amphetamine provided as a mixture of different amphetamine salts. As a once-daily, medication, Adderall XR is currently the most widely prescribed amphetamine-based ADHD treatment in the USA.

Table 1
A summary of current and earlier drugs used in the treatment of ADHD

Generic drug name	Neurotransmitter target	Pharmacological mechanism(s)	Registered trade names
dl-Amphetamine	Norepinephrine (NE)+ Dopamine (DA)	Release, neuronal and vesicular reuptake inhibition, MAO inhibition	Benzedrine
d-Amphetamine	NE+DA	Release, neuronal and vesicular reuptake inhibition, MAO inhibition	Dexedrine, Dexedrine Spansules[a]
l-Amphetamine	NE+DA	Release, neuronal and vesicular reuptake inhibition, MAO inhibition	Cydril
"Mixed salts" amphetamine (3:1 mixture of *d*- and *l*-isomers)	NE+DA	Release, neuronal and vesicular reuptake inhibition, MAO inhibition	Adderall, Adderal XR[a]
Lysdexamphetamine	NE+DA	Release, neuronal and vesicular reuptake inhibition, MAO inhibition (*d*-Amphetamine prodrug)	Vyvanse
dl-Methylphenidate[b] (*erythro* + *threo* isomers)	NE+DA	Neuronal reuptake inhibitor	Centedrine
dl-threo-Methylphenidate	NE+DA	Neuronal reuptake inhibitor	Ritalin, Ritalin SR[a], Metadate CD[a], Concerta[a], Daytrana[a]
d-threo-Methylphenidate	NE+DA	Neuronal reuptake inhibitor	Focalin, Focalin XR[a]
Atomoxetine	NE	Neuronal reuptake inhibitor	Strattera
Guanfacine[c]	NE	α_2-Adrenoceptor agonist	Intuniv[a]
Bupropion[d]	DA	Neuronal reuptake inhibitor	Welbutrin, Welbutrin SR[a]

[a] Extended release formulation.
[b] Product withdrawn.
[c] Drug in pre-registration for ADHD.
[d] Not approved as an ADHD treatment.

Methylphenidate was first synthesised in 1944 (Panizzon, 1944), but its psychostimulant properties were not recognised for almost a decade (Meier et al., 1954). Centedrin (a 4:1 racemate of *erythro*- and *threo*-methylphenidate isomers) was the first methylphenidate-based ADHD medication. However, after Szporny and Görög (1961) reported that *erythro*-methylphenidate was almost totally devoid of CNS activity, all racemic methylphenidate products were formulated as 1:1 racemates of the *d*- and *l*-enantiomers of *threo*-methylphenidate. It was with *dl-threo*-methylphenidate that the landmark National Institute of Mental Health (NIMH) Multimodal Treatment study of children with ADHD (MTA) trial was performed (1999). The outcome of this clinical trial was that treatment with methylphenidate was significantly superior to behavioural therapy for children with ADHD (MTA Cooperative Group, 1999) and firmly established psychostimulant pharmacotherapy as the treatment strategy of choice in ADHD (Heal and Pierce, 2006). In a move to improve on the benefit/risk profile of racemic *threo*-methylphenidate, *d-threo*-methylphenidate, which is the more active enantiomer, has been developed as the single enantiomer products, Focalin and Focalin XR (Table 1).

Atomoxetine is a highly selective, norepinephrine reuptake inhibitor (Bolden-Watson and Richelson, 1993). Although atomoxetine was initially evaluated as an antidepressant, clinical candidate (Chouinard et al., 1984), it was subsequently found to be an effective treatment for ADHD (Spencer et al., 1998; Michelson et al., 2001, 2002), and it was approved for the treatment of this disorder in the USA in 2002.

In summary, therefore, there are 3 major pharmacological and chemical classes of established ADHD drug to be explored, viz amphetamine's enantiomers (β-phenylethylamine monoamine releasers/reuptake inhibitors), *threo*-methylphenidate's enantiomers (methyl α-phenyl-2-piperidineacetate, psychostimulant catecholamine reuptake inhibitors) and atomoxetine ((−)-*N*-methyl-3-phenyl-3-(*o*-tolyloxy)-propylamine, a "classical" selective norepinephrine reuptake inhibitor). With a recent approvable decision from the Food and Drugs Administration (FDA) for the use of guanfacine in the treatment of ADHD, α_{2A}-adrenoceptor agonists will soon be added to this list. The chemical structures of the isomers of amphetamine, *erythro*- and *threo*-methylphenidate, and atomoxetine are shown in Fig. 1.

5. The spontaneously hypertensive rat as an animal model of ADHD

The SH rat is an inbred, genetic strain derived from the Wistar Kyoto (WKY) rat and is one of the best validated and established rodent models of hypertension (e.g. Setescak et al., 1984; Palkowitz et al., 1994). The SH rat is also hyperactive and impulsive and these behavioural traits are present prior to the onset of hypertension, which does not develop until 10–12 weeks of age. Although the SH rat develops these traits before it becomes hypertensive, they persevere into adulthood (Adriani et al., 2003). Analogous to patients with ADHD, SH rats have altered

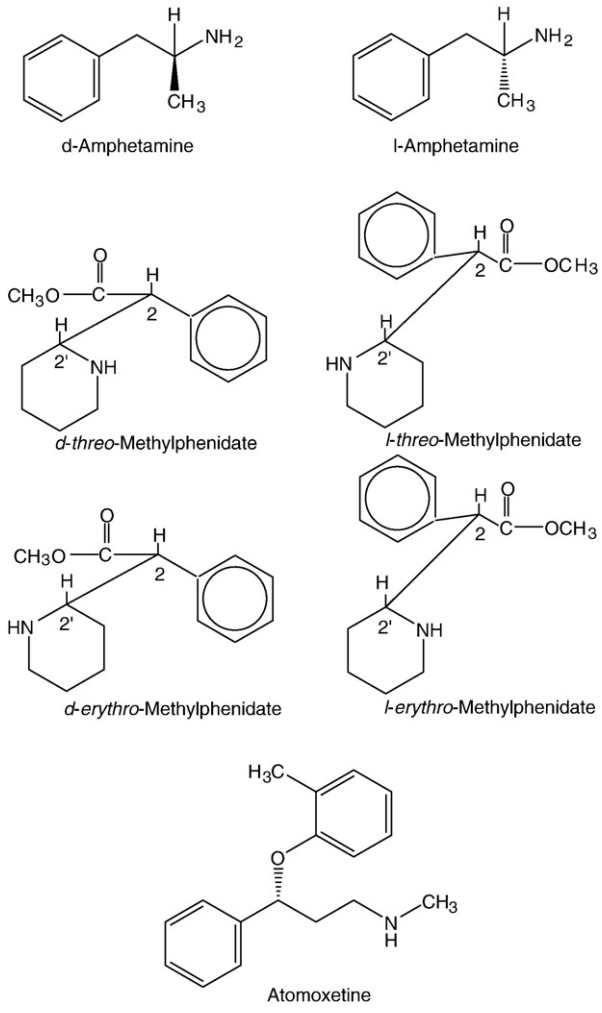

Fig. 1. Chemical structures of the major drugs used to treat ADHD.

reinforcement mechanisms involving the mesolimbic and mesostriatal dopaminergic systems (Russell et al., 1995; Russell, 2000) and the number of their dopamine transporter sites has been shown to be increased in the caudate-putamen (Watanabe et al., 1997). Adriani et al. (2003) demonstrated that SH rats could be divided into impulsive and non-impulsive subpopulations. They reported that the impulsive subpopulation had lower norepinephrine levels in the cingulate cortex and median PFC when compared with the non-impulsive subgroup. However, no differences in norepinephrine turnover were found.

SH rats show impaired performance in conditional avoidance tasks, autoshaping, and spatial learning tasks (e.g. Hecht et al., 1978; Knardahl and Karlsen, 1984; Sutterer et al., 1980, 1981) including the radial-arm maze (Hernandez et al., 2003; Levin et al., 1996; Mori et al., 1995; Nakamura-Palacios et al., 1996; Wyss et al., 1992) and the Morris water maze (Gattu et al., 1997a,b). SH rats have also been shown to be impulsive in various behavioural tests. Studies have reported that the ability of drugs used clinically to treat ADHD to improve these behaviours in the SH rat is inconsistent (e.g. Wultz et al., 1990; Sagvolden 2006, submitted for publication; Adriani et al., 2003, 2004; van den Bergh et al., 2006; Bizot et al., 2007; Sagvolden and XU 2008). Therefore, although the SH rat is a viable model of ADHD, the data also highlight the fact that the predictive validity of this rat strain as a test of the efficacy for drugs to treat ADHD is highly dependent on the behavioural test employed.

