Involvement of Norepinephrine in the Control of Activity and Attentive Processes in Animal Models of Attention Deficit Hyperactivity Disorder

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SUMMARY

Functional and morphological studies in children affected by Attention Deficit Hyperactivity Disorder (ADHD) suggest a prefrontal cortex (PFc) dysfunction. This cortical region is regulated by subcortical systems including noradrenergic (NEergic), dopaminergic (DAergic), cholinergic, serotonergic, and histaminergic pathways. A wealth of data in humans and in animal models demonstrates altered dopamine (DA) regulation. Drugs that modulate norepinephrine (NE) transmission are also effective in ADHD patients, thus leading to the hypothesis of a NEergic disorder. This review covers the regulation of PFc functions by NE and the interaction between the NE and DA systems, as suggested by pharmacological, electrophysiological, morphological, and gene knock out (KO) studies. A negative feedback between NE and DA neurons emerges from KO studies because KO mice showing increased (NE transporter (NET) KO) or decreased (DBH and VMAT2 KO) NE levels are respectively associated with lower and higher DA levels. Locomotor activity can be generally predicted by the DA level, whereas sensitivity to amphetamines is by NE/DA balance. Some animal models of ADHD, such as spontaneously hypertensive rats (SHR), show alterations in the PFc and in the DA system. Evidence about a correlation between the NE system and hyper-locomotion activity in such animals has not yet been clarified. Therefore, this review also includes recent evidence on the behavioral effects of two NET blockers, reboxetine and atomoxetine, in two animal models of ADHD: SHR and Naples High Excitability rats. As these drugs modulate the DA level in the PFc, certain effects are likely to be due to a rebalanced DA system. We discuss the significance of the results for theories of ADHD and make suggestions for future experimentation.

KEYWORDS

ADHD, norepinephrine system, dopamine system, norepinephrine transporter inhibitors, prefrontal cortex, motor activity, attention, behavior, review

INTRODUCTION

The grayish-blue colored small pigmented region of the fourth ventricle floor, the *locus coeruleus* (LC), includes a cluster of about 1600 neurons per nucleus in rats, several thousand in monkeys, and 10,000 to 15,000 in humans (Foote et al., 1983; for review see Berridge & Waterhouse,

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2003). These neurons contain norepinephrine (NE), as shown by Dahlstrom and Fuxe in 1964 (1964), and are the origin of widespread nerve terminals in the mammalian brain and spinal cord (Loughlin et al., 1982; Fallon & Loughlin, 1982). Norepinephrine neurons give rise to two major bundles, the dorsal and ventral one. The former (dorsal tegmental bundle) originates from LC neurons and terminates in many regions of the forebrain, cerebellum, and spinal cord (Cerbone & Sadile, 1994). The latter or central tegmental tract originates from diffuse NE cell groups in the pons and medulla and terminates in the hypothalamus and spinal cord (Mason, 1981).

Norepinephrine LC cells fire monotonically (2-4Hz in the tonic mode) in relation to stages of sleep and waking, with the highest rates occurring during wakefulness (Robbins, 1997). Moreover, LC cells respond to novel or noxious stimuli, conditioned stimuli, and appetitive events (Robbins, 1997; Bouret & Sara, 2002). They play, in fact, regulatory effects on attention and arousal, fear and anxiety, information storage and locomotor activity (for review see Mason, 1981; Cerbone & Sadile, 1994; Sadile, 1996; Robbins, 1997).

Several drugs interfere with the NE system. The psychostimulant amphetamine and the dopamine (DA) transporter blocker, methylphenidate, inhibit LC firing (Lacroix & Ferron, 1988). Interestingly, both drugs are largely used in the treatment of a disorder characterized by hyperactivity and inattention, known as attention deficit hyperactivity disorder (ADHD). As a consequence, the possibility that ADHD represents a NEergic disorder has been recently discussed (Biederman & Spencer, 1999).

Attention deficit hyperactivity disorder is a neurodevelopmental problem diagnosed in 1 to 3 percent of children (Castellanos& Tannock, 2002; Sergeant et al., 2003). The main features are "a persistent pattern of inattention and/or hyper-activity/impulsivity that is more frequently displayed and more severe than in individuals at a comparable level of development" (American Psychiatric Association (APA), 1994). The disorder can occur before the 7th year of age and in different environments. Different variants have been identified: hyperactive-impulsive, inattentive, and combined (APA, 2004).

