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Central Nervous System Biogenic Amine Targets for Control of Appetite and Energy Expenditure

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Central biogenic amine systems have long been studied for their effects on feeding behavior, energy balance, and maintenance of body weight. Those monoaminergic systems that use dopamine (DA), norepinephrine (NE), and serotonin (5-hydroxytryptamine, 5-HT) as neurotransmitters have been the main targets of study. A number of antiobesity medications that affect monoaminergic activity have appeared on the market and/or in clinical trials. Early examples of such agents are the so-called CNS stimulants, e.g., the amphetamines, phentermine, ephedrine, etc. These agents release monoamines from neuronal stores, and their antiobesity activity seems to be tied most closely to their ability to release NE. Inhibitors of neuronal reuptake of NE or 5-HT have been shown to reduce feeding and weight gain both preclinically and clinically. However, the magnitude and sustainability of such effects in clinical trials has generally not been great enough to register or label these agents for the treatment of obesity. Sibutramine, however, is an exception. This compound is metabolized in vivo to produce metabolites that have varying degrees of inhibition of NE, 5-HT, and/or DA uptake. Sibutramine is the only drug affecting monoaminergic systems currently approved for the long-term control of obesity. Research continues on serotonergic and histaminergic systems to determine if targets such as the 5-HT_{2C} and H_3 receptors may be suitable for developing antiobesity agents. Because the clinical antiobesity effects of monoaminergic drugs have been modest, future directions include looking at combinations of different monoaminergic mechanisms and/or combinations of monoaminergic drugs with non-monoaminergic mechanisms.

Key Words: Obesity; monoamines; biogenic amines; serotonin; norepinephrine; dopamine.

Introduction

Central biogenic amine systems have a long history of study as targets for regulating feeding behavior, energy balance, and maintenance of body weight. A number of compounds from this broad class have been in clinical trials, and some have seen extensive use in humans as marketed therapeutic agents. The term biogenic amine is typically applied to those monoaminergic neuronal systems that use dopamine (DA), norepinephrine (NE), serotonin (5-hydroxytryptamine, 5-HT), or histamine as neurotransmitters, and these will be the focus of this review.

The so-called classical monoamine neurotransmitters mentioned above may not be the only monoamines that can affect feeding and energy expenditure. For example, epinephrine is produced by the adrenal gland and can be released by increased activity of the sympathetic nervous system to affect peripheral energy expenditure. There is evidence for a minor CNS localization of epinephrine-containing neurons, but little is known about the possible functional significance of this *(1)*. Likewise, trace amines have been postulated to have roles in CNS function similar to the classical monoamine transmitters. However, even with the recent cloning of the trace amine receptors, little is known about the possible physiologic roles of these molecules *(2)*. Because of the paucity of information regarding these systems as antiobesity targets, they will not be addressed in the present discussion.

Over the past two decades, a vast number of monoaminergic receptors and transporters have been cloned, expressed, and pharmacologically characterized (Table 1). In many cases, genes encoding these proteins have been deleted in mice to reveal their physiological importance, especially in the areas of feeding and metabolism. Some of these findings have been very instructive. For example, using gene-deletion techniques, the $5HT_{2C}$ receptor was found to play an important role in body weight maintenance *(3)*. In subsequent studies, the receptor was found to be the target for norfenfluramine, the major metabolite of the antiobesity compound fenfluramine (see below). These results provided the probable mechanism of action for this compound and this target became the major focus of a number of pharmaceutical companies. Despite this success, there are obviously roles for many of these transporters and receptors that are currently unknown. For instance, little is known about

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Table 1
Potential Human Monoamine Drug Targets for the Treatment of Obesity: Major Receptor Subtypes, Transporters and Enzymes

Dopamine receptors	Serotonin receptors	Norepinephrine/ epinephrine receptors	Histamine receptors	Trace amine receptors	Transporters	Metabolizing enzymes
D_1	5-HT_{1A}	α_{1A}	H_1	TAAR1	DAT	MAO-A
D_2	5-HT_{1B}	α_{1B}	H_2	TAAR2	NET	MAO-B
D_3	5-HT_{1D}	α_{1D}	H_3	TAAR5	SERT	COMT
D_4	5-ht_{1E}	α_{2A}	H_4	TAAR6	VMAT1	
D_5	5-ht_{1F}	α_{2B}		TAAR8	VMAT2	
	5-HT_{2A}	α_{2C}		TAAR9		
	5-HT_{2B}	β_1				
	5-HT_{2C}	β_2				
	5-HT_3	β_3				
	5-HT_4					
	5-ht_{5A}					
	5-ht_{5B}					
	5-ht_6					
	5-HT_7					

The serotonin receptors with the lower case designation have been cloned but their physiologic functions have not been established. In addition, multiple other variants of these receptors have been proposed based on alternative splicing, pseudogenes, species differences, and repeats. These receptor variants can exhibit differing pharmacology, neuroanatomical and synaptic localizations and/or functional coupling. Table summarizes data found in the following reviews *(2,4–9)*. Abbreviations: TAAR, trace amine receptor; DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter; VMAT, vesicular monoamine transporter; MAO, monoamine oxidase types A and B; COMT, catechol-*O*-methyltransferase.

the trace amine receptors including the endogenous ligand(s) for many of the members. Even within well-researched targets such as the transporters, there is a lively debate on the relative contributions each of these sites make to the efficacy of anorectic agents. What is obvious is that there are substantial drug discovery opportunities within this large family of proteins.