6. The pharmacological profile of ADHD drugs as monoamine reuptake inhibitors and monoamine-releasing agents *in vitro*

One key issue that still causes some confusion is the common description of drugs as being "both monoamine-releasing agents and reuptake inhibitors". It is undoubtedly true that monoamine-releasing agents impede the clearance of monoamines from the synaptic cleft because as competitive substrates for the monoamine reuptake transporters, they compete with the monoamines for access into the presynaptic terminal. However, the releasers do not have an extraneuronal site of action like "classical" reuptake inhibitors. As demonstrated below, in assays to measure the uptake of [^{3}H] monoamines into synaptosomes *in vitro*, the monoamine-releasing agents are generally relatively low potency, competitive inhibitors in comparison with high affinity "classical" reuptake blockers.

Table 2 summarises data reporting the inhibition of [^{3}H]monoamine uptake into rat brain synaptosomes *in vitro* by various ligands. A general "rule of thumb" when dealing with Ki values is <1 nM=very potent; 1–10 nM=potent; 10–100 nM=moderate; 100–1000 nM=weak; 1000–10,000 nM=very weak and >10,000 nM=inactive. To illustrate the points made above, both enantiomers of amphetamine are considerably less potent as [^{3}H]monoamine uptake inhibitors than the "classical" reuptake blockers, e.g. atomoxetine, and their actions are essentially confined to the inhibition of norepinephrine and dopamine reuptake. *d*-Amphetamine has been investigated extensively and is generally accepted to be a "weak" dopamine reuptake inhibitor with a Ki value of ~100 nM. Unusually for a releasing agent, *d*-amphetamine is a "moderately potent" inhibitor of norepinephrine reuptake ([^{3}H] norepinephrine reuptake, Ki=34 nM) (Richelson and Pfenning, 1984). As discussed later, *d*-amphetamine does also display some characteristics of a norepinephrine reuptake inhibitor at low dose *in vivo*, which may explain some of the differences between the pharmacology of the two enantiomers of amphetamine. With Ki values ranging from 1.4 to 3.8 μM, *d*-amphetamine can be considered to be only a very weak inhibitor of 5-HT reuptake. Direct comparisons of the enantiomers of amphetamine, reveal that *l*-amphetamine is 3.2 to 7-fold (Richelson and Pfenning, 1984; Kula and Baldessarini, 1991; Easton et al., 2007) less potent than *d*-amphetamine as a dopamine reuptake inhibitor. In contrast, it is only 1.8-fold less potent against norepinephrine (Richelson and Pfenning, 1984). Like the *d*-isomer, *l*-amphetamine is not a 5-HT reuptake inhibitor.

If one considers the "classical" reuptake blockers first, it is evident that these drugs are either "potent" or "very potent" inhibitors of [^{3}H] monoamine uptake (Table 2). By comparison, the catecholamine uptake Ki values of *dl-threo*-methylphenidate and its enantiomers are considerably lower than those of the "classical" reuptake inhibitors, including atomoxetine. Thus, *dl-threo*-methylphenidate, is a reuptake inhibitor that is "moderate/weak" for norepinephrine, "weak" for dopamine and inactive for 5-HT. The reuptake Ki values of *d-threo*-methylphenidate are slightly lower than those of the parent racemate, whilst *l-threo*-methylphenidate is a "very weak" *in vitro* inhibitor of [^{3}H]norepinephrine uptake. Patrick et al. (1987) is the only study to have compared directly the relative potencies of the enantiomers of *threo*-methylphenidate as catecholamine reuptake inhibitors, and for both dopamine and norepinephrine, they observed that *d-threo*-methylphenidate was approximately 10x more potent than *l-threo*-methylphenidate. These results indicate that for this particular pharmacological mechanism, at least, *dl-threo*-methylphenidate comprises one highly active, i.e. *d-threo*-methylphenidate, and one very weakly active, i.e. *l-threo*-methylphenidate, enantiomer.

The release of monoamines is the other key pharmacological action of amphetamine and related drugs and this mechanism has been investigated extensively *in vitro*. The methodologies employed have not undergone such radical modification as those for measuring [^{3}H]monoamine uptake. Consequently, a wider spread of literature material is acceptable for review. That having been said, an extensive search of the literature revealed only three studies (Heikkila et al., 1975; Holmes and Rutledge, 1976; Easton et al., 2007) that have rigorously compared the [^{3}H]monoamine release profiles of the *d*- and *l*-isomers of amphetamine and two of these investigations were limited to determining their effects on [^{3}H]dopamine and [^{3}H]

Table 2
Inhibition of [^{3}H]monoamine uptake into rat brain synaptosomes *in vitro* by various drugs used to treat ADHD and by comparator reuptake inhibitors

Drug	Reference	Inhibition of [^{3}H]monoamine uptake (Ki=nM)		
		[^{3}H] Dopamine	[^{3}H] Norepinephrine	[^{3}H]5-HT
Amphetamine enantiomers				
d-Amphetamine	1	82	50	1840
	2	34	39	3830
	3	225	–	–
	4	132	45	1441
	5	78	–	–
	6	206	55	–
l-Amphetamine	1	380	90	10,000
	3	720	–	–
	6	1435	259	–
Methylphenidate enantiomers				
dl-threo-Methylphenidate	1	160	40	22,000
	6	341	238	–
	7	281	103	>1000
d-threo-Methylphenidate	8	270	150	–
	9	1300	100	–
l-threo-Methylphenidate	9	11,000	1200	–
d-erythro-Methylphenidate	8	140,000	33,000	–
Reuptake inhibitors				
Atomoxetine	6	2355	21	–
	10	1400	1	43
GBR 12935	2	4	277	289
Desipramine	1	5200	1	340
	2	5946	8	350
Paroxetine	10	1700	33	0.73

– = Not tested; 1 = Richelson and Pfenning (1984); 2 = Rothman et al. (2001); 3 = Kula and Baldessarini (1991); 4 = Heal et al. (1998a); 5 = Rowley et al. (2000); 6 = Easton et al. (2007); 7 = Andersen (1989); 8 = Ferris et al. (1972); 9 = Patrick et al. (1987); 10 = Bolden-Watson and Richelson (1993).

Table 3
Ability of drugs used to treat ADHD and comparator reuptake inhibitors to release [^{3}H]monoamines from rat brain slices or synaptosomes *in vitro*

Drug	Reference	Superfused slices	Release of [^{3}H]monoamines		
			[^{3}H]Dopamine	[^{3}H]Norepinephrine	[^{3}H]5-HT
Amphetamine enantiomers					
d-Amphetamine	1	Yes	✓	✓	✓
	2	No	✓	✓	✓
	3	No	✓	✓	✓
	4	No	✓	✓	–
	5	Yes	✓	–	–
	6	No	✓	–	–
	7	Yes	✓	–	–
l-Amphetamine	3	No	✓	✓	✓
	4	No	✓	✓	–
	5	Yes	✓	✓	–
Methylphenidate enantiomers					
dl-threo-Methylphenidate	5	Yes	X	X	–
	7	Yes	✓	–	–
	8	Yes	✓	–	–
	9	Yes	✓	–	–
	10	No	✓/X	✓/X	✓/X
	11	No	X	X	–
d-threo-Methylphenidate	11	No	X	X	–
	12	No	X	X	–
l-threo-Methylphenidate	11	No	X	X	–
d-erythro-Methylphenidate	12	No	X	X	–
Reuptake inhibitors					
Atomoxetine	5	Yes	X	✓	–
GBR 12935	2	No	X	X	X
Desipramine	2	No	X	X	X
Fluoxetine	2	No	X	X	X
	13	Yes	–	–	X

At concentrations ≤10^{-5} M ✓ = Causes release; X = Inactive; ✓/X = Equivocal result; – = Not tested. 1 = Heal et al. (1998b); 2 = Rothman et al. (2001); 3 = Holmes and Rutledge (1976); 4 = Heikkila et al. (1975); 5 = Easton et al. (2007); 6 = Azzaro et al. (1974); 7 = Russell et al. (1998); 8 = Vickroy and Johnson (1982); 9 = Heal et al. (1996); 10 = Wall et al. (1995); 11 = Patrick et al. (1987); 12 = Ferris et al. (1972); 13 = Heal et al. (1998a).