The contributions of NE and DA neuro-transmissions to the motor and cognitive symptoms of ADHD have been investigated in rodent and primate models and in humans as well (Castellanos et al., 1996). Dopamine and NE interact at their nuclei of origin and at shared target sites, such as the prefrontal cortex (PFc) (Berridge & Waterhouse, 2003), wherein the control of DA and NE is very complex. Interestingly, the PFc has shown typical functional alterations in ADHD across many different studies (see below).

The functions of the PFc and its regulation by DA and NE are here reviewed in two animal models, spontaneously hypertensive (SHR) and Naples High Excitability rats (NHE; see also for review Viggiano et al., 2002; Viggiano et al., 2003b).

2. THE PFC IN ANIMAL MODELS OF ADHD

2.1 Functional anatomy and alterations

The existence of a PFc in rodents has been widely debated (Brown & Bowman, 2002; Uylings et al., 2003). In particular, the human PFc can be divided into different subregions, which share a common input represented by the mediodorsal thalamic nucleus (Uylings et al., 2003). In rats, however, the latter projects only to the medial and orbital frontal lobe, but not to dorsolateral regions.

The PFc has been involved in attention, decision making (Mulder et al., 2003), temporal organization of behavior (Fuster, 2000), working memory (Goldman-Rakic, 1996), and reversal learning (McAlonan & Brown, 2003).

Functional subdivision of the PFc in rats, mainly based on lesion studies, comprises three

main fields: medial (dorsal and ventral), orbitofrontal, and dorsolateral. Lesions of the rat medial PFc impair attention and attentional set shift (Brown & Bowman, 2002; Uylings et al., 2003), reduce anxiety, and increase novelty-induced locomotor activity (Deacon et al., 2003). Lesions of the dorsomedial (anterior cingulate) PFc impair discriminative accuracy, whereas the ventromedial (infralimbic) region sustains inhibitory control (Chudasama et al., 2003). Orbitofrontal lesions impair behavioral flexibility, leading to perseverative tendencies, thus decreasing performance in various learning and memory tasks (Chudasama et al., 2003; Vafaei & Rashidy-Pour, 2004). Lesions of the dorsolateral PFc lead to shifting strategy alterations, impairing behavioral flexibility in rats. Conversely, a corresponding lesion in humans impairs working memory.

The activity of the PFc is modulated by a set of subcortical systems comprising acetylcholine-, DA-, 5-hydroxytryptamine (5-HT)-, NE-, and histamine-containing neurons (Robbins, 1997; Uylings et al., 2003). Recently, a sixth system has been characterized, originating from the lateral hypothalamus, which releases glutamate and orexin (also called hypocretin) as neuromodulators (Torrealba et al., 2003). All derive from a relatively small number of neurons with a terminal arborization that allows the innervation of the entire forebrain and spinal cord. These subcortical systems partially share target regions and influence each other in a very complex manner. In particular, NE neurons from the LC project to almost the entire neocortex, and the NE terminals are denser in layers IV and V, although the laminar distribution is less evident in rodents (Foote et al., 1987). Th is widespread input to the cortex differs from the DA input, which is restricted at the cortical level to the medial PFc (Bjorklund & Lindvall, 1984). The only cortical areas that project back to the LC are the same medial PFc in rats and the dorso-lateral PFc in primates (Arnsten, 1997). Interestingly, the orexin system also interacts with both DA (Fadel & Deutch, 2002) and NE systems (Monda et al., 2004). Finally, the PFc receives input also and projects back to the reticular formation (Newman et al., 1989; Gritti et al., 1997).

Electrophysiological evidence shows that PFc neurons are activated during the delay period of a delayed-response trial (see Goldman-Rakic, 1996), thus suggesting its involvement in working memory functions. Recent data would also suggest a direct involvement in reward-directed learning. In fact, neurons in the PFc increase their firing rate at the presentation, whereas they reduce the firing rate from the conditioned-stimulus presentation up to the reward delivery, after the task has been learnt (Mulder et al., 2003). Thus, the PFc has access to reward stimuli. In fact, a loop has been anatomically and functionally described, involving the PFc, striatum, globus pallidum, and substantia nigra (the striatal output), the thalamus (ventromedial nucleus), and back to the PFc (Uylings et al., 2003). This circuit is thought to link motivationally relevant stimuli to motor areas (Kalivas et al., 1993). The electrophysiological data reported above rather suggest, however, that PFc neurons encode for prediction in reward delivery and transfer this information directly to the VTA, where the error in prediction is calculated (Williams et al., 2004).

