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Norepinephrine transporter inhibitors and their therapeutic potential

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Abstract

The norepinephrine transporter (NET) is located in the plasma membrane of noradrenergic neurons, where it functions to take up synaptically released norepinephrine (NE). The NET thus serves as the primary mechanism for the inactivation of noradrenergic signaling. Some potent and selective or mixed NET inhibitors (*e.g.*, reboxetine and atomoxetine) have been successfully developed to treat a variety of mental disorders such as depression and attention deficit hyperactivity disorder (ADHD). However, to date, only a very limited number of NET-selective inhibitors are available. New potent and selective NET inhibitors may find application in the treatment of mental disorders or in PET imaging, and may enhance our basic understanding of these illnesses. In the present review, both previously reported and newly designed NET inhibitors, as well as their therapeutic and imaging potential, will be discussed. Two types of molecules, the conformationally constrained tropanes and the piperidine-based cocaine/modafinil hybrid ligands, represent new leads and provide good opportunities for discovering novel potent and selective NET inhibitors that are useful as therapies and imaging agents for the NET.

Introduction

Mental disorders are common to all countries and cause immense human suffering, social exclusion, disability and poor quality of life. They also increase mortality and cause staggering economic and social costs. It is estimated that 1 in every 4 people has a mental disorder. Most often these are not diagnosed or treated because of gaps which exist in diagnostic technologies. The combined costs of mental illness, including lost productivity, lost earnings due to illness and social costs, are estimated to total at least USD 113 billion annually (1,2). It is anticipated that these problems will increase considerably in the years to come as a result of an aging population and other factors.

In the central nervous system (CNS), monoamines such as dopamine (DA, **1**), serotonin (5-HT, **2**) and norepinephrine (NE, **3**) (Fig. 1) have an important modulatory role in neurotransmission and are involved in numerous physiological functions and pathological conditions (3,4). Selective or mixed monoamine transporter (DAT, SERT or NET) inhibitors (*e.g.*, GBR-12909, mazindol, fluoxetine, paroxetine, reboxetine, amoxapine, desipramine, imipramine, bupropion and nisoxetine) have been developed to treat a variety of brain-related disorders, including depression, cocaine dependence, addiction and abuse, ADHD, anxiety disorders, mood disorders, personality disorders, psychosexual disorders, schizophrenia, eating disorders, premenstrual dysphoria, Parkinson's disease, Alzheimer's disease, bipolar disorder, chronic pain, migraine, epilepsy, multiple sclerosis, stroke, trauma, mania, obsessive-compulsive disorder, obesity and narcolepsy (5-17). However, important issues regarding the selectivity and mechanisms of action of these drugs remain unresolved. Research with novel ligands that vary both in their selectivity profiles and potency at each of the three monoamine transporter sites may be of value in unraveling the relevant pharmacological mechanisms, and thus aid in the discovery of new therapies with fewer side effects.

In contrast to the substantial body of research on DAT-selective ligands, fewer studies have focused on the structure-activity relationships (SAR) relevant to designing ligands having other patterns of transporter selectivity, particularly NET-selective ligands (6,10,18). The present review focuses on NET inhibitors and their use as imaging ligands, as well as their therapeutic potential in the treatment of mental illness.

Role of norepinephrine and the norepinephrine transporter

Norepinephrine, also known as noradrenaline, is a neurotransmitter found in the sympathetic nervous system and biosynthesized from the amino acid tyrosine, which is sequentially hydroxylated to generate dihydroxyphenylallanine (Dopa), decarboxylated to produce dopamine, and hydroxylated to form NE (19). As one of the crucial neurochemical messengers in the CNS, NE plays an important role in human physiology and pathology, and is involved in mood regulation, sleep regulation, expression of behavior and the general degree of alertness and arousal (20). Norepinephrine also exerts central control over the endocrine system and autonomic nervous system. Outside the CNS and adrenal glands, NE is located in the sympathetic nerve endings, and the NE content of a particular tissue reflects the extent of its sympathetic innervation (21).