norepinephrine release. There are some technical aspects of [^{3}H] monoamine release experiments that also merit comment. Since the slices are preloaded with [^{3}H]monoamines, their disposition in the presynaptic compartments, i.e. the cytosolic and vesicular storage pools, does not conform to the physiological situation. Moreover, strategies to prevent the vesicular storage of the [^{3}H]monoamines using reserpine, as employed by Rothman et al. (2001), can influence the outcome of the experiments. Whilst reserpinisation does not affect the rank order of potency of drugs to release individual monoamines, it does bias the results when comparing the effects of a single drug across all three neurotransmitters. The reason is that the size of the easily released cytosolic pool differs between dopaminergic, norepinephrinergic and serotonergic neurones. Artificially boosting the size of the cytosolic pool by vesicular destruction makes releasing agents appear disproportionately potent against norepinephrine, because for this monoamine the cytosolic pool is very small (Florin et al., 1994). For this reason, the [^{3}H]monoamine release results shown in Table 3 indicate only whether drugs are active as releasing agents or not.

d-Amphetamine evokes the release of all three monoamines and this is in spite of the fact that its substrate affinity defined in the [^{3}H] monoamine uptake experiments indicates it is catecholamine-selective (Table 3). *l*-Amphetamine also releases [^{3}H]dopamine, [^{3}H] norepinephrine and [^{3}H]5-HT. Comparing the relative potencies of *d*- and *l*-amphetamine, Heikkila et al. (1975) and Easton et al. (2007) reported that the *d*-isomer was ~4-fold more potent than the *l*-isomer to release [^{3}H]dopamine. In contrast *l*-amphetamine was either more potent or equipotent with the *d*-isomer as a releaser of [^{3}H] norepinephrine (Heikkila et al., 1975; Easton et al., 2007). These data are, therefore, entirely consistent with the substrate affinities of the two enantiomers of amphetamine for these two monoamine transporters.

In comparison to amphetamine's enantiomers, which are very potent and powerful releasers of monoamines, neither *dl-threo*-methylphenidate nor its *d*- or *l*-isomers increased the release of [^{3}H] monoamines from preloaded synaptosomes. In contrast, the majority of superfusion experiments report that high concentrations of *dl-threo*-methylphenidate can increase basal release of [^{3}H]dopamine and [^{3}H] norepinephrine from rat brain slices *in vitro*. However, this effect is usually observed only at non-pharmacologically relevant concentrations of 10^{-5} M–10^{-3} M (Azzaro et al., 1974; Holmes and Rutledge, 1976; Vickroy and Johnson, 1982; Heal et al., 1996; Russell et al., 1998; Easton et al., 2007). In support of this hypothesis, classical monoamine reuptake inhibitors, which are devoid of monoamine-releasing properties, have been observed to increase the basal overflow of [^{3}H]monoamines from rat brain slices in superfusion experiments at these supra-pharmacological concentrations (Heal et al., 1998a; Easton et al., 2007).

The other pharmacological action of the amphetamines that may contribute to increased synaptic concentrations of monoamines is their ability to inhibit monoamine oxidase (MAO). Mantle et al. (1976) reported that both *d*- and *l*-amphetamine were inhibitors of membrane-bound liver MAO; the Ki values were 20 μM and 70 μM for the *d*- and *l*-isomers, respectively.

7. Neurochemistry of the SH rat *in vivo*

As described earlier in this review, the SH rat has been proposed by several groups to be a genetic strain that displays the core behavioural and cognitive deficits present in ADHD (Sagvolden et al., 1992a,b; Sagvolden, 2000; Adriani et al., 2003; Oades et al., 2005), although not all researchers in the area support this view (van den Bergh et al., 2006). However, in spite of the fact that the SH rat was first proposed as a model of ADHD more than 15 years ago (Sagvolden et al., 1992a,b), the majority of experiments performed in this rat strain have been

behavioural to phenotype its psychological and cognitive disturbances (e.g. Wultz and Sagvolden, 1992; Sagvolden et al., 1992b; Adriani et al., 2003) and the effects on these deficits of drugs used in ADHD (Sagvolden et al., 1992a; Mook and Neuringer, 1994; Sagvolden, 2006). In addition, *ex vivo* neurochemistry has been used, particularly superfusion experiments, to define the profile of [^{3}H]monoamine release from slices of various brain regions (e.g. de Villiers et al., 1995; Russell et al., 1995, 1998; Russell, 2000; Russell and Wiggins, 2000). Whilst experiments performed *ex vivo* make an important contribution to our understanding of the neurochemistry of the SH rat, they do not provide a time-resolved, quantitative index of extraneuronal neurotransmitter concentrations *in vivo*, or an assessment of the dynamic effects of drugs upon them. With the exception of the *in vivo* microdialysis studies described here, we are aware of only three other investigations by Linthorst et al. (1991), Carboni et al. (2003) and Fujita et al. (2003) that have explored the neurochemistry of conscious, freely-moving SH rats using the technique of *in vivo* microdialysis, and none which have employed the technique of *in vivo* voltammetry.

In our dual-probe, microdialysis experiments, we compared the neurochemistry of the SH rat with that of the outbred, SD strain. The latter was selected as a comparator rather than the WKY strain because it has aberrant behavioural and neurochemical traits (Drolet et al., 2002). Moreover, the majority of microdialysis experiments investigating the effects of drugs, like amphetamine, methylphenidate and atomoxetine, have been performed in outbred SD rats (Zetterström et al., 1983; Sharp et al., 1987; Maisonneuve et al., 1992; Cadoni et al., 1995; Miele et al., 2000; Rowley et al., 2000; Géranton et al., 2003a,b, 2004). We have used a sampling time of 24 h after probe implantation as this is widely thought to be the optimal time to avoid gliosis around the microdialysis probe, intracranial infection and progressive neuropathy.

Monoamine "efflux" is the extraneuronal concentration of neurotransmitter that results from the difference between the two active processes of release and reuptake, and as a consequence, basal efflux is a useful surrogate of monoaminergic tone. In turn, changes in efflux produced by drugs can be used as a surrogate of their influence on monoaminergic neurotransmission. Our experiments revealed that, compared with the SD rat, the basal efflux of norepinephrine was 26% lower (P=0.035) in the prefrontal cortex (PFC) of the SH rat, whereas basal dopamine efflux in the striatum was 78% higher (P=0.0007) (Cheetham et al., 2007). The latter finding is consistent with the earlier report of increased basal dopamine efflux in the nucleus accumbens shell of SH rats compared to WKY controls (Carboni et al., 2003), and furthermore, the reported increased intrinsic level of locomotor activity in SH rats (Knardahl and Sagvolden, 1979; McCarty and Kopin, 1979; Myers et al., 1982; Moser et al., 1988; Wultz et al., 1990; Sagvolden et al., 1992b; Wultz and Sagvolden, 1992; Sagvolden et al.,