Interestingly, the process of error prediction is possibly altered in ADHD, thus suggesting a direct involvement of PFc modulation in this disorder. More direct evidence of an involvement of the PFc in ADHD is the following:

- reduced volume of right PFc (Hynd et al., 1990; Castellanos et al., 1996; Filipek et al., 1997; Pueyo et al., 2000; Mostofsky et al., 2002),
- reduced activation of right prefrontal metabolism during response inhibition tasks (Rubia et al., 2000; Langleben et al., 2001), and
- reduced [F^{18}]-DOPA uptake ratios in the medial PFC.

Moreover, the analysis of the PFc in animal models of ADHD reveals similar alterations in the

PFc. In fact, rats selected over a mixed population for their impulsivity in an attentive task show a lower metabolic activation of the PFc (Barbelivien et al., 2001). In addition, the PFc of SHR compared with Wistar Kyoto (WKY) control rats shows (a) a reduced number of tyrosine hydroxylase (TH) fibers (King et al., 2000) and (b) increased NE activity in the PFc (Russell, 2000).

2.2 Modulation of PFc functions by NE and DA

The PFc receives projections from both the VTA and the LC and sends back projections to the VTA and to regions where dendrites of NE LC cells are present (Jodo et al., 1998). In fact, several data (see e.g. Jodo et al., 1998) show that the PFc is a major source of excitation of the LC.

Strikingly, DA does not modulate NE release in the PFc (Vanderschuren et al., 1999), whereas NE can modulate DA release (Bymaster et al., 2002). In fact, the excitation of LC neurons increases the release of DA in the PFc, but this effect does not occur through a direct excitation of DA neurons or terminals (Kawahara et al., 2001). A mechanism responsible in part for this effect is represented by the reuptake of DA by the NE transporter (NET) (Carboni et al., 1990). As a consequence, the NE terminals in the PFc would regulate the resting levels of DA through the NET. This offers an interpretation of the increase NE and DA levels in the PFc after treatment with drugs that block the NET, such as reboxetine and atomoxetine (Bymaster et al., 2002). In fact, the effects of psychostimulants such as D-amphetamine on DA outflow can be partially controlled by NE receptors (Darracq et al., 1998). The NE and DA systems, however, can also interact at the nuclei of origin and in other target sites.

3. Dopamine and norepinephrine interactions

Direct connections have been found between the VTA and the LC. In fact, DA neurons in the VTA express α-2c NE receptors (Lee et al., 1998), and there is a tonic release of NE in the VTA (Reith et al., 1997). Indeed, morphological evidence supports a direct projection from the LC to the VTA (Phillipson, 1979). In addition, direct projections from the VTA to the LC exist (Swanson, 1982) and are able to excite NE neurons (Deutch et al., 1986).

The nucleus accumbens is the target of DA neurons from the VTA and NE neurons from the nucleus tractus solitarius (NST) (Berridge & Waterhouse, 2003). Thus, local injection of adrenoceptor agonists modulates DA release (Yavich et al., 1997), and, conversely, DA modulates the release of NE (Vanderschuren et al., 1999). These interactions at the nuclei of origin and target sites and the DA reuptake by the NET make it difficult to disentangle the behavioral effects of NE and DA drugs. In particular, inferences about the pathogenesis of ADHD based on the efficacy of drugs acting on the NE system should be advanced with caution. To this aim, the behavioral effects of genetic manipulation of the NE system and the changes in the NE system evidenced in animal models of ADHD are discussed.

4. BIOCHEMICAL/BEHAVIORAL EFFECTS OF CHANGES IN THE NE SYSTEM

4.1 Embryogenesis

The use of knock out (KO) technology has been used to explain many functional aspects of NEergic neurons (for review see Carson & Roberson, 2002; see Table 1 for a summary). The LC neurons are strictly regulated during their development, although the regulatory cascade is not yet fully defined. Homeobox gene Phox2a-deficient mice show an absence of the LC and defects in sensory/ autonomic ganglia (Morin et al., 1997). Therefore, the homeodomain protein