The NET is located in the plasma membrane of noradrenergic neurons, where it functions to take up synaptically released NE. The NET thus serves as the primary mechanism for the inactivation of noradrenergic signaling (22-24). Reuptake of NE by the NET protein (also known as uptake 1) is the primary mechanism by which the biological effects of NE in the synapse are terminated (Fig. 2). The inactivation process through the NET is critical in preventing an excessive increase in the NE concentrations in the synaptic cleft, which regulate adrenergic neurotransmission in the brain, as well as the removal of NE from the heart and other peripheral organs (21,25). Reuptake of NE is competitive with a variety of naturally occurring amines and drugs. Drugs of abuse (*e.g.*, cocaine) and antidepressants (*e.g.*, desipramine, venlafaxine, reboxetine, bupropion) block the transport of NE and thereby cause an elevation in the synaptic concentrations of NE and potentiation of the activation of postsynaptic receptors (21,26,27). The above neurobiology of the NE system and other accumulating evidence support the view that NE plays an important role in the CNS and, although details of its mechanism of action remain unknown, NE is likely to play a role in various psychiatric disorders, including major depression and ADHD.

Previously reported norepinephrine transporter inhibitors

The chemical structures of a number of previously reported NET-selective or mixed NET inhibitors are shown in Figure 3. Among them, reboxetine (4), a racemic mixture of the (*R,R*)- and (*S,S*)-enantiomers, is the first potent, selective and specific NE reuptake inhibitor that has been marketed as an antidepressant (14). The K_i values of reboxetine are 1.1, 129 and $> 10,000$ nM for rat NET, SERT and DAT, respectively (28). Its (*S,S*)-enantiomer is even more potent and selective at the NET (29). The methyl analogue of reboxetine, 2-[(2-methoxyphenoxy)phenylmethyl]morpholine (methyl-norethylreboxetine, MeNER, 5), was reported to be 3 times more potent than reboxetine itself in inhibiting the reuptake of [^3H]-NE in rat synaptosomes (30). The potent and selective NET inhibitor (*R*)-nisoxetine (6) has K_i values of 0.46, 158 and 378 nM at rat NET, SERT and DAT, respectively (31). Its iodo analogue 2-I-(*R*)-NXT (7) has more potent NET activity, with a K_i value of 0.03 nM (32). (*R*)-Thionisoxetine (8), the sulfur analogue of nisoxetine, has potent NET activity similar to (*R*)-nisoxetine itself (33). (*R*)-Tomoxetine (atomoxetine, 9) differs structurally from (*R*)-nisoxetine (6) by a methyl group at the 2-position instead of a methoxy group. Its K_i values are 5,77 and 1451 nM at cloned human NET, SERT and DAT, respectively (34). Desipramine (10) has K_i values of 7.36, 163 and $> 10,000$ nM at rat NET, cloned human SERT and rat DAT, respectively