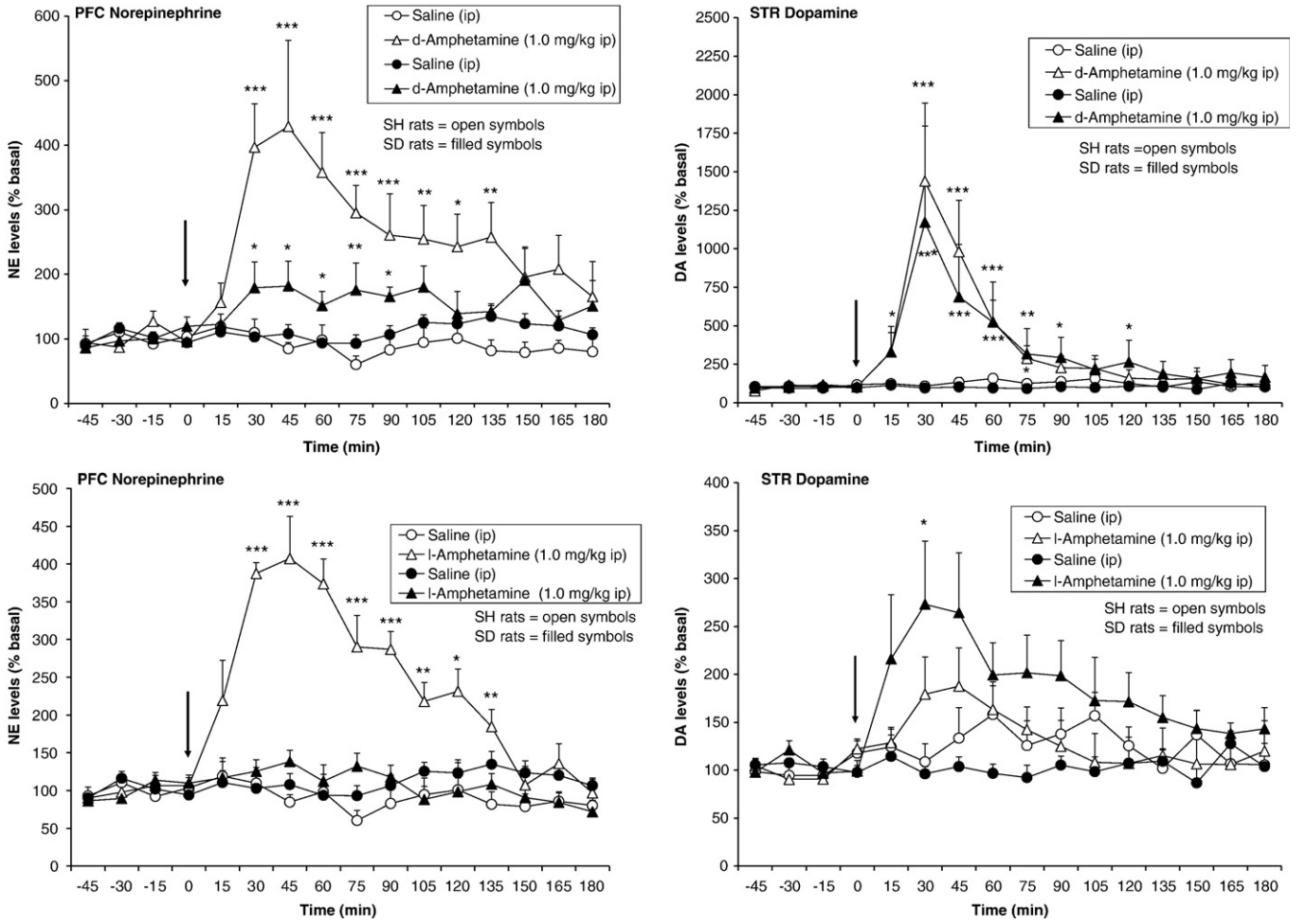

Fig. 2. A comparison of the effects of amphetamine's isomers on cortical norepinephrine and striatal dopamine efflux in SH and SD rats. A comparison of the effects of *d*- and *l*-amphetamine (1.0 mg/kg, ip) on the extracellular concentrations of norepinephrine in the prefrontal cortex (PFC; left-hand panels) and dopamine in the striatum (STR; right-hand panels) of freely-moving SH and SD rats. Values are mean±SEM (n=7–16 rats). The vertical arrow indicates administration of drug or saline. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ significantly different from appropriate saline-treated controls according to ANCOVA with Williams test for multiple comparisons (norepinephrine) or *t*-test for multiple comparisons (dopamine). Data taken from Cheetham et al. (2007).

1993; Adriani et al., 2003; van den Bergh et al., 2006; Cheetham et al., 2007). When the effects of the *d*- and *l*-isomers of amphetamine on the extraneuronal concentrations of prefrontocortical norepinephrine and striatal dopamine were compared in SH and SD rats, some fundamental and profound differences in the actions of these monoamine-releasing drugs were observed. As shown in Fig. 2, at a dose of 1 mg/kg, *d*-amphetamine and *l*-amphetamine evoked significantly ($P<0.05$) greater increases in norepinephrine efflux in the PFC of the SH rat than in the SD, whereas at this dose, there was no difference in the striatal dopamine efflux between the two rat strains (Cheetham et al., 2007). Moreover, when the catecholaminergic effects of the amphetamine isomers were tested over a range of doses, i.e. *d*-amphetamine at 0.3, 1 and 3 mg/kg and *l*-amphetamine at 1, 3 and 9 mg/kg, other differences became apparent. In the SH rat, the extraneuronal concentration of norepinephrine in the PFC was dose-dependently elevated by *l*-amphetamine, but in the SD rat, an inverted U-shaped dose-response was observed with *l*-amphetamine producing its maximum effect at a dose of 3 mg/kg. Identical phenomena were observed when the actions of *l*-amphetamine on striatal dopamine efflux were compared in SH and SD rats (Cheetham et al., 2007).

As predicted, the difference in the shapes of the dose-response curves in these two rat strains had a marked impact on the relative magnitude of the catecholaminergic responses to *d*- and *l*-amphetamine. Thus, the AUC (area under the curve) values for prefrontocortical norepinephrine efflux evoked by *d*-amphetamine were significantly greater in the SH rat at doses of 1 and 3 mg/kg and greater increases with *l*-amphetamine were also observed at 1 and 9 mg/kg (Cheetham et al., 2007). Striatal dopamine efflux in the SH rat was significantly greater than that in the SD with *l*-amphetamine at a dose of 9 mg/kg (Cheetham et al., 2007). In other respects, i.e. onset of drug action, time to peak effect and duration of action, the action of amphetamine's enantiomers on prefrontocortical norepinephrine and striatal dopamine efflux did not differ between these two rat strains (Fig. 2).

In contrast to these findings, Linthorst et al. (1991) reported that the basal efflux of dopamine in the striatum of SH rats was decreased by about 35% compared with WKYs. Moreover, these authors also showed that release of this catecholamine was also more susceptible to D_2 autoreceptor modulation in the SHRs. Although the findings are unequivocal, it is important to consider the microdialysis technique that was used to generate them. Even at the time these experiments were performed, transcranial microdialysis was considered to be unsuitable for three reasons. First, the high degree of non-specific damage caused to the brain by the transcranial probe. Second, the uncertainty of the neuroanatomical locus of sampling. Finally, the highly invasive surgery employed, especially when micorodialysis sampling was performed in conscious animals only 24 h later. Non-specific damage is often reflected in high basal efflux levels of monoamines. In our experiments, we obtained a basal striatal dopamine efflux of about 20 fmol/20 µl. By contrast Linthorst et al. (1991) reported basal efflux levels approximately 10-fold higher than this value, i.e. 288±35 fmol/30 µl (190 fmol/20 µl). Whilst the data of Linthorst et al. (1991) cannot be discounted, their findings would need to be replicated using modern microdialysis techniques before they could be considered to be definitive.

When our findings are taken together with the results of Carboni et al. (2003), they indicate that the SH rat has hyperfunctional basal dopaminergic tone in both the nigrostriatal and mesolimbic systems, together with potentially hypofunctional norepinephrinergic tone in the PFC. If one accepts the premise that the SH rat models some of the neurochemical deficits responsible for ADHD, the results indicate that the probable hypofunctionality of norepinephrinergic tone in the PFC together with their increased responsiveness to amphetamine's isomers are consistent with the observation that drugs increasing norepinephrinergic neurotransmission in the PFC are efficacious in ADHD treatment. Moreover, the increased responsiveness of the SHR to the actions of amphetamine's isomers, and in the case of *l*-amphetamine, the linearity of its dose-response characteristics provide an explanation of why these drugs should be especially efficacious in the treatment of this behavioural and cognitive disorder.

8. Comparison of *in vivo* with *in vitro* neurochemical studies

The observations from *in vivo* microdialysis experiments showing that basal norepinephrinergic tone in the PFC is probably hypofunctional in the SH rat, whilst basal dopaminergic tone in the striatum and nucleus accumbens is hyperfunctional (Carboni et al., 2003; Cheetham et al., 2007) are precisely the opposite of the hypothesis proposed by Russell and colleagues on the basis of experiments investigating [^{3}H]monoamine release from brain slices *in vitro* (Russell, 2002; Russell et al., 2005). However, when the results from these *in vitro* experiments are examined in detail, it is evident that the data are open to more than a single interpretation. The closest analogy to a basal neurotransmitter efflux *in vivo* is the effect of either electrical or K^+ stimulation of [^{3}H]monoamine release from brain slices *in vitro*. In their superfusion experiments, Russell et al. (2000) reported that K^+-evoked release of [^{3}H]norepinephrine in PFC slices from SH rats was less susceptible to inhibition by activation of presynaptic α_2-adrenoceptors than from WKYs. However, K^+-evoked [^{3}H]norepinephrine release from PFC slices was unaltered in SH rats versus WKYs, as was [^{3}H]norepinephrine release in the presence of the α_2-adrenoceptor antagonist, idazoxan. As they stand, the data do not necessarily argue for hyperfunction of the mesocortical norepinephrinergic system, and in fact, a reduced sensitivity of presynaptic α_2-adrenoceptors could be explained as a compensatory decrease in auto-inhibitory control to counterbalance reduced norepinephrine turnover in this brain region.