TABLE 1

Knockout and transgenic mice for norepinephrine system

Gene (--=KO; ++=over-expression)	DA levels	NE levels	Sensitivity to amphetamines	Locomotor activity	References
Phox2a --				No development of NE system	(Morin, 1997)
Mash-1 --				No development of NE system	(Hirsch, 1998)
TH --	<	<		<	(Kobayashi, 1995; Zhou, 1995)
TH -- + DBH	<	-		<	(Zhou, 1995)
DBH --	>	<	>	-	(Thomas, 1997)
DBH ++		-			(Kobayashi, 1994)
VMAT2 --	>		>		(Takahashi, 1997)
NET --	<	>	>	<	(Xu, 2000)
Orct3 --	-	-		-	(Zwart, 2001)
COMT	>	-			(Gogos, 1998)
MAO-A	>	>			(Cases, 1998)
MAO-B	-	-			(Carson, 2002)
α 1b --	-	<	>	>	(Drouin, 2002)
α 1b ++		<		<	(Zuscik, 2000)
α 1d --				Impaired response to noxious stimuli	(Tanoue, 2002)
α 2a --		>		<	(Schramm, 2001; Lahdesmaki, 2002; Davies, 2003)
α 2c --	<		>	>	(Sallinen, 1999)
α 2c++				<	(Sallinen, 1999)

KO = knockout, DA = dopamine, NE = norepinephrine
-: no changes; >: increased; <: decreased

Phox2a controls NEergic traits during development, possibly through an interaction with Mash-1, a gene transiently expressed in the central nervous system. In fact, the targeted mutation of Mash-1 also causes the absence of central and peripheral NE neurons (Hirsch et al., 1998). Knockout (KO) mice have been useful for dissecting the cascade that controls the NE phenotype.

4.2 Synthesis and clearance

Norepinephrine is synthesized from L-dihydroxyphenylalanine (L-DOPA), which is first converted to DA by the enzyme tyrosine hydroxylase (TH) and then to NE, through the action of the enzyme DA-beta-hydroxylase. Therefore, perturbations in TH (see below, TH KO) will affect the synthesis of both NE and DA. In fact, TH KO mice lack both DA and NE (Kobayashi et al., 1995; Zhou, 1995a). The animals survive embryogenesis but die at 3 to 4 weeks of age from severe hypoactivity and hypophagia. The synthesis of NE, however, can be normalized by the transgenic expression of TH under the control of the DA-b-hydroxylase promoter, thus generating pure DA KO mice. Such mice survive, displaying normal NE synthesis, but make no DA (Zhou, 1995a) and are severely hypoactive. Unfortunately, no data are available about their sensitivity to amphetamines.

Interestingly, the induction of a mutation in the TH gene, which leads to a 40% reduction in TH activity, leads to a lowered learning performance and memory formation. In these animals, spatial learning and LTP are normal, and the performance in behavioral tasks can be restored by stimulating NE activity (Kobayashi et al., 2000). Therefore, low levels of NE reduce motivation and performance in learning tasks.

On the other hand, DA beta-hydroxylase (DBH) KO mice, which selectively lack NE (Thomas & Palmiter, 1997), do not show alterations in locomotor activity or learning, although they swim more slowly and show more rapid extinction. These animals show characteristic somatic problems such as ptosis, reduced body mass, hypotension, increased embryonic mortality, and deficient thermogenesis (Carson & Roberson, 2002). Interestingly, DA levels are elevated in most tissues (Thomas et al., 1998). The mice are hypersensitive to the behavioral effects of amphetamine (Weinshenker et al., 2002) and susceptible to seizure-inducing stimuli (Szot et al., 1999). Reboxetine and other antidepressants have no effect in DBH KO mice (Cryan et al., 2001), which confirms that all the indirect effects of these drugs necessitate the release of NE. Unfortunately, no data are available about the effectiveness of reboxetine or other NET blockers in DA KO mice, which could disentangle the NE-dependent effects from the DA-dependent effects.

Conversely, the hyperexpression of DBH in transgenic mice is not accompanied by changes in NE levels (Kobayashi et al., 1994). Therefore, it is possible that the hypolocomotor phenotype of TH KO mice is, rather, due to the changes in DA levels or to the lack of compensatory DA changes in DBH mice.

The vesicular monoamine transporter (VMAT2) stores monoamines, and particularly NE, from the neuronal cytoplasm into vesicles. VMAT2 KO mice die by the second week after birth and show increased mortality, possibly from arrhythmias (Takahashi et al., 1997). Heart rate, body temperature, NET, DA transporter (DAT), adrenergic, and DAergic receptors are normal, but DA levels are increased, as is the sensitivity to amphetamines.