(31). Both nortriptyline (**11**) and protriptyline (**12**) have a similar tricyclic structure as desipramine. Nortriptyline is a more potent inhibitor of the NET ($K_i = 3.4$ nM) than the SERT ($K_i = 161$ nM) (35). Amoxapine (**13**) is a tricyclic dibenzoxazepine antidepressant that is chemically similar to the antipsychotic agent loxapine. In animal tests, amoxapine and its metabolites block reuptake of the neurotransmitter NE, with little effect on 5-HT (36). Maprotiline (**14**), which differs from other typical tricyclic antidepressants by its tetracyclic structure, is a highly selective inhibitor of NE reuptake in the brain and peripheral tissues, but has a notable lack of 5-HT uptake inhibition (37). Mazindol (**15**) is a dual NET/DAT inhibitor with K_i values of 3.2, 27.6 and 153 nM at NET, DAT and SERT, respectively (38). The marketed antidepressant bupropion (**16**), is chemically unrelated to tricyclic or tetracyclic selective serotonin reuptake inhibitors (SSRIs) or other known antidepressants. Preclinical and clinical data demonstrate that bupropion and its metabolites inhibit the reuptake of NE and DA in humans without affecting the release or transport of other neurotransmitters and without binding to other neurotransmitter receptors (39). Duloxetine (**17**) has the same type of chemical structure as nisoxetine and is a dual NET/SERT inhibitor with K_i values of 7.5, 0.8 and 240 nM at NET, SERT and DAT, respectively (40,41). As a selective NET/SERT inhibitor, venlafaxine (**18**) simultaneously affects both 5-HT and NE, which are implicated in depression and anxiety (42,43). Indatraline (**19**) is a potent but nonselective triple NET/SERT/DAT inhibitor with potential for the treatment of drug abuse, having K_i values of 5.8, 0.42 and 1.7 nM at NET, SERT and DAT, respectively (44).

Newly developed norepinephrine transporter-selective ligands

Tropane-based ligands as norepinephrine transporter inhibitors

As part of our research program aimed at the discovery of possible medications for cocaine abuse, depression and other psychiatric disorders, we have explored a new series of conformationally constrained tropane analogues in an effort to establish how best to “dial in” certain levels of transporter selectivity. Most of the potent tropane-based monoamine transporter inhibitors, including cocaine (**20**, Fig. 4), are believed to have at least three major interactions with the transporter binding sites: an ionic or H-bonding interaction of the basic nitrogen, a dipole-dipole or H-bonding interaction of the ester group of the ligand, and an interaction of the aryl group of the ligand with a lipophilic binding pocket (10,18). Based upon our preliminary SAR studies on conformationally constrained tricyclic tropanes (**21**), the lipophilic aromatic substituent at the 9-position appears to be a crucial determinant of selectivity (8,9,12,45,46). The different shape and size of the lipophilic recognition pocket that interacts with the aryl ring(s) of these tropanes are major determinants of a ligand’s transporter activity at either the SERT or the NET, and these studies have led to the discovery of analogues selective for the SERT, such as compounds **22** and **23** (12), as well as analogues selective for the NET, such as the biaryl compounds **24–30** (8) and the thio-phenes derivatives **31–34** (9, 12) (Fig. 4, Table I). Compound **23** has a K_i value of 0.06 nM at the SERT and K_i values of > 10,000 nM at both the DAT and the NET (12). The biphenyl analogue **24**, on the other hand, is a potent and selective NET inhibitor with a K_i value of 12 nM and about 50-fold selectivity over both the SERT and the DAT (45). Modifications on the substituents on the outer phenyl ring led to a significant improvement in potency and selectivity. Both ligands **25** and **29**, like (*R*)-nisoxetine, display remarkable nanomolar to subnanomolar potency at the NET and > 100-fold selectivity versus both the SERT and the DAT. Ligand **30** is a potent and dual NET/SERT inhibitor with a selectivity profile similar to duloxetine and venlafaxine. The thienyl tropane ligand **33** is a potent NET inhibitor somewhat like nortriptyline.

Piperidine-based ligands as norepinephrine transporter inhibitors

Previously, we reported on the synthesis and pharmacology of several 3,4-substituted piperidine-based cocaine analogues (47). After extensive *in vitro* studies, together with *in*

vivo assays of locomotor activity and self-administration and drug discrimination studies, the DAT/NET-selective ligand nocaïne (**35**) was chosen for advancement to human clinical studies for the treatment of cocaine addiction (48,49). Nocaïne has both lower potency and efficacy than cocaine in increasing locomotor activity in rodents and produces partial methamphetamine-like discriminative stimulus effects, although it is fully cocaine-like in cocaine-trained animals. Moreover, it shows reduced reinforcing effects in nonhuman primates. Nocaïne dose-dependently antagonizes cocaine-induced locomotor activation and potentiates the discriminative stimulus effects of a low dose of cocaine.