In the case of the hypothesis that the dopaminergic neurotransmitter systems in the brains of the SH rat are hypofunctional, there is a greater degree of divergence between results obtained *in vitro* using the superfusion methodology, those from *in vivo* microdialysis and some *ex vivo* studies. Although it has been reported that single-pulse, electrically-evoked release of [^{3}H]dopamine from PFC slices is reduced in the SH rat relative to WKY controls (Russell et al., 1995, 1998), the efflux of this monoamine was actually significantly greater in the SH rats on repeated electrical stimulation, i.e. S_2/S_1 (Russell et al., 1995, 1998). Electrically-evoked release of [^{3}H]dopamine from striatal slices was similarly attenuated after a single stimulation (Linthorst et al., 1990; Russell et al., 1995, 1998, 2000), but was unchanged on repeated stimulation (Russell et al., 2000). The experimental findings are similarly contradictory on the topic of the sensitivity of the D_2 autoreceptor control of striatal dopamine release. Linthorst et al. (1990) reported increased D_2 receptor-mediated control of electrically-evoked [^{3}H]dopamine release in SH rats compared with WKYs, whilst Russell et al. (1995, 2000) observed no difference. In the nucleus accumbens, basal efflux of [^{3}H]dopamine was unchanged in the SH rat relative to the WKY, as was electrically-stimulated release of this neurotransmitter (Russell et al., 1995, 1998, Russell, 2003), including a lack of difference when the core and shell regions of this brain area were examined separately (Russell, 2003). Together, these *in vitro* data indicate that although the mesocortical and nigrostriatal dopaminergic systems may appear hypofunctional, dopaminergic tone is likely to be enhanced in the PFC and at least unchanged in the striatum of the SH rat under conditions of high levels of neuronal firing. Moreover, the *in vitro* studies have revealed no evidence to support the view that the mesolimbic dopaminergic system in the SH rat is hypofunctional. Thus, the *in vitro* findings are generally equivocal in their support of the hypothesis for dopaminergic hypofunctionality in the brain of the SH rat. Moreover, they are also at variance with other *in vivo* and *ex vivo* observations. It is well known that the SH rat is hyperactive (Knardahl and Sagvolden, 1979; McCarty and Kopin, 1979; Myers et al., 1982; Moser et al., 1988; Wultz et al., 1990;

Table 4
A comparison of the effects of various catecholaminergic drugs on prefrontocortical norepinephrine and striatal dopamine efflux determined in SH and SD rats

Drug (mg/kg)	Norepinephrine in prefrontocortex				Dopamine in striatum			
	SH rats		SD rats		SH rats		SD rats	
	Peak effect (%) of baseline	Duration of effect (min)	Peak effect (%) of baseline	Duration of effect (min)	Peak effect (%) of baseline	Duration of effect (min)	Peak effect (%) baseline	Duration of effect (min)
d-Amphetamine								
0.3 mg/kg	186±51	≤90	–	–	731±259	≤75	–	–
1.0 mg/kg	429a±133	≤135	182±38	≤90	1439±506	≤75	1173±625	≤120
3.0 mg/kg	649a±87	≤135	198±39	≤90	4898±1912	≥180	1606±391	≥180
l-Amphetamine								
1.0 mg/kg	407[a]±56	≤135	NS	NS	NS		273±66	≤30
3.0 mg/kg	393±89	≤135	246±49	≤105	644±208	≤75	792±374	≤150
9.0 mg/kg	1069[a]±105	≥180[a]	157±24	≤120	3294[a]±691	≥180[a]	459±107	≤150
d-+*l*-Amphetamine								
2.0 mg/kg	–	–	185±24	≤75	–	–	644±218	≤150
d-threo-MPH								
1.0 mg/kg	179±36	≤105	–	–	337±45	≤75	–	–
3.0 mg/kg	276±45	≤135	–	–	513±97	≤225	–	–
10.0 mg/kg	449±51	≥240	–	–	1042±117	≤225	–	–
l-threo-MPH								
10.0 mg/kg	NS	–	–	–	200±24	≤45	–	–
dl-threo-MPH								
10.0 mg/kg	376.3±65	≥240	–	–	389±152	≤210	–	–
20.0 mg/kg	469±73	≥240	–	–	729±232	≤210	–	–

n=4–12 observations; – = Not examined; NS = No significant change.
MPH = methylphenidate.
[a] AUC value significantly greater than corresponding value in SD rats.

Sagvolden et al., 1992b; Wultz and Sagvolden, 1992; Sagvolden et al., 1993; Adriani et al., 2003; van den Bergh et al., 2006; Cheetham et al., 2007) and it has previously been demonstrated that locomotor activity levels correlate strongly with extraneuronal dopamine concentrations in the nucleus accumbens (Rowley et al., 2000). Increased dopamine turnover in the brains of SH rats measured either *ex vivo* (McKeon and Hendley, 1988) or *in vivo* (Carboni et al., 2003; Cheetham et al., 2007) could also explain why there is a compensatory increase in the number of dopamine reuptake transporter sites in the nucleus accumbens and striatum of young (pre-hypertensive) SH rats (Watanabe et al., 1997). Increased dopaminergic tone in ADHD is also indicated by the finding that [^{18}F]L-DOPA uptake into midbrain dopaminergic neurones is markedly enhanced in children with ADHD (Ernst et al., 1999) and reports of an increased number of dopamine reuptake transporters in the striatum of adult patients with ADHD (Dougherty et al., 1999). The proposal that the SH rat suffers from a combination of dopaminergic hyperfunction together with norepinephrinergic hypofunction in the brain is also consistent with the clinical observations and neurochemical hypothesis of ADHD proposed by Oades (2002).

9. Pharmacological profiles of ADHD drugs revealed by microdialysis in SH, WKY and outbred rats

Although numerous studies using *in vivo* microdialysis have been performed with amphetamine and methylphenidate, experiments to define their pharmacological profiles using the freely-moving SH rat as an animal model of ADHD have been conducted by only two research groups, viz our own and that of Carboni et al. (2003). Moreover, the number of *in vivo* microdialysis studies that have been conducted on the individual enantiomers of these stimulants is very limited even in outbred rats. We have performed the only experiments on these compounds in the SH rat. As a number of marketed ADHD drugs are either racemic compounds or fixed-ratio mixtures of isomers (Table 1), both enantiomers of amphetamine and *threo*-methylphenidate are likely to provide contributions to the efficacy and side-effects of such products, and as such, a greater understanding of their pharmacological profiles *in vivo* is essential. Only the research group from Lilly has performed microdialysis experiments with atomoxetine and they were performed in SD rats (Bymaster et al., 2002; Swanson et al., 2006).

Our results show that, in the SH rat, *d*-amphetamine powerfully enhances the efflux of both norepinephrine in the PFC and dopamine in the striatum (Fig. 2; Table 4). At a dose of 1 mg/kg, the increase in dopamine efflux is ~3-fold greater than that of norepinephrine, but the potentiating effect of this drug on norepinephrine efflux is substantially longer in duration. For both catecholamines, *d*-amphetamine has a rapid onset of action producing its peak effects on these neurotransmitters ~30 min after administration. As shown by the data in Table 4, the dose-responsiveness of striatal dopamine efflux after administration of *d*-amphetamine is very steep in the SH rat with a marked increase in the magnitude occurring at a dose of 3 mg/kg, whereas for norepinephrine the dose-responsiveness is much more gradual. *l*-Amphetamine shows more balance in its ability to increase extraneuronal concentrations of cortical norepinephrine and striatal dopamine (Fig. 2; Table 4). Its profile contrasts with that of the *d*-isomer, because at low dose, i.e. 1 mg/kg, *l*-amphetamine produces a substantial increase in norepinephrinergic neurotransmission in the PFC without significantly increasing striatal dopamine efflux, whereas at higher doses this balance of effect is totally reversed (Table 4). Another important difference between the pharmacological profiles of amphetamine's isomers is the very marked increase in both cortical norepinephrine and striatal dopamine efflux that occurred at the highest dose tested, i.e. 9 mg/kg. When one considers these results in terms of the relative abilities of amphetamine's isomers to potentiate norepinephrinergic and dopaminergic neurotransmission in the CNS, the data from the SH rat suggest that at low doses, which are those most appropriate to the clinical use of amphetamine in ADHD, for

racemic amphetamine, the *d*- and *l*-isomers are likely to contribute equally to potentiating norepinephrinergic neurotransmission in the PFC. In contrast, it is the *d*-isomer that is predicted to provide most of the increase in dopaminergic drive.

Based on *in vitro* Ki values (Table 2), it is evident that *d-threo*-methylphenidate is ~10-fold more potent as a norepinephrine and dopamine reuptake inhibitor than the *l-threo*-isomer. In the microdialysis experiments in the SH rat, *d-threo*-methylphenidate dose-dependently increased the efflux of norepinephrine in the PFC and dopamine in the striatum (Kulkarni et al., 2006; Fig. 3). In terms of percentage increases, the effect on striatal dopamine was approximately twice as large as that on prefrontocortical norepinephrine at each of the doses tested; however, the increase in norepinephrine efflux was more gradual and more sustained than that of dopamine. The onset of action of *d-threo*-methylphenidate was rapid for both catecholamines with peak effects occurring between 30 and 60 min after dosing.