Clearance for NE is accomplished by the NE reuptake system or NET, by monoamine oxidases (MAO-A and MAO-B), and by catechol-O-methyltransferase (COMT) enzymes. Although the NET is the predominant transporter in neuronal tissue, a second process termed uptake-2 is responsible for the removal of NE, accomplished by the protein extraneuronal monoamine transporter (EMT) or the organic cation transporter 3 (OCT-3). Finally,

COMT methylates NE, thus forming normetanephrine, whereas the intraneuronal degradation of NE is accomplished by MAO-A and MAO-B.

Mice carrying a functional deletion of the NET (Xu et al., 2000) are viable and fertile. Disruption of the NET gene prolongs the clearance of NE and elevates its extracellular levels. Intracellular levels of NE are decreased in NET KO mice, however, suggesting that NE storage levels in neurons is controlled by reuptake rather than by synthesis. These animals are hypothermic. Moreover, they are less active than controls in an open field. This is accompanied by lower resting levels of DA, and supersensitive postsynaptic D2/D3 DA receptors, thus increasing the behavioral effects of amphetamine.

Knock out mice for the Orct3 gene, which encodes for the EMT (extraneuronal monoamine transporter, see above), display normal general behavior and normal levels of DA and NE (Zwart et al., 2001). Mice lacking COMT show increased levels of DA but not of NE (Gogos et al., 1998). Monoamine oxidase-A KO mice show increased levels of NE, 5-HT, DA in the CNS (Cases et al., 1998), and increased aggression. Conversely, MAO-B KO mice do not show differences in NE, 5-HT, and DA levels (Grimsby et al., 1997). All this evidence is in agreement with the hypothesis that MAO-A is the only oxidase in NE neurons in the LC.

In summary, lesions that reduce the extracellular NE level (DBH KO, VMAT2 KO mice) induce the compensatory hyperDAergic system, normal locomotor activity but hyperresponsivity to amphetamines. On the contrary, lesions that increase the intracellular NE level (NET KO mice) also decrease the DA level, accompanied by low locomotor activity and hypersensitivity to amphetamines. Finally, lesions that decrease both DA and NE levels (TH KO mice) reduce locomotor activity.

Thus, the NE and DA systems are regulated in a negative feedback manner. The levels of DA correlate with locomotor activity, whereas the responsivity to amphetamines is the outcome of a balance between the two systems. In fact, selective and extensive depletion of NE in neonatal rats by 6-OHDA in presence of a DAT inhibitor leads to increased expression of DA, motor hyperactivity, and distractability (Raskin et al., 1983; Carli et al., 1983).

4.3 Receptors

The NE receptors have been classified in three classes: alpha$_1$ receptors, which are at postsynaptic sites, alpha$_2$ receptors, both pre- and postsynaptic, and three different beta receptors, which are mainly postsynaptic but can also act presynaptically to facilitate NE release (O'Donnell, 1993; Murugaiah & O'Donnell, 1995). A fourth beta receptors isoform has been hypothesized recently (Oostendorp et al., 2000), The fourth group, beta receptors, represents an important interface between the sympathetic nervous system and the cardiovascular system (the so called beta-adrenergic axis; see Naga Prasad et al., 2001). As far as beta receptor KO mice are concerned, they are not included in this review because most of their alterations pertain to the heart.

Three α_1 subtypes (α_{1a}, α_{1b}, α_{1d}) and four α_2-receptor subtypes ($\alpha_{2a\text{-}d}$) are described. These are also expressed on glial cells, and their expression is different according to the brain region, α_{1a} being expressed mainly in the ventral part of the brain, α_{1b} in the thalamus, amygdala and raphe nuclei and α_{1d} in the cortex and hippocampus (see also Tanoue et al., 2002). Here, synapses are present on both pyramidal and nonpyramidal neurons, on which the α_{2a} receptor is the predominant NE receptor isoform (Aoki et al., 1998). Mice lacking the α_{1ab}, α_{1b}, α_{1d}, α_{2a}, α_{2b}, α_{2c} adrenergic receptor subtypes and a mouse line containing a point mutation in the α_{2a} receptor have been developed. Moreover, lines overexpressing the α_{1a}, α_{1b}, α_{1d} receptors under the control of different promoters have been studied (Sallinen et al., 1999; Tanoue et

al., 2002; Drouin et al., 2002). Only one subset of these lines has been behaviorally tested. None of the α_1 subtypes is required for embryologic development, as these KO mice do not show gross abnormalities.