Modafinil (Provigil[®], **36**), a non-amphetamine-like wake-promoting agent, is approved for the treatment of narcolepsy. However, modafinil has been linked to side effects which include nausea, infection, nervousness, anxiety and/or insomnia. It shows low but selective affinity for the DAT in canine brain membranes (50). Modafinil's mode of action is complex and still uncertain, although studies suggest that it increases wakefulness by activating α_1 -adrenergic transmission (51) or hypothalamic cells that contain the peptide hypocretin (52), or that it may work by modulating GABAergic tone (53). Other research suggests that the presynaptic activation of DA transmission is a key pharmacological event in mediating the wake-promoting effects of currently available CNS stimulants and that it is critical for the pharmacological control of wakefulness, while activation of the NE system is critical for rapid eye movement (REM) sleep regulation (54,55).

Since valuable therapies have emerged from compounds exhibiting varying levels of transporter selectivity, we sought to examine the effect of creating hybrid molecules combining structural features of both nocaïne and modafinil (56). Specifically, we explored the effect of replacement of the hydrolyzable ester function of nocaïne with the same type of sulfur-containing side-chain as found in modafinil. This modification of one of the key pharmacophore elements of nocaïne was anticipated to further reduce its reinforcing properties, while possibly improving its half-life (Fig. 5).

From the resulting SAR data (Table I), we learned that replacement of the hydrolyzable ester function of nocaïne with the sulfur appendage found in modafinil leads to a substantial enhancement in the NET-inhibitory potency for many of the ligands relative to their activity at the DAT. In addition, some of the ligands display unique profiles of transporter selectivity and potency. Like mazindol, the alcohol **38** exhibits remarkable potency at the NET ($K_i = 0.94$ nM) and the DAT ($K_i = 16$ nM), as well as 170- and 10-fold selectivity *versus* the SERT, respectively. The inverse benzoyl ester **39** is a good SERT/NET inhibitor with potencies of 6.7 and 4.5 nM, respectively. Interestingly, the amide analogue **40** exhibits outstanding activity, with approximately 1 nM potency at all three monoamine transporters, similar to indatraline. Ligand **41** is another promising SERT/NET inhibitor with potencies of 4.5 and 0.68 nM, respectively. The sulfoxide amide analogue **42** is a dual NET/DAT ligand with low potency at SERT. Interestingly, the *N*-demethylated ligand **43** exhibits a high level of activity at the NET, with a K_i value of 0.56 nM.

In order to assess the bioavailability and initial cocaine-like behavioral activity of compound **38**, we compared two doses of cocaine and **38** in an open-field locomotor activity study. Ligand **38** was more potent than cocaine and appeared to be more efficacious as well (56). Further behavioral testing of several additional ligands is currently under way.

Therapeutic potential of norepinephrine transporter inhibitors

For more than four decades, NE has been postulated to play an important, possibly primary, role in the pathophysiology and subsequent treatment of mood disorders. To date, a number of potent and selective or mixed NET inhibitors have been marketed as antidepressants. In the past, the tricyclic antidepressants (TCAs) such as desipramine (Norpramin), nortriptyline

(Aventyl, Pamelor), protriptyline (Vivactil) and amoxapine (Asendin) were the first-choice medications for typical depression (57). The first generation of antidepressants, such as maprotiline, are not widely used in the U.S. due to the emergence of newer, more effective antidepressants. However, TCA use is fairly common in other countries. A new generation of antidepressants resulted from the discovery of SSRIs. Although SSRIs such as fluoxetine and paroxetine are very effective antidepressant drugs with substantially reduced severe side effects compared to the older tricyclic antidepressants, they are not universally effective and can also have bothersome side effects of their own, such as anxiety, sleep disturbances, weight gain, sexual dysfunction and gastrointestinal disturbances (58). Pharmacologically and chemically unrelated to TCAs or SSRIs, reboxetine (Edronax) is the first truly selective NET inhibitor and is currently being marketed as an antidepressant in over 50 countries, including Europe. The selectivity of reboxetine for the NET and its benign side effect profile result in the drug being well tolerated (14,59).