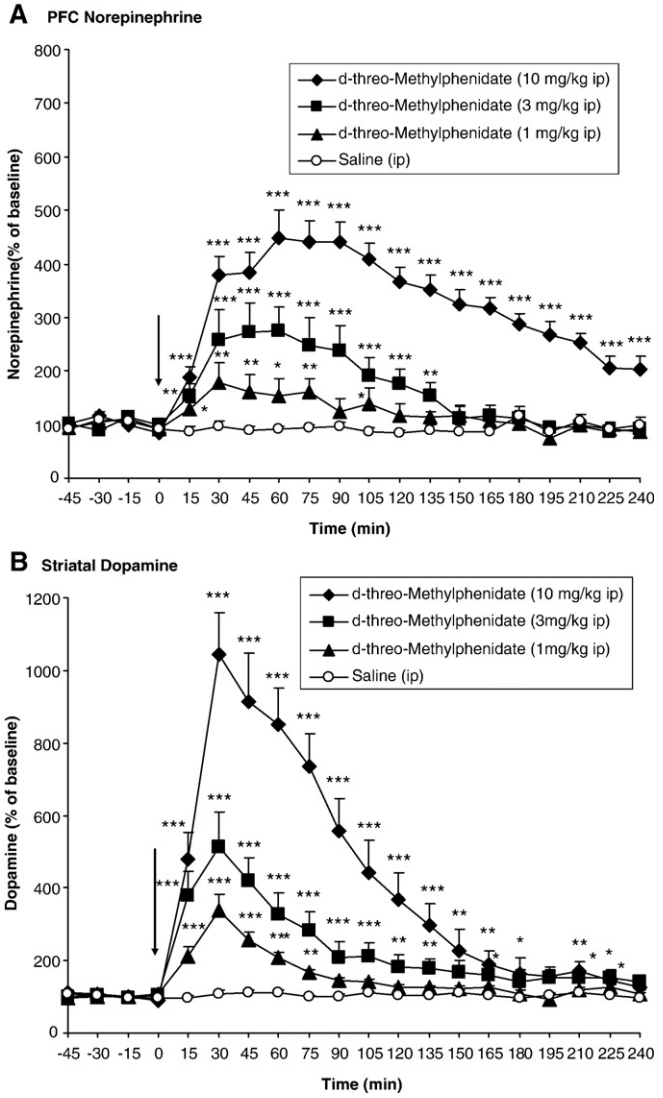

Fig. 3. Effect of *d-threo*-methylphenidate on cortical norepinephrine and striatal dopamine efflux in SH rats. Effects of *d*- and *l-threo*-methylphenidate on the extracellular concentrations of (A) norepinephrine in the prefrontal cortex (PFC) and (B) dopamine in the striatum. Each point represents the mean percentage of baseline ± SEM (n = 8–13 rats). The vertical arrows indicate the time of injection of drug or saline. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ significantly different from saline-treated controls according to ANCOVA with Williams test for multiple comparisons. Data taken from Kulkarni et al. (2006).

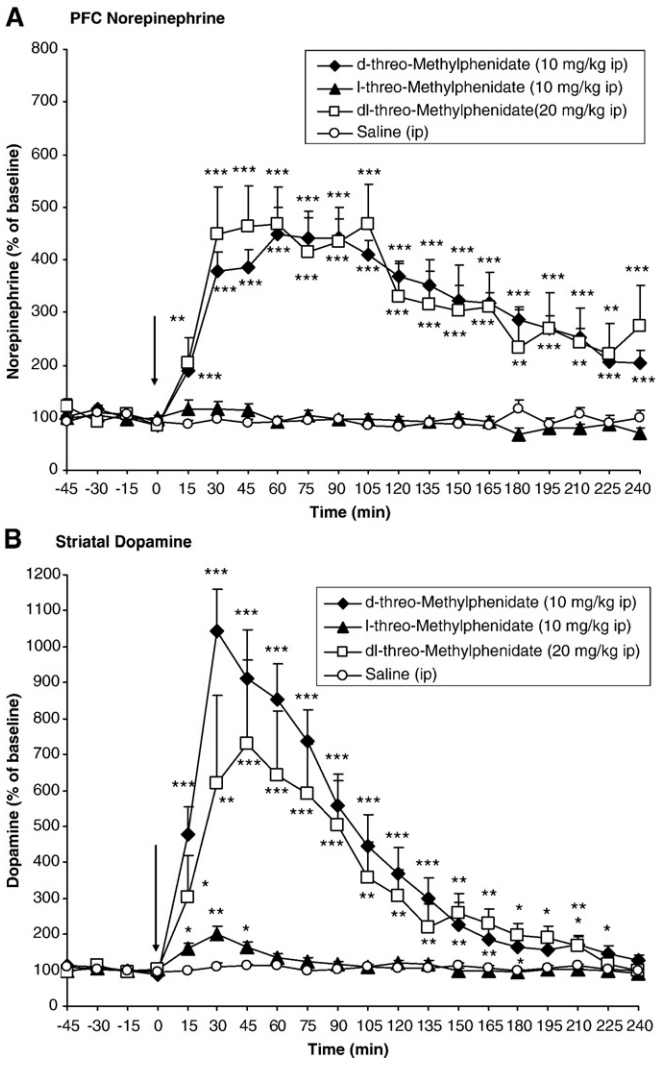

Fig. 4. A comparison of the effects of *threo*-methylphenidate's isomers on cortical norepinephrine and striatal dopamine efflux in SH rats. Effects of *dl-threo*-methylphenidate and its isomers on the extracellular concentrations of (A) norepinephrine in the prefrontal cortex and (B) dopamine in the striatum. Each point represents the mean percentage of baseline ± SEM (n = 8–13 rats). The vertical arrows indicate the time of injection of drug or saline. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ significantly different from saline-treated controls according to ANCOVA with Williams test for multiple comparisons. Data taken from Heal et al. (2006).

Based on the reuptake inhibition Ki values, *l-threo*-methylphenidate was initially tested only at the highest dose of 10 mg/kg. Consistent with the potency difference between it and the *d*-isomer that was observed *in vitro*, *l-threo*-methylphenidate evoked a small increase in striatal dopamine efflux (200 ± 24%, $P<0.01$) and a small non-significant increase in extraneuronal norepinephrine (117 ± 15%) (Table 4). In pharmacodynamic terms, i.e. relative effect on striatal dopamine and prefrontocortical norepinephrine, maximum effect size, and onset and duration of action, the *l*-isomer is a much less potent pharmacological clone of *d-threo*-methylphenidate. This opinion was confirmed when the effects of 20 mg/kg of *dl-threo*-methylphenidate on prefrontocortical norepinephrine and striatal dopamine were compared to the perspective contributions provided by 10 mg/kg doses of the individual *d*- and *l*-isomers (Fig. 4).

As well as measuring basal efflux, Carboni et al. (2003) also determined the effects of low doses of amphetamine and methylphenidate on dopamine efflux in the nucleus accumbens shell of the SH rat using the WKY as the control strain. Since the enantiomeric specificity of the drugs was not stated in the manuscript, it is assumed