Mice KO for α_{1b} show increased reaction to novelty and novelty-induced hyperactivity (Spreng et al., 2001) although other reports failed to confirm this evidence (Drouin et al., 2002). Moreover, KO mice do not display changes in the DA system, but the levels of NE in the PFc are lower than those in wild-type mice. The locomotion-stimulating effects and the increase in DA levels induced by d-amphetamine-morphine are decreased in these animals (Drouin et al., 2002; Auclair et al., 2002). This reduction might depend on a lower efflux of DA after stimulation (Battaglia et al., 2003).

Conversely, mice overexpressing the α_{1b} receptor have an age-dependent loss in horizontal deambulation and a reduced number of rearings, which can improve after treatment with adrenergic blockers or with L-DOPA (Zuscik et al., 2000). Moreover, this phenotype is accompanied by seizures and neurodegeneration, and NE plasma levels are reduced.

Mice lacking the α_{1d} receptor show an impaired response to noxious stimuli (Tanoue et al., 2002). Mice lacking the α_{2a} receptor show normal locomotor activity (Schramm et al., 2001) but a reduced number of rearings in open field (Lahdesmaki et al., 2002).The resting levels of NE are normal, but the NE turnover is increased (Lahdesmaki et al., 2002), and the NE LC neurons are hypertrophic (Davies et al., 2003). Anxiety tests in these animals suggest that α_{2a} is stress protecting (Schramm et al., 2001).

Targeted inactivation of the α_{2c} receptor leads to normal or increased locomotor activity, and super-sensitivity to amphetamine (Sallinen et al., 1999). Moreover, these mice exhibit lower DA turnover. Conversely, transgenic mice overexpressing α_{2c} receptors show normal or reduced activity and increased stress susceptibility. Anxiety tests in these animals suggest that α_{2c} mediates stress susceptibility (Schramm et al., 2001).

Interestingly, methylphenidate, a drug that blocks the DA transporter and is widely used in ADHD pharmacotherapy, decreases the collected reward in an operant conditioning task in normal mice, but has opposite effect in α_{2c} receptor KO mice (Ihalainen et al., 2001). This is in agreement with the increase in spontaneous locomotor activity induced by amphetamine in these animals. It is plausible that the alteration in DA metabolism can explain the different effect of MPH (see also the companion paper by Viggiano et al., 2004 - this issue).

In summary, both α_{1b} and α_{2c} receptors control locomotor activity; KO mice for these receptors show increased activity, whereas transgenic over-expressing mice show decreased activity. Their influence on DA and NE levels is different. The α_{1b} KO mice do not show changes in DA level but have a lower NE level than the wild-type, whereas α_{2c} KO mice show a lower DA turnover. The sensitivity to amphetamines is again reflected by the balance between DA and NE levels.

5. NOREPINEPHRINE SYSTEM CHANGES IN BEHAVIORAL MODELS OF ADHD

Many data are available about the NE system in an animal model of ADHD, the SHR. These mice are hyperactive and impulsive in comparison with their WKY controls. The evidence from LC neurochemistry does not show an alteration in NE levels (Kaehler et al., 2000), although the turnover can be elevated. The firing rate of these neurons is normal (Conti et al., 1997) or lower (Olpe et al., 1985). The TH levels are increased in the medulla (Kumai et al., 1996, 2003) and around vessels (Gradin et al., 2003) but decreased in the PFc (King et al., 2000). Recent data also report lower amounts of TH in a bounded postnatal period (Leo et al., 2003). The NE levels are normal in the

solitary tract (Dev & Philip, 1996) but elevated in the median preoptic area (Tanaka et al., 2002), striatum and brainstem (Howes et al., 1984). The release of NE per varicosity might be increased (Jimenez-Altayo et al., 2003). The α_1 receptors in the cortex of SHR are increased (Hellstrand & Engel, 1980), a datum that contrasts with the hypoactivity of α_{1b} over-expression in mice. Some of these alterations might explain the elevated blood pressure in these animals, but do not correlate with the hyperactivity (Reja et al., 2002).