Some dual NET inhibitors display a unique clinical profile. As a dual NET/DAT ligand, bupropion (Wellbutrin) has demonstrated efficacy comparable to that of other antidepressants without the side effects of SSRIs (39). As a dual NET/SERT inhibitor, venlafaxine (Effexor) represents a new class of antidepressants and has a higher efficacy rate and the lowest dropout rate when compared to TCAs and SSRIs (60). Another dual NET/SERT inhibitor, duloxetine (Cymbalta), approved by the FDA in 2004, is a well-tolerated and even more effective antidepressant than venlafaxine (61).

The potent NET inhibitor atomoxetine (Strattera) is an effective and generally well-tolerated treatment for adults with ADHD (62). As a nonstimulant, it is the first ADHD treatment to be approved specifically for adult use based on its efficacy in well-controlled adult trials. Studies illustrate that atomoxetine carries a negligible risk of abuse or diversion and is a valuable new treatment option for adults with ADHD, particularly for patients at risk of substance abuse or those who do not wish to take a controlled substance (63).

Furthermore, certain mixed NET inhibitors have been used for the treatment of other neuropsychiatric and neurodegenerative disorders. As a dual NET/DAT inhibitor, mazindol (Sanorex, Mazanor) has been used as an appetite suppressant in the treatment of obesity (64). Bupropion is also marketed under the name Zyban by GlaxoSmithKline as a drug for smoking cessation and has been used very successfully to treat nicotine addiction directly (65).

Norepinephrine transporter-selective PET and SPECT imaging ligands

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging methods have been established as powerful tools to map the distribution of proteins targeted by selective radioligands in the healthy and diseased brain. While providing a valuable diagnostic tool in the short run, knowledge gathered from imaging the NET would eventually lead to a better understanding of disease processes involving this transporter, and hence to improved therapies or even disease prevention. [^{11}C]-Desipramine has been prepared but preliminary *in vivo* data obtained with PET imaging showed that the degree of specific binding in the monkey brain was too low to allow for visualization of the NET (66,67). [^{11}C]-Nisoxetine has been used for *in vivo* studies of the NET, but only displayed moderate specific binding in mice (68). An iodo derivative of tomoxetine has also been prepared as a potential imaging ligand, but demonstrated no specific binding *in vivo* in rat brain and a very high lung uptake (69,70). Recent research suggested that [^{11}C]-(*S,S*)-MeNER, an analogue of reboxetine, may be a useful imaging agent for the NET *in vivo* and a lead compound for further development (71–74). *In vitro* autoradiography of rat brain sections using [^{11}C]-(*S,S*)-MeNER showed the ligand's localization at the locus coeruleus, pendunculopontine nucleus, bed nucleus of the stria terminal and hypothalamus, regions with a high density of NET. Imaging studies in

baboons and monkeys suggested that [^{11}C]-(*S,S*)-MeNER localized in the lower brain stem and thalamus. The midbrain to striatum ratio in nonhuman primates is 1.4-1.6 (72,73). More recently, a radioiodinated ligand, (*R*)-*N*-methyl-(2-[^{125}I]-iodophenoxy)-3-phenylpropylamine ([^{125}I]-2-INXT, [^{125}I]-MIPP), was prepared and the data suggest that it may be useful for mapping NET binding sites in the brain (75,76).