that they were racemates, i.e. *dl*-amphetamine and *dl-threo*-methylphenidate. Both stimulants evoked dose-related increases in extraneuronal dopamine in this brain region with a dose of 0.5 mg/kg of amphetamine producing a similar sized increase in dopamine efflux to 1 mg/kg of methylphenidate. For both stimulants, the magnitude of the increase in efflux was significantly greater in SH than WKY rats. Consistent with our results obtained in the PFC and striatum, the onset of pharmacological action of amphetamine and methylphenidate was rapid with maximum elevations being observed at 40–60 min after dosing. The other experiment that was performed determined the effect of reverse dialysis of 30 or 60 mM K^+ on dopamine release, and here, a significantly reduced dopaminergic response was observed in the SH rat (Carboni et al., 2003). When these and our own data are viewed together, a hypothesis can be constructed relating to the implications of dopaminergic hyperfunctionality in the SH rat. Thus, the increased dopamine turnover, which has been observed both *ex vivo* (McKeon and Hendley, 1988) and *in vivo* (Carboni et al., 2003), produces a compensatory increase in the number of dopamine reuptake transporters in the striatum and nucleus accumbens of the SH rat (Watanabe et al., 1997). When the SH rat is challenged with either a competitive dopamine reuptake transporter substrate, e.g. amphetamine or one of its isomers, or a competitive dopamine reuptake transporter blocker, e.g. *threo*-methylphenidate or one of its isomers, their pharmacological actions are magnified by the increased number of dopamine transporters in the brain of the SH rat (Carboni et al., 2003; Cheetham et al., 2007). Although the newly synthesised/releasable pool of dopamine is decreased, consistent with the reduction in K^+-evoked release of dopamine (Carboni et al., 2003), amphetamine can potentiate dopamine efflux by releasing this monoamine from the vesicular storage pool (Sulzer and Rayport, 1990; Floor and Meng, 1996). For *threo*-methylphenidate, which potentiates firing-dependent release of catecholamines (Butcher et al., 1991), auto-inhibitory controls are an important factor also to be considered. *In vivo* microdialysis experiments have revealed that although overall D_2 receptor auto-inhibitory control of exocytotic dopamine release is enhanced in the striatum of the SH rat relative to the WKY (Linthorst et al., 1991), D_2 auto-receptor control of dopamine efflux at the terminal level in the striatum and nucleus accumbens is generally unaltered. A minor increase was reported in quinpirole-induced inhibition of dopamine efflux at only 2/9 time-points in the nucleus accumbens (Fujita et al., 2003). Under this circumstance, increased efflux of dopamine is not, therefore, subject to significantly greater control, which in turn, results in an enhanced efflux of dopamine in response to administration of the firing-dependent increase in dopamine overflow produced by *threo*-methylphenidate, and perhaps also, low doses of amphetamine.

Although our data revealed that norepinephrinergic systems in the PFC of the SH rat are likely to be hypofunctional, constructing a hypothesis to explain why amphetamine's isomers evoke greater release of this catecholamine is much more speculative because of the paucity of complimentary data. If decreased norepinephrine efflux (equating with turnover) is coincident with increased levels of norepinephrine storage in the presynaptic terminals as reported by de Villiers et al. (1995), it would provide a much larger pool of norepinephrine for *d*- or *l*-amphetamine to release by reverse-transport, thereby resulting in increased norepinephrine efflux in response to these compounds.

Studies to compare the pharmacological profiles of the enantiomers of amphetamine or *threo*-methylphenidate using *in vivo* microdialysis are relatively uncommon being restricted to a single study on the former (Kuczenski et al., 1995) and two on the latter (Aoyama et al., 1996; Ding et al., 1997). Having taken the potency difference between amphetamine's *d*- and *l*-enantiomers into account in the dose selection for microdialysis experiments, i.e. 2 mg/kg *d*-amphetamine versus 6 mg/kg *l*-amphetamine, Kuczenski et al. (1995) observed in outbred rats that both isomers potentiated the efflux of striatal dopamine and 5-HT, together with hippocampal norepinephrine. At the doses employed, the effects of *d*- and *l*-amphetamine on the efflux of the catecholamine neurotransmitters were either very similar or superimposable and their findings are consistent with those we obtained in SD rats reported in Table 4. Despite having Ki values for the inhibition of [^{3}H]5-HT uptake *in vitro* in the low micromolar range (Table 2), Kuczenski et al. (1995) observed that both amphetamine enantiomers nonetheless evoked substantial increases in 5-HT efflux *in vivo*. This demonstrates the promiscuous profile of these competitive reuptake transporter substrate drugs, and the often underestimated effects they produce by this very low affinity release mechanism. Aoyama et al. (1996) and Ding et al. (1997) studied identical doses of *d*- and *l-threo*-methylphenidate, i.e. 2.5 mg/kg, on striatal dopamine efflux in outbred, Wistar and SD rats, respectively. These experiments revealed that, in comparison to the *l*-enantiomer, *d-threo*-methylphenidate is much more powerful in its ability to enhance striatal dopamine efflux. These findings are in agreement with data reporting their relative potencies as inhibitors of [^{3}H] dopamine uptake *in vitro* (Table 2) and have been confirmed and extended *in vivo* by Heal et al. (2006) (see Fig. 4 and Table 4). Although racemic *threo*-methylphenidate has been reported also to inhibit [^{3}H] 5-HT uptake *in vitro* in the low micromolar range (Table 2), unlike amphetamine's enantiomers, the monoaminergic actions of this drug are restricted to the potentiation of catecholamine efflux *in vivo* (Kuczenski and Segal, 1997). Berridge et al. (2006) recently explored the effects of low dose *dl-threo*-methylphenidate on the extraneuronal concentrations of catecholamines in the PFC and dopamine in the nucleus accumbens of freely-moving SD rats. Using both the intraperitoneal and oral routes of administration, these researchers showed that racemic *threo*-methylphenidate elevated both norepinephrine and dopamine in the PFC. At low doses (≤1 mg/kg, ip), the effect of the drug on dopamine efflux was more pronounced in the PFC than in the nucleus accumbens, which is consistent with it being able to improve vigilance and cognitive performance at doses lower than those causing behavioural activation (Berridge et al., 2006).

The requirement for intact neuronal firing is another key differentiator between the respective mechanisms of *threo*-methylphenidate and amphetamine. In this regard, it has been shown to be a prerequisite for the actions of the former (Butcher et al. 1991), but not for a high dose of the latter (Westerink et al., 1987). One caveat to this generalisation relates to *d*-amphetamine's mode of action to enhance norepinephrine efflux in the PFC where *in vivo* microdialysis experiments have demonstrated that this effect is at least partially firing-dependent (Géranton et al., 2003a). In the case of *d*-amphetamine-mediated dopamine efflux, our research into the literature revealed no information on the subject of whether its actions are also partially firing-dependent at low doses.

Atomoxetine is the other major catecholaminergic drug that is currently employed to treat ADHD. Although no *in vivo* microdialysis experiments with atomoxetine have been performed in the SH rat, data from experiments in outbred, SD rats have been reported (Bymaster et al., 2002; Swanson et al., 2006). In the PFC, this potent, selective, norepinephrine reuptake inhibitor (Bolden-Watson and Richelson, 1993; Bymaster et al., 2002) evoked a moderate and sustained (≥4 h) increase in norepinephrine efflux that was maximal at ~1 h (Bymaster et al., 2002; Swanson et al., 2006). The pharmacodynamic profile of atomoxetine is consistent with the criteria for a classical reuptake inhibitor as described by Gundlah et al. (1997). Thus, the effect on norepinephrine efflux was gradual in onset (Bymaster et al., 2002; Swanson et al., 2006) and sustained over many hours (Bymaster et al., 2002; Swanson et al., 2006). There was a ceiling to the maximum effect that was not overcome by increasing the dose or concentration of atomoxetine (Bymaster et al., 2002) and the drug's ability to potentiate norepinephrine efflux was clearly firing-dependent (Swanson et al., 2006). Atomoxetine produced similar increases in the extraneuronal concentrations of norepinephrine

Table 5
A comparison of the catecholaminergic profiles of various ADHD drugs determined by *in vivo* microdialysis in SH rats compared with their efficacy in the treatment of ADHD

Factor	Monoamine-releasing agent	Stimulant reuptake inhibitors			Classical reuptake inhibitor
	d-Amphetamine	*dl*-MPH	*d*-MPH	*l*-MPH	Atomoxetine
PFC norepinephrine efflux	+++	++	++	NS	+[a]
Dose ceiling effect	No	No	No	ND	Yes[a]
PFC dopamine efflux	ND	ND	ND	ND	++/+[a]
Dose ceiling effect	ND	ND	ND	ND	Yes[a]
Striatal dopamine	++++	++	++++	+	0[a]
Dose ceiling effect	No	No	No	ND	NA
Efficacy in ADHD	+++	+++	+++	0/ND	++

[a]Data only available in SD rats taken from Bymaster et al. (2002), Swanson et al. (2006).
Classification of effects on catecholamine efflux (% of baseline): +=0–300%; ++=301–600%; +++=601–1000%; ++++=>1000%.
Classification of efficacy in ADHD (response rates): +=≤50%; ++=51–60%; +++=~70%.
ND = Not determined; NA = not applicable.

in the occipital cortex, hypothalamus, hippocampus and cerebellum (Swanson et al., 2006). This drug and another selective norepinephrine reuptake inhibitor, reboxetine (Bymaster et al., 2002), also increased dopamine efflux in the PFC, although the former was without effect in other brain regions, i.e. striatum, nucleus accumbens, occipital cortex and hypothalamus (Bymaster et al., 2002; Swanson et al., 2006). These authors suggested this observation was due to these drugs preventing dopamine reuptake into norepinephrinergic nerve terminals in the PFC. Bymaster et al. (2002) also demonstrated that atomoxetine had a catecholamine-specific profile because it did not alter the extraneuronal concentration of 5-HT *in vivo*.