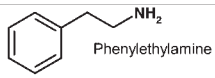
Central Nervous System Stimulants

The use of antiobesity agents targeting central biogenic amine systems in humans began with the introduction of the amphetamines, compounds that are noted for their CNS stimulant properties. Compounds in this class are often also referred to as sympathomimetics, i.e., their effects mimic certain effects of stimulating the sympathetic nervous system, e.g., increased heart rate, increased blood pressure, etc. As early as 1938, the weight-reducing effects of benzadrine (amphetamine sulfate) were being reported in the literature (for a review see ref. *10*), and over the years a number of amphetamine or amphetamine-like agents have been approved for use in the treatment of obesity. Table 2 shows a list of the agents that are currently approved for therapeutic use in the United States. Phenylethylamine is shown for comparison as all of these agents contain this substructure within their overall structure.

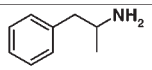
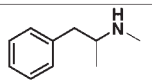
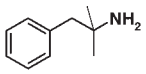
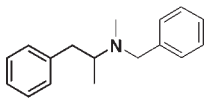
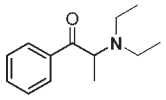
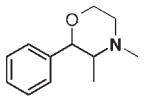
The use of the amphetamines in obesity has been limited by the CNS stimulant properties and the peripheral cardiovascular effects (e.g., tachycardia and increased blood pressure). In addition, tolerance to the anorectic effects of the amphetamines can occur. Of greatest concern, however, is the abuse potential of these compounds, and both amphetamine and methamphetamine have a high propensity for abuse. This has limited their therapeutic use and has led to periods of large-scale abuse in society. Subsequent to the introduction of the amphetamines, newer agents were developed that retained the weight-reduction properties of amphetamine with reduced CNS stimulation and abuse liability. Table 2 illustrates, based on their controlled substance classification, that the newer agents do have less of an abuse liability.

The stimulants listed in Table 2 appear to produce their anorectic (and other) effects through actions on the NE, DA, and/or 5-HT neurotransmitter systems. Over the years there have been debates about which aspects of their overall pharmacology are most likely the basis for their in vivo effects. In some cases the parent compound is responsible for the effects; in other cases, it appears to be a metabolite or metabolites that are the active component. In either case, it appears, in general, that the active compound is transported into neurons primarily by one or more of the monoamine transporters. The vesicular monoamine transporter 2 (VMAT2, Table 1)

Table 2
CNS Stimulant Drugs Approved for Obesity Treatment in the US



Phenylethylamine

Generic Name	Structure	Controlled Substance Class	Year Approved in U.S.
Amphetamine		2	1939*
Methamphetamine		2	1943*
Phentermine		4	1959
Benzphetamine		3	1960
Diethylpropion		4	1959
Phendimetrazine		3	1959

*These compounds were originally approved for indications other than obesity.

as well as the monoamine transporters appear to be intimately involved in the process of increased synaptic release of monoamines *(11)*. Because the stimulants are actively transported by the monoamine transporters, these agents act as competitive inhibitors of the uptake of the native neurotransmitters. Therefore, the stimulants increase synaptic monoamines by a combination of inhibiting both VMAT2 and reuptake inhibition. Once inside the neuron, the active compound causes release of the stored neurotransmitter, which then interacts with extracellular receptors to initiate the biologic response.

Over the last few years, a systematic examination of these older compounds has revealed much about their differences in interacting with the monoaminergic neurons, which may, in turn, explain their biologic differences. Table 3 summarizes the effects of these agents on the uptake and release of the monoamines as measured in vitro using synaptosomal preparations.

Amphetamine and methamphetamine appear to have the broadest effect across the three major monoamine systems. The compounds are generally most potent on uptake and release at the norepinephrine transporter (NET) and at norepinephrine (NE) release, followed closely by effects on dopamine (DA) (Table 3). While it is well established that amphetamines can affect serotonin (5-HT) release, this effect seems to occur at much higher concentrations than required to affect NE uptake and release. Compared to the other agents listed in Table 3, amphetamine and methamphetamine have a much more potent effect on DA uptake and release. This may provide an explanation for the much greater abuse liability of these two compounds compared to the other stimulants. The central DA neurons are well known to play a key role in the brain's reward pathway, and likewise, it is clear that increased DA signaling in selected brain regions is involved in the abuse potential of many drugs, such as cocaine and amphetamine (for a review see ref. *15*). Owing to concerns about abuse liability and tolerance to the anorectic activity, amphetamine and methamphetamine are approved only as short-term adjuncts in a regimen of weight reduction based on caloric restriction for patients refractory to alternative therapy (e.g., repeated diets, group programs, other drugs).