Conclusions

The discovery of new potent and selective NET inhibitors is important as it is expected to advance the diagnosis and treatment of mental disorders and enhance our basic understanding of these illnesses. However, to date, only a very limited number of NET-selective inhibitors are available. As described in the present review, two types of molecules, the conformationally constrained tropanes and the piperidine-based cocaine/modafinil hybrid ligands, represent new leads and offer opportunities for finding novel potent and selective NET inhibitors that are useful as imaging agents and therapies. Extensions of the present work through SAR, *in vivo* studies and radiolabeling for PET/SPECT are anticipated to be of great value in the development of diagnostic tools that may yield insights into the role of the NET in disease processes, and eventually lead us to the development of therapies for those diseases.

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Fig. 1.
Chemical structures of the monoamines DA, 5-HT and NE.

Fig. 2.

Diagram of a noradrenergic axonal terminal showing the release and reuptake of norepinephrine (NE). **a.** NE is synthesized from tyrosine via hydroxylation to form dihydroxyphenylalanine (Dopa), decarboxylation to form dopamine, and hydroxylation to form NE, and **b** stored in vesicles. **c.** As a result of an appropriate stimulus (not shown), NE is released into the synaptic cleft. **d.** Released NE activates the adrenergic receptors located on the postsynaptic membrane (α_1 , β_1 and β_2) and also the **e** presynaptic membrane (α_2 and β_2) and causes **f** postsynaptic reactions such as protein kinase activation and protein phosphorylation. **g.** The NET is responsible for reuptake of NE in the synaptic cleft and terminates its action. **h.** After reuptake by the NET, a small portion of the NE is restored in vesicles (following uptake by the vesicular amine transporter 2, VMAT₂); **i** the rest is metabolized in the mitochondria by the enzyme monoamine oxidase (MAO), and **j** the end product dihydroxyphenylglycol (DHPG) is released into the circulation. **k.** A small portion of the synaptic NE leaks into the circulation, or **l** is taken up by another system (uptake 2) and **m** metabolized to form normetanephrine (NMN). Because 70–90% of the synaptic NE is taken up by the NET, blockade of NET is likely to produce a shift towards the NMN pathway and away from the DHPG pathway. A high ratio of plasma NMN to DHPG might prove useful in measuring this blockade.

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Fig. 3.
Chemical structures of selective or mixed NET inhibitors.

Fig. 4.
Tropane-based ligands as NET inhibitors.

Fig. 5.
Piperidine-based ligands as NET inhibitors.

Table 1

Inhibition of monoamine transporters ($K_i \pm \text{SEM}$ (nM))^a

Compound	[³ H]-DA uptake	[³ H]-5-HT uptake	[³ H]-NE uptake
Cocaine	423 ± 147	155 ± 0.4	108 ± 3.5
Nocaine	233 ± 62	8490 ± 1430	252 ± 43
Modafinil ^b	3800	-	> 10,000
24	477 ± 81	614 ± 105	12 ± 1
25	787 ± 234	1336 ± 7	6.5 ± 2.1
26	236 ± 76	239 ± 37	9.7 ± 2.7
27	646 ± 29	138 ± 23	5 ± 2
28	556 ± 19	1166 ± 425	8.8 ± 0.5
29	106 ± 6	295 ± 36	0.82 ± 0.2
30	228 ± 28	1.2 ± 0.7	1.3 ± 0.3
31	130 ± 27	53 ± 2	9.7 ± 2.8
32	368 ± 2	29 ± 1.6	5.0 ± 1.3
33	403 ± 20	179 ± 38	4.9 ± 0.2
34	371 ± 11	531 ± 51	10 ± 0.5
38	16 ± 5	158 ± 5	0.94 ± 0.27
39	68 ± 22	6.7 ± 1.5	4.5 ± 1.2
40	1.0 ± 0.2	1.1 ± 0.4	0.8 ± 0.1
41	83 ± 1	4.5 ± 0.8	0.68 ± 0.25
42	55 ± 8	1,795 ± 67	12 ± 1
43	51 ± 16	13 ± 3	0.56 ± 0.09

^aData are mean ± standard error of at least three experiments, as described in Ref. 8.

^bData taken from Ref. 54.