The data presented in Table 2 raise the question about why ADHD subjects respond to pharmacological agents that modify the NE system. In order to address this point, we will show data about the biochemical and behavioral effects of NET blockers and revise these effects from the view-point of an alteration in the attentional networks.

6. NET BLOCKADE IN HYPERACTIVE RATS

6.1 Norepinephrine and dopamine systems

Reboxetine and atomoxetine are potent and selective inhibitors of the NET (Wong et al., 2000). In contrast, the affinity of the psychostimulant methylphenidate is higher for the DAT than for the NET (Bymaster et al., 2002). Reboxetine and atomoxetine exert a similar action on the levels of NE and DA in the PFc, but differ as regards the effects on 5-HT levels. In fact, both reboxetine and atomoxetine enhance DA output in the PFc, but not in the accumbens (Kuczenski & Segal, 1995; Wong et al., 2000). Possibly this effect is dependent on a blockade of DA reuptake by the NET in the PFc, a mechanism absent in the striatum, where NE terminals are few (Carboni et al., 2003). It is possible, however, that indirect mechanisms intervene, as α_2-adrenergic antagonists also increase DA release in terminal fields (Shi et al., 2000; Linner et al., 2001).

The increased DA level is accompanied by an increased burst firing but not an average firing frequency of DA cells in the VTA (Linner et al., 2001). Similar changes might be responsible for altered reward perception, with a mechanism similar to methylphenidate (see also companion paper (Viggiano et al., 2004 - this issue).

Similarly, the blockade of NET by these drugs increases the levels of NE (Kuczenski & Segal, 1995). High doses lead to a reduction of the firing rate of LC NE cells and 5-HT cells in a dose-dependent relation (Wong et al., 2000), an effect similar to that of other stimulants (Aston-Jones & Bloom, 1981). Interestingly, α_2-adrenergic antagonists and methylphenidate (Kuczenski & Segal, 2001) also enhance NE release (Thomas, 1991). Reboxetine does not lead to increased extracellular levels of 5-HT (Kuczenski & Segal, 1995).

Similar studies with atomoxetine show that specific PFc modulators have a different sensibility to these drugs. In fact, NE is already increased at low doses (0.3 mg/kg), whereas DA is increased at medium doses (1 mg/kg) and 5-HT at high doses (3mg/kg) (Bymaster et al., 2002). The maximal release occurs 1 hour after injection. Interestingly, the effects on DA are evident only in the PFc, as no changes can be detected in the striatum.

6.2 Behavior

Nowadays, low-doses of AMPH-like stimulants and methylphenidate are the main pharmacological treatment of ADHD. These drugs ameliorate the symptoms of inattentiveness, hyperactivity, and impulsivity. Unfortunately, about 10 to 30 percent of ADHD children are either non-responders or intolerant to psychostimulant therapy (Barkley, 1977), which may reflect the heterogeneous neural substrates of ADHD.

Other therapies primarily affect the NE system, thus indirectly modulating the DA system. In fact, NE α_2-agonists like guanfacine (Hunt et al., 1995) are also effective in the treatment of the disorder.

TABLE 2

Changes of the norepinephrine system in SHR rats

LC	
NE content in LC	- (Kaehler, 2000)
Release of NE in LC	- (Kaehler, 2000)
NE turnover in LC	> (Kaehler, 2000)
Firing rate of LC neurons	- (Conti, 1997) < (Olpe, 1985)
NE in striatum, brainstem	> (Howes, 1984)
Release of 5-HT	> (Reja, 2002)
TH in medulla oblongata	> (Kumai, 1996)
TARGETS	
NE content of Nucleus of the solitary tract	- (Dev, 1996)
TH+ fibers in frontal cortex	< (King, 2000)
NE release in Median preoptic area	> (Tanaka, 2002)
Inhibition of NE Release by α-2 receptors	< (Russell, 2000)
NE release per varicosity	> (Jimenez-Altayo, 2003)
TH fibers around vessels	> (Gradin, 2003)
TH activity in adrenal medulla	> (Reja, 2002; Kumai, 2003)
Alfa-1 receptors in cortex	> (Hellstrand, 1980)
Correlation of TH gene expression of blood pressure	Positive (Reja, 2002)
-: no changes; >: increased; <: decreased	

TABLE 3

Behavioral effects of Reboxetine				
	SHR	WKY	NHE	NRB
Number of rearings	-	< (-47%)	< (-19%)	-
Horizontal activity	< (-25%)	< (-40%)	-	-
Duration of rearings	> (+26%)	> (+17%)	-	> (+22%)
Behavioral effects of Atomoxetine				
Number of rearings	not tested	not tested	< (-27%)	-
Horizontal activity	not tested	not tested	< (-17%)	-
Duration of rearings	not tested	not tested	-	-
Legend -: no changes; >: increased; <: decreased				

Finally, antidepressants like reboxetine and atomoxetine have also been used. In particular, atomoxetine (tomoxetine, LY139603) is effective in treating ADHD in adults (Spencer et al., 1998).