When viewing all of the results from the microdialysis studies performed in SH, WKY and outbred rats, some important conclusions can be drawn about the respective mechanisms of the ADHD drugs and the pharmacodynamics of their effects *in vivo*. In terms of catecholaminergic actions, *d*-amphetamine has the most pronounced dopaminergic profile of the drugs tested with very powerful effects on this monoamine in both SH and SD rats. On the other hand, the *l*-enantiomer of amphetamine has a more balanced effect on both catecholamines, which is particularly apparent from the experiments performed in the SH rat. Consistent with their monoamine-releasing mechanism of action, the effects of amphetamine's enantiomers on the catecholamines are fast in onset, of considerable magnitude, and in the SH rat, there is no response ceiling to the effects of these compounds when doses are increased.

dl-threo-Methylphenidate and its *d*-enantiomer are intriguing drugs because although their actions are firing-dependent like the classical reuptake inhibitors, they are much more potent and powerful as potentiators of catecholamine efflux than would be predicted from their Ki values as inhibitors of [^{3}H]norepinephrine and [^{3}H]dopamine uptake *in vitro* (Table 2). In the SH rat, there is clearly no ceiling to the increase in catecholamine efflux produced by administration of either *dl*- or *d-threo*-methylphenidate. Moreover, this phenomenon is not peculiar to the SH rat because Kuczenski and Segal (1997) observed dose-dependent increases in the magnitude of dopamine and norepinephrine efflux in outbred rats; albeit in the latter case, it was the AUC rather than the maximum efflux that was increased at higher doses. Furthermore, in comparison to *d*-amphetamine, although *dl-threo*-methylphenidate is less potent, it nonetheless produced increases in the efflux of these catecholamines which were not markedly different from those seen after administration of a reasonably high dose of *d*-amphetamine (Kuczenski and Segal, 1997). In contrast to amphetamine where both enantiomers are powerful catecholaminergic drugs with a difference in their relative effects on norepinephrine and dopamine, in racemic *threo*-methylphenidate, the *d*- and *l*-enantiomers have similar catecholaminergic profiles with the exception that the former is ~10-fold more potent *in vitro* (Table 2) and *in vivo* (Aoyama et al., 1996; Ding et al., 1997; Kulkarni et al., 2006).

Compared with amphetamine's enantiomers, racemic *threo*-methylphenidate and its *d*-isomer, the selective norepinephrine reuptake inhibitor, atomoxetine, evokes a moderate increase in prefrontocortical norepinephrine efflux which is response-limited. Moreover, atomoxetine's ability to potentiate dopaminergic function is restricted to a secondary prevention of its uptake into PFC norepinephrinergic neurones.

Of the above drugs, amphetamine's enantiomers are the only ones that directly potentiate 5-HT neurotransmission (Table 3; Kuczenski et al., 1995; Heal et al., 1998a).

10. Clinical implications

To explore whether the catecholamine effects of ADHD drugs defined by *in vivo* microdialysis predict their relative efficacy in the treatment of ADHD, we have chosen *d*-amphetamine, *dl-threo*-methylphenidate, its enantiomers and atomoxetine as examples. For these drugs, there is sufficient preclinical and clinical information to make such comparisons. The results presented in Table 5 indicate that the most efficacious drugs to treat ADHD have powerful effects to increase norepinephrinergic and dopaminergic neurotransmission. Moreover, the data also suggest that amphetamine and *threo*-methylphenidate increase the concentration of norepinephrine in the PFC to at least the same extent as the selective reuptake inhibitor, atomoxetine. Another important factor to emerge is that the effects of the stimulants to potentiate the efflux of norepinephrine and dopamine does not have a low ceiling (Kuczenski and Segal, 1997; Ding et al., 1997; Kulkarni et al., 2006; Heal et al., 2006), which enables them to evoke peak increases that are often in excess of 1000% of baseline (see Table 4). This contrasts sharply with the pharmacological actions of atomoxetine. In terms of defining the pharmacological criteria to achieve optimal efficacy for a catecholaminergic drug in the treatment of ADHD, we cannot deduce from these experiments whether it is the magnitude of effect that the drugs are able to produce, the spread of effect across norepinephrine and dopamine, or a combination of both. To answer that question would require a stimulant reuptake inhibitor drug like *threo*-methylphenidate, which had an action exclusively on either dopamine or norepinephrine, to be investigated in the clinic. A releasing agent, cf amphetamine, would not be useful in this regard, because to date, it has not been possible to

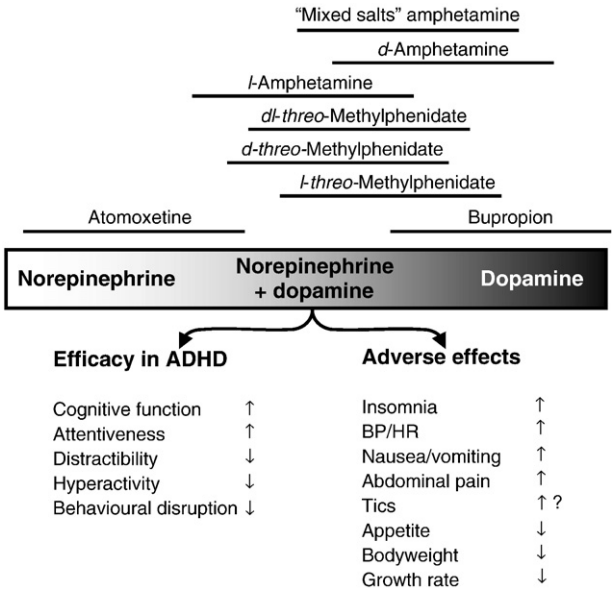

Fig. 5. Catecholaminergic profiles of the major drugs used in the treatment of ADHD.

restrict the actions of ß-phenylethylamine releasing agents to a single monoamine neurotransmitter. One conclusion that can be drawn from the pharmacological profile of atomoxetine is that simultaneously potentiating dopaminergic and norepinephrinergic neurotransmission in the PFC does not yield the same degree of clinical efficacy as drugs that also increase dopaminergic function in the limbic and striatal areas. Alternatively, viewing the question from the opposite perspective, it can be hypothesised that it is the ability of the stimulants to increase striatal and mesolimbic dopaminergic signalling together with catecholaminergic function in the PFC which provides optimum efficacy in ADHD therapy. This would be consistent with the involvement of multiple neurotransmitter systems in circuits linked to the PFC and striatum in the aetiology of ADHD as postulated by Arnsten and others (Durston, 2003; Arnsten and Dudley, 2005; Russell et al., 2005).

If the same exercise of attempting to translate pharmacology into clinical effect is performed using information from *in vitro* experiments (Tables 2 and 3), the results would have led to some misleading conclusions because the power of the stimulants is markedly underestimated by *in vitro* measurements of uptake inhibition and release.

11. Overall conclusions

In summary, *in vivo* microdialysis experiments have provided a very different perspective on the mode of action of catecholaminergic drugs used to treat ADHD (Fig. 5), and in addition, have suggested which pharmacological properties are likely to be required by such drugs to achieve optimum efficacy in the clinic. Moreover, in the concept of a norepinephrine-selective methylphenidate analogue, they have also suggested one direction for the development of novel, non-stimulant drugs for the treatment of ADHD that will possibly deliver efficacy equivalent to that of the stimulants. For many years, the SH rat has been used as a model of the behavioural and cognitive deficits of ADHD, to characterise the pharmacology of existing drugs for the treatment ADHD and to screen for novel clinical candidates in this therapeutic indication. On this front, the *in vivo* microdialysis studies, which have been performed initially by Carboni et al. (2003) and latterly in our laboratories, have revealed that the neurochemical abnormalities in this strain of rat are precisely the opposite of those that had been postulated on the basis of *in vitro* data. In fact, the observation that dopaminergic neurotransmission is hyperfunctional, whilst norepinephrinergic neurotransmission is probably hypofunctional, fits much more closely with the hyperactive phenotype of the SH rat. Finally, the experiments performed with the enantiomers of amphetamine, and latterly *threo*-methylphenidate, demonstrate that pharmacodynamic effects of drugs reported from experiments in WKY and outbred rat strains, e.g. SDs, do not necessarily translate to the SH rat. If one accepts that the SH rat does model some of the core behavioural and cognitive deficits present in ADHD, and for this reason is a useful model for the study of drugs for the treatment of this disorder, then the conclusion from this review is that further *in vivo* microdialysis work performed in the rat will help to develop our understanding of ADHD and of drugs used in its treatment.

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