Phentermine is a close congener of amphetamine, differing only by having an additional methyl group on the carbon adjacent to the amino function. This small change, however, significantly alters potency and selectivity at monoamine uptake and release. Proportionally, this decrease in potency is greatest for DA uptake and release. Phentermine has been reported to produce weight loss similar in magnitude to amphetamine [for a review see Samuel and Burland *(10)*], but it appears to have significantly less propensity for abuse than amphetamine. Given the differences in potency for

Table 3
Comparison of Monoamine Uptake Inhibition and Release by CNS Stimulant Compounds

	NET		DAT		SERT	
	Uptake IC_{50}, n*M*	Release EC_{50}, n*M*	Uptake IC_{50}, n*M*	Release EC_{50}, n*M*	Uptake IC_{50}, n*M*	Release EC_{50}, n*M*
(+) Amphetamine	38.9 ± 1.8	7.07 ± 0.95	34 ± 6	24.8 ± 3.5	3830 ± 170	1765 ± 94
(+) Methamphetamine	48.0 ± 5.1	12.3 ± 0.7	114 ± 11	24.5 ± 2.1	2137 ± 98	736 ± 45
Phentermine	244 ± 15	39.4 ± 6.6	1580 ± 80	262 ± 21	13,900 ± 510	3511 ± 253
Diethylpropion	1810 ± 1500	>10,000	14,990 ± 540	>10,000	31,000 ± 28,000	>10,000
N-dealkylated diethylpropion	360 ± 29	99.3 ± 6.6	1014 ± 80	>1000	3840 ± 240	2118 ± 98
Phendimetrazine	8300 ± 445	>10,000	19,000 ± 537	>10,000	>100,000	>100,000
(+)-Phenmetrazine*	240 ± 24	37.5 ± 4.3	359 ± 23	87.4 ± 7.8	>10,000	3246 ± 263
(−)-Phenmetrazine*	388 ± 54	62.9 ± 9.5	1669 ± 189	415 ± 45	>10,000	>10,000

Values are given as the mean ± S.D. from the listed references. *The parent compound phendimetrazine is metabolized to phenmetrazine. Potencies were available for the enantiomers of phenmetrazine but not for the enantiomers of the parent compound. Data are taken from refs. *12–14*.

monoamine uptake and release, it is tempting to suggest that the lowered likelihood for abuse compared to amphetamine might be due to the decreased potency for DA release relative to NE release, while the maintenance of the antiobesity effect could be due to the relatively higher potency for releasing NE.

Phentermine is currently approved as a short-term (a few weeks) adjunct in a regimen of weight reduction based on exercise, behavioral modification, and caloric restriction in the management of exogenous obesity for patients with an initial body mass index ≥30 kg/m^2, or ≥27 kg/m^2 in the presence of other risk factors (e.g., hypertension, diabetes, hyperlipidemia). While clinical data on the potential long-term effectiveness of phentermine alone are sparse, studies of 6–9 mo duration have shown retention of phentermine's antiobesity effects (for reviews see refs. *10* and *16*). Generally, phentermine appears to be relatively well tolerated. It can produce side effects consistent with its catecholamine-releasing properties, e.g., tachycardia, increased heart rate, increased alertness, but the incidence and magnitude of these appear to be less than with the amphetamines.

Benzphetamine represents yet another modification of the amphetamine backbone, and, as with the other compounds discussed in this section, it is classified as a sympathomimetic and CNS stimulant. A review of the literature shows a surprising lack of experimental data on the possible mechanism of action of benzphetamine. With the bulky benzyl group attached to the amino function of the molecule (see Table 2), one might wonder whether the parent molecule could actually affect monoaminergic systems directly or whether a metabolite might be the active component. At least one study has identified amphetamine and methamphetamine as metabolites of benzphetamine in humans *(17)*. The side effect profile of benzphetamine has been described as similar to that of phenmetrazine *(10)*, and the product literature indicates general side effects and precautions to use that are similar to those for amphetamine (see product insert, http://www.pfizer.com/download/uspi_didrex.pdf). The clinical use of benzphetamine appears limited.

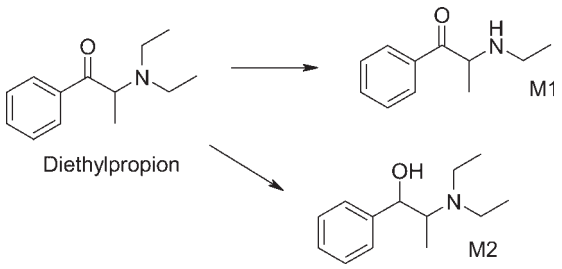


Fig. 1. Major metabolites of diethylpropion.

Diethylpropion is another amphetamine analog for which there has been a debate about the mechanism of action. In humans, diethylpropion is rapidly and extensively metabolized, producing two major metabolites (Fig. 1).

Yu et al. *(14)* evaluated diethylpropion in vitro for effects on monoamine uptake and release of DA, finding that it produced no significant effect. This prompted them to conclude that it might be acting as a prodrug. In contrast, the N-dealkylated metabolite (M1 in Fig. 1) was relatively potent at causing the release of NE, being about 10 times more potent at releasing NE than DA (Table 3). Like the parent molecule, the reduced metabolite (M2) also had essentially no effect on either the uptake or release of the monoamine neurotransmitters *(14)*. The relatively greater effect on NE systems than DA may explain the inability of diethylpropion to substitute for cocaine in clinical trials and its apparent lower abuse liability than the amphetamines *(18)*. However, its actions as a NE releaser are consistent with its side effect profile, including CNS stimulation and mild changes in heart rate and blood pressure.

Phendimetrazine, a sympathomimetic/CNS stimulant type of anorexiant, represents a structural departure compared to the other marketed compounds in this class, i.e., the amino

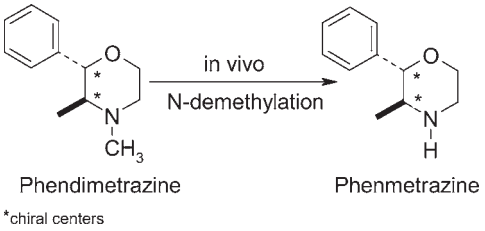


Fig. 2. Metabolism of phendimetrazine to phenmetrazine.

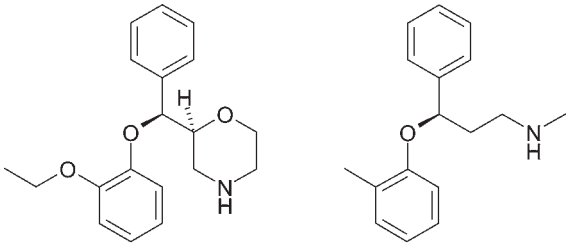


Fig. 3. Examples of selective NE reuptake inhibitors.

group is incorporated into a morpholino ring (see Table 2). After oral administration to humans, phendimetrazine is extensively demethylated to form phenmetrazine (Fig. 2) *(19)*. Differences in the in vivo effects of phendimetrazine and phenmetrazine that depend on the route of administration suggested the possibility that phendimetrazine might actually be a prodrug and that one of its metabolites is the active compound (for a review see ref. *13*). This led Rothman et al. *(13)* to examine phendimetrazine and its demethylated metabolites for effects on the uptake and release of DA, NE, and 5-HT. Phendimetrazine itself produced no effect on either uptake or release of these monoamines. Phendimetrazine is a racemic mixture of the *trans* configuration, producing both *trans* isomers of phenmetrazine when metabolized in vivo. Unlike the parent compound, both of these isomers affected NE and DA release *(13)* (Table 3). The (+) isomer appeared to be the more potent isomer. It appears likely, therefore, that the biologic effects of phendimetrazine are due primarily to the effects of the metabolite phenmetrazine on central NE and DA release.

Like the other compounds discussed in this section, phendimetrazine can produce CNS stimulation, increased blood pressure, and tolerance develops to the anorectic effects.

Summary: The Central Nervous System Stimulants

A unifying feature of these molecules appears to be their ability to release NE. The anorectic activity seen with these compounds would thus seem likely due to this effect on NE, which is consistent with current knowledge about central NE systems and feeding behavior (for a review see ref. *20*). Release of NE seems likely also to be associated with the major side effects of these compounds, including the CNS stimulation and increases in blood pressure. The development of tolerance to the anorectic effect of these drugs also suggests that monotherapy targeting NE systems in a non-selective fashion may not produce sustained weight loss.

Other Approaches to Altering Norepinephrine Systems

Selective NE Reuptake Inhibitors (NRI)

If NE releasing agents have anorectic effects, then do other mechanisms that increase synaptic levels of NE also have effects on feeding? One mechanism known to selectively increase synaptic levels of NE is through the use of so-called selective NE reuptake inhibitors (NRI) (Fig. 3). However, the literature around the effects of NRIs on feeding behavior is sparse. Jackson et al. *(21)*, looking at the effect of compounds on cumulative food intake by freely feeding rats during an 8-h dark period, concluded that the NRI nisoxetine, at doses up to 30 mg/kg, had no significant effect on food intake. Conversely, Gehlert et al. *(22)*, using the NRI LY368975 [(R)-thionisoxetine], found a significant reduction in food intake by food-deprived rats and a reduction in consumption of sweetened condensed milk by freely fed rats. Currently, two NRIs are used for the treatment of humans. These are reboxetine, available in Europe for the treatment of depression, and atomoxetine, available in both Europe and the United States for the treatment of ADHD. There is minimal information regarding the effects of these compounds on body weight in humans. In clinical trials of atomoxetine in normal weight adults lasting up to 10 wk, decreased weight was reported in 2% of atomoxetine-treated compared to 1% of placebo-treated individuals. Ten percent of individuals receiving atomoxetine reported decreased appetite, while 3% of placebo-treated individuals reported decreased appetite (http://www.strattera.com/1_6_hcp/1_6_1_prescribing.jsp, prescribing information). Thus, atomoxetine appears to produce weight decrease in a few normal weight individuals. However, evaluation of NRIs in obese patients will be required to assess their true potential for treating human obesity.

Because of autoregulatory mechanisms, uptake inhibitors typically cannot increase synaptic levels of monoamines as much as the releasing agents can (for examples see refs. *23* and *24*). Thus, it might be argued that the increase in synaptic NE that can be produced by NRIs may not be sufficient to produce the anorectic effects seen with the NE releasing agents.

Serotonin (5-Hydroxytryptamine, 5-HT) System Targets

There is a large literature showing the interactions of central serotonergic systems in eating behavior (for reviews see refs. *25* and *26*). Given that serotonergic projections provide relatively dense innervation of most areas of the CNS and that there are at least 14 distinct 5-HT receptor subtypes (Table 1), it is not surprising that sometimes the conclusions regarding the CNS effects of serotonergic drugs can be confusing and even contradictory.

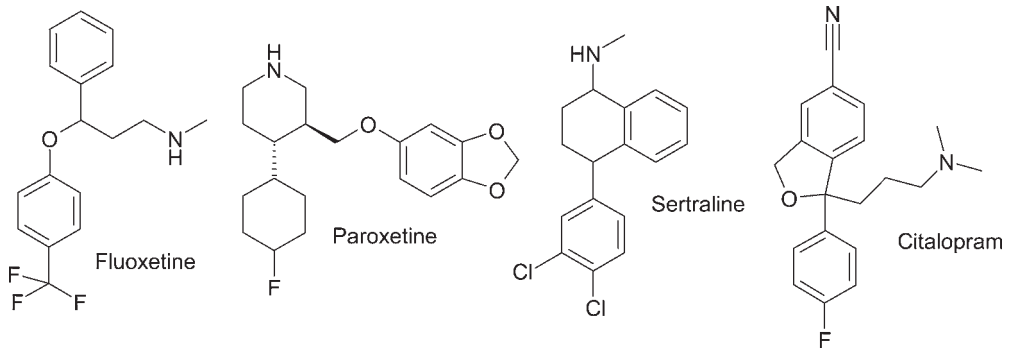


Fig. 4. Examples of selective serotonin reuptake inhibitors.

Fig. 5. Fenfluramine and its metabolite norfenfluramine.

Selective Serotonin Reuptake Inhibitors (SSRI)

Fluoxetine is perhaps the most studied of the SSRIs (Fig. 4) when it comes to feeding behavior and weight gain in preclinical models. Preclinical animal studies have reported that fluoxetine can decrease feeding and weight gain (see for example, refs. *27* and *28*). However, most of the animal studies have been carried out over relatively short time periods. Clinical studies with fluoxetine have produced a mixed picture. In a meta-analysis of obesity trials, Li et al. *(29)* found seven studies that reported weight loss outcomes of fluoxetine treatment at 6 mo, with six of the seven studies showing statistically significant weight loss. However, in six studies that followed fluoxetine administration for 12 mo only three of the six studies reported statistically significant weight loss. The authors' conclusion from the meta-analysis was that fluoxetine probably promotes weight loss for at least 6 mo when given along with recommendations for an appropriate diet. Given the very large numbers of patients that have been treated with the SSRIs, one would expect that, if there were a readily observable and persistent weight-reducing effect, it would have been reported. Because this does not appear to be the case, it is not surprising that weight reduction is an indication that has not been actively pursued for the SSRIs.

Fenfluramine and the 5-HT$_{2C}$ Receptor

Fenfuramine is a compound affecting serotonergic systems that has been studied for a number of decades. It made its first appearance in the scientific literature in the 1960s and was approved by the FDA for the short-term treatment of obesity in 1973. Fenfluramine is a substituted amphetamine and, like amphetamine, causes the release of monoamine neurotransmitters. In vivo, fenfluramine is extensively metabolized to norfenfluramine (Fig. 5), which is also a monoamine releasing agent. Both fenfluramine and norfenfluramine release 5-HT and NE *(30)*. At high doses, fenfluramine can also produce a long-lasing depletion of brain 5-HT in animal models *(31)*.

Starting in the early 1990s, as the multitude of 5-HT receptors were discovered and characterized, it became increasingly clear that the decrease in feeding and decreased weight gain seen with serotonergic compounds could be attributed to actions at specific 5-HT receptor subtypes (for a review see ref. *26*). Unlike fenfluramine, norfenfluramine is a potent agonist at the 5-HT$_{2C}$ receptor subtype. An accumulation of data from pharmacologic studies in animals and humans and from the use of transgenic animals has suggested that activation of the 5-HT$_{2C}$ receptor by norfenfluramine is responsible for most of the antiobesity effects of fenfluramine in humans (for reviews see refs. *26* and *32*).

Use of fenfluramine to treat human obesity dramatically increased after the publication of a series of papers in 1992 *(33)*, demonstrating the effects seen when fenfluramine was used in combination with phentermine, popularly known as phen-fen. In 1996, the FDA approved the *d*-enantiomer of fenfluramine for the long-term treatment of obesity. However, the upsurge in the usage of fenfluramine brought with it the realization that chronic use could result in the development of cardiac valvulopathy *(20)*, and so fenfluramine (both *d*-fenfluramine and the racemic form) was withdrawn from the market in 1997. Currently, it is hypothesized that the fenfluramine-induced valvulopathy might be due to activation of the 5-HT$_{2B}$ receptor by the metabolite norfenfluramine *(34)*. The success of phen-fen suggests that combination therapy with a NE-releasing agent and a 5-HT-releasing/5-HT$_{2C}$ agonist agent might produce a better weight-loss effect than either mechanism alone. However, it does not appear that a conclusive head-to-head comparison of the combination with the individual agents has been done in humans.

Combinations of Pharmacologic Activity

A common topic in discussions of pharmacologic treatment of obesity is the biologic redundancies that are designed to keep organisms, including humans, eating. Also common are discussions about combining different pharmacologic

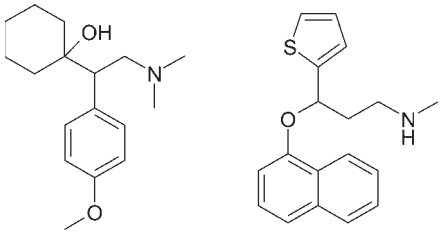


Fig. 6. Examples of dual 5-HT/NE reuptake inhibitors.

mechanisms in attempts to increase the magnitude of the antiobesity effect. In some cases, as described above for phenfen, separate molecules are combined. In other cases, such as described below, single molecules are endowed with multiple pharmacologic effects.

Dual Serotonin/Norepinephrine Reuptake Inhibitors (SNRI)

While neither selective NE uptake inhibition nor selective 5-HT uptake inhibition has yielded a successful antiobesity agent, it has been suggested that perhaps the combination of these mechanisms might produce a significant effect. Venlafaxine and duloxetine (Fig. 6) are two agents that combine 5-HT and NE reuptake inhibition *(35,36)* and are approved for the treatment of major depression. Venlafaxine is also approved for the treatment of generalized and social anxiety disorders, and duloxetine is also approved for the pain of peripheral diabetic neuropathy and urinary incontinence. Neither compound is approved for use in the treatment of obesity. However, in preclinical studies, a SNRI (nisoxetine) combined with a SSRI (fluoxetine) produced an increase in metabolism (oxygen consumption) that neither compound could achieve on its own *(37)*, suggesting that combining the two mechanisms may be important for weight loss.

According to the product literature, venlafaxine (see: www.wyeth.com/products/wpp_products/featured_az.asp#d, prescribing information) showed small effects on body weight in normal weight individuals during clinical trials. It is reported that over a period of several weeks a loss of 5% or more of body weight occurred in 6% of patients treated with venlafaxine compared with 1% of patients treated with placebo. In trials lasting 4–8 wk, the overall incidence of measurable weight loss was listed as 1%. In four 8-wk trials in pediatric patients (6–17 yr of age) venlafaxine-treated patients lost an average of 0.45 kg while patients receiving placebo gained on average 0.77 kg.

In trials for major depressive disorder, normal weight patients treated for up to 9 wk with duloxetine had a mean weight loss of approx 0.5 kg, compared to a mean weight gain of about 0.2 kg in patients receiving placebo. In trials for pain due to diabetic neuropathy that lasted for up to 13 wk, the duloxetine-treated patients showed a mean weight loss of about 1.1 kg, compared with a mean weight gain of about 0.2 kg, in the placebo-treated patients (see: http://www.cymbalta.com/index.jsp, prescribing information).

The relatively small effects of venlafaxine and duloxetine on body weight in humans appear consistent with what has been seen with either SSRIs or NRIs when used alone.

Bupropion

Bupropion is a marketed compound approved for the treatment of major depressive disorder and as an aid to smoking cessation treatment. It is also used off-label to enhance weight loss. Structurally, bupropion is related to diethylpropion (compare Figs. 1 and 7). Although bupropion appears in the scientific literature as early as the 1970s, there continues to be considerable discussion and debate about its likely mechanism(s) of action. This is compounded by the fact that bupropion is metabolized in vivo (Fig. 7) to produce compounds that likely have biologic activity as well *(38–41)*.

Much of the preclinical work with bupropion has focused on its actions as an inhibitor of the neuronal uptake of NE and DA. Bupropion is generally considered to be slightly more potent at inhibiting DA uptake than NE uptake, but the apparent potency of bupropion and its metabolites at NET and DAT varies across literature reports (Table 4). At the very least, it is clear that bupropion and its metabolites have relatively low potency/affinity at DAT and NET.

The mechanism(s) of action of bupropion in vivo of appears complex as well, with evidence for interactions on both NE and DA systems *(45,46)*. In humans, little is known about the possible mechanisms of action. However, Learned-Coughlin et al. *(47)* have reported an in vivo study of the human DAT using positron emission tomography (PET). In this study, the subjects were given therapeutically relevant doses until steady-state plasma levels were achieved. Under these conditions, the administration of bupropion resulted in an average DAT occupancy of 25–26% that persisted for at least 24 h after dosing. Thus, it is conceivable that at least a portion of bupropion's overall human pharmacology results from this blockade of DA transport. In this PET study, it was also noted that steady-state plasma levels showed AUC levels of the metabolite hydroxybupropion to be about ninefold higher than the levels for the parent compound.

To add confusion to the understanding of bupropion's effects, it has been reported that bupropion can antagonize certain types of cholinergic nicotinic receptors *(48)*. Additional in vitro studies examining both bupropion and its metabolites have confirmed that these compounds can antagonize nicotinic receptors at therapeutically relevant concentrations, depending on the form of nicotinic receptor examined and the metabolites studied *(40,41)*. While some in vivo effects of nicotine can be blocked by bupropion *(41)*, other studies have shown generalization to the nicotine cue in a drug discrimination paradigm *(40)*. Thus, it continues to be safe to say that the in vivo actions of bupropion (and its metabolites) are complex and not completely understood.

Much of the clinical work regarding bupropion's effects on weight gain appears to be anecdotal, with few controlled

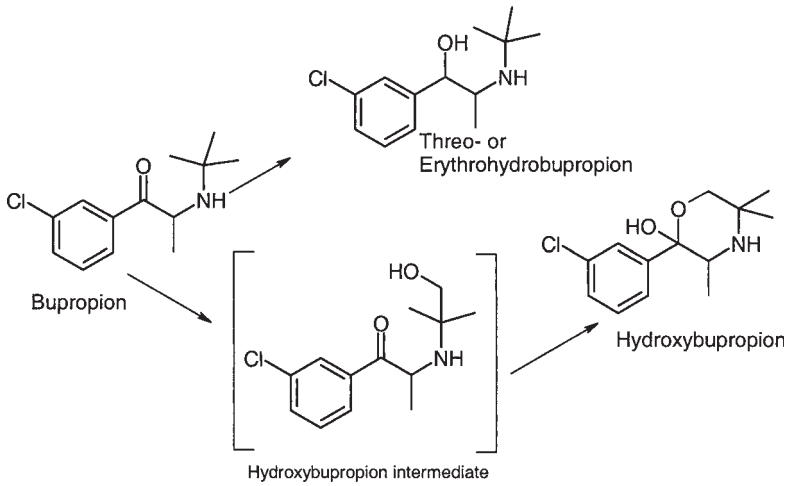


Fig. 7. Bupropion and its metabolites.

Table 4
Activity of Bupropion to Inhibit Monoamine Uptake

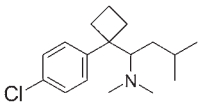
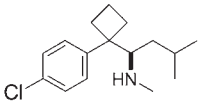
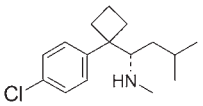
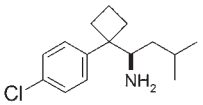
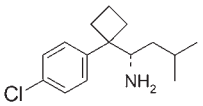
	Potency			
Uptake Studies	Rat DAT IC_{50} (μ*M*)	Rat NET IC_{50} (μ*M*)	Rat SERT IC_{50} (μ*M*)	Reference
Bupropion	2	5	58	*42*
Hydroxybupropion	23	7	105	*42*
Threohydrobupropion	47	16	67	*42*
Bupropion	1.9	2.2	>30	*43*
(*S,S*)hydroxybupropion	9.3	1.1	>30	*43*
(*R,R*)hydroxybupropion	>100	>30	>100	*43*
Bupropion	0.55	1.9		*41*
(2*S*,3*S*)hydroxybupropion	0.79	0.52		*41*
(2*R*,3*R*)hydroxybupropion	>10	>10		*41*
	Affinity			
Binding Studies	Human DAT, K_i (μ*M*)	Human NET, K_i (μ*M*)	Human SERT, K_i (μ*M*)	
Bupropion	0.56	>10	>10	*44*
Bupropion	1.02	>10	>10	*40*
(*S,S*)hydroxybupropion	1.3	3.9	>10	*40*
(*R,R*)hydroxybupropion	>10	>10	>10	*40*

clinical trials. In a meta-analysis of clinical trials, Li et al. *(29)* were able to identify three trials that could be included in the analysis. Two trials were of 6 mo duration and one was of 12 mo duration. The meta-analysis combined the results for all three trials, i.e., mixing the results of the 6- and 12-mo studies, and found a pooled random-effects estimate of weight loss in bupropion-treated patients to be 2.77 kg over that of placebo.

Sibutramine

Racemic sibutramine hydrochloride monohydrate is a mixed SERT and NET inhibitor that is approved for the treatment of obesity in conjunction with dietary management. While sibutramine was originally evaluated in the 1980s as a potential antidepressant, efforts were redirected toward development as an anorectic agent (for a review see ref. *49*). This was most likely the result of the serendipitous finding that it produced weight loss, particularly in obese, depressed patients. Sibutramine is approved in a number of countries around the world including North and South America, Europe, and Africa. In conjunction with a low calorie diet, sibutramine produced a sustained weight loss in 1- and 2-yr clinical trials. In general, sibutramine is well tolerated by patients with dry mouth, anorexia, insomnia, con-

Table 5
Comparison of Sibutramine and Its Metabolites
on Monoamine Uptake In Vitro*

	Inhibition of uptake, IC_{50} (nM)		
	NET	DAT	SERT
 (*RS*)-sibutramine	350	1200	2800
 (*R*)-desmethylsibutramine	4	12	44
 (*S*)-desmethylsibutramine	870	180	9200
 (*R*)-didesmethylsibutramine	13	8.9	140
 (*S*)-didesmethylsibutramine	62	12	4300

*Data taken from (*50*). Absolute configurations taken from (*51*).

stipation, and headache being the most common side effects. Sibutramine also appears to have a low potential for abuse liability, although it does increase heart rate and blood pressure and is contraindicated for patients with histories of cardiovascular diseases.

In preclinical studies, sibutramine produces a dose-dependent decrease in food intake (for review see ref. *52*). This effect can be reduced by 5-$HT_{2A/2c}$ and β_1-adrenergic receptor antagonists and completely inhibited using prazocin, an α_1-adrenergic receptor antagonist *(21)*. These results along with others suggest the suppression of feeding by sibutramine is mediated by the combined actions on SERT and NET *(52,53)*. In subchronic study of obese Zucker rats, sibutramine was found to decrease food intake initially but the total food intake over 2 wk was not significantly lower than vehicle-treated controls *(37)*. In a subsequent study, sibutramine was found to dose-dependently increase oxygen consumption and body temperature indicating increased thermogenesis (for a review see ref. *54*). This effect appeared to require central occupancy of SERT and NET and was mediated by brown adipose tissue thermogenesis via the β_3 receptor *(37,55,56)*. Therefore, the antiobesity effects of sibutramine in rats are likely the result of both decreased food intake and increased metabolism. Because adult humans do not have abundant brown fat, it is not clear if increased energy expenditure is a significant contributor to weight loss in a clinical setting *(57)*.

Because racemic sibutramine is a mixture a two enantiomers and there are two active metabolites of each enantiomer, considerable effort has been expended to understand the pharmacological properties of these compounds. As detailed in Table 5, both (*R*)-desmethylsibutramine and (*R*)-didesmethylsibutramine are considerably more potent at all three monoamine transporters when compared to (*R/S*)-sibutramine using in vitro assays. On the other hand, (*S*)-desmethylsibutramine is less potent than (*R/S*)-sibutramine at NET and SERT but more potent at DAT. (*S*)-didesmethylsibutramine complicates the picture further by exhibiting high potency at DAT, moderate affinity for NET and relatively low affinity for SERT. In vivo, both (*R*)-enantiomers exhibited greater anorectic potency than their corresponding (*S*)-enantiomers as well as (*R/S*)-sibutramine. Racemates of the sibutramine metabolites have also been reported to increase metabolism as well *(37)*. Because sibutramine and its metabolites have some affinity for DAT in vitro and in vivo *(58)*, the effects of the enantiomers of sibutramine on locomotor activity have been evaluated. In these studies, all the enantiomers of sibutramine increased locomotor activity in a dose-dependent manner indicating occupancy of DAT *(50)*. However, the duration of food intake suppression exceeded the dura-

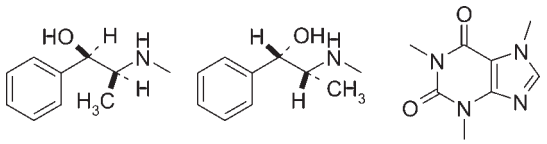


Fig. 8. Ephedrine and caffeine.

tion of the locomotor increases suggesting the antiobesity actions are dissociated from the DAT occupancy. Several of the sibutramine enantiomers are currently under clinical development by Sepracor.

Ephedrine and Caffeine

Another approach to using multiple pharmacologies for obesity treatment has been the use of ephedrine (a sympathomimetic/CNS stimulant) in combination with caffeine (Fig. 8). This combination was most commonly achieved through the use of dietary supplements, i.e., plant materials containing ephedrine and caffeine, so that the product could be marketed without the need of a prescription. Much has been written about the use of ephedrine alone or in combination with caffeine to treat obesity, but it is difficult to determine the mechanisms of the effects from the hype that surrounds the use of these compounds.

Although ephedrine can be made synthetically, it and related compounds are natural constituents of a number of different plant species, especially of the family Ephedraceae (e.g., *Ephedra sinica*, *E. equisetina*, and *E. gerardiana*). The plants of this group are often collectively referred to as ephedra or ma huang (also written as mahuang). Ephedrine contains two chiral centers; thus, four stereoisomers are possible. Over the years, ephedrine has been described as both a direct acting (i.e., direct adrenergic receptor agonist) and an indirect acting (i.e., NE-releasing) sympathomimetic drug. However, a careful characterization of ephedrine and its enantiomers by Rothman