To study the differential effects of NET blockers in normal and hyperactive rats, we used two animal models of hyperactivity and attention deficit, previously characterized by a hyper-functioning mesocorticolimbic (SHR) or meso-cortical (NHE) DA system (Viggiano et al., 2000, 2003a,b). To this aim, male SHR and NHE rats, with WKY and Naples Random Bred (NRB) rats as their respective controls, received daily i.p. injections of the NE reuptake inhibitor reboxetine (Pharmacia Upjohn, Milano, Italy, 10mg/kg) or atomoxetine (Lilly, 1mg/kg) for 14 days. Rats were tested in a spatial novelty (Làt-maze) 90 min after the last injection of drug or vehicle. The behavioral test lasted for 30 minutes, during which it was videotaped and analyzed off-line for indices of activity (traveled distance, rearing frequency) and non-selective attention (scanning durations).

In SHR and control rats (WKY), reboxetine induced a significant reduction in traveled distance compared with vehicle-treated controls (–25% and –40% respectively). This reduction mainly pertained to the first 15 minutes of exploration. In contrast, reboxetine did not reduce the traveled distance in NHE rats nor in NRB controls.

Reboxetine reduced the orienting frequency only in WKY rats (–47% compared with vehicle), without effects on SHR rats. In contrast, the drug reduced the frequency of rearings in NHE (–19%) but not in NRB rats. Reboxetine increased the duration of rearing episodes in both SHR and WKY rats (+25% and +17% respectively). In contrast, the drug increased the duration of rearings in NRB (+22%) but not in NHE rats. Therefore, the net effects were different if we consider the number of rearings, the horizontal exploratory activity, or the duration of rearing episodes. The number of rearing was decreased, but only in NHE and WKY rats, whereas SHR and NRB rats did not show any change. In SHR and WKY rats, reboxetine reduced horizontal activity and prolonged scanning time. In contrast, in NHE and NRB rats reboxetine increased rearing durations in the control line, whereas it decreased the rearing frequency in NHE rats. Similarly, atomoxetine did not modify the horizontal and vertical activity of NRB rats, whereas it reduced the number of rearings (–27%) and the traveled distance (–17%), without effects on scanning durations in NHE rats (see Table 3).

The data suggest different activity of the NE system in the SHR versus NHE lines. In fact, SHR and NHE rats have different responses, reflecting different neural substrates. The differential sensitivity of these two lines to reboxetine could be due to a different involvement of the DA and NE systems in these animals. Although the effects of the NET blockade are similar in both the hyperactive SHR and the control rats, the main effects are evident in NHE rats, but not in their NRB controls.

The overall results would suggest that the NE system is similar in SHR and WKY rats, whereas differences exist between NHE and NRB rats. Alternatively, the interaction between the NE and DA systems is altered in NHE rats. These conclusions are in agreement with previous suggestions that an alteration of the NE system in adult SHR rats is indeed linked to the hypertensive phenotype but not to hyperactivity.

As both the NE and the DA systems are involved in arousal, attention, and cognitive functions, the drugs may act on two different pathways, thus changing the level of motivation (Wong et al., 2000). In fact, the LC-NE system is sensitive to novel environmental stimuli. An enhanced NE neurotransmission in a novel environment (see Làt maze) decreases attention to an individual object, increasing the scanning of the environment (Berridge & Waterhouse, 2003).

Therefore, the lower frequency of rearings and the increase of their duration in NHE rats may

indicate a lower firing rate of LC neurons, whereas the changes in SHR and WKY rats can be explained by the involvement of other systems, such as DA. The data lead to the suggestion that the positive effects of NEergic drugs may be due to an indirect action on the DA system. Nonetheless, further research is warranted by these findings to disentangle this intricate issue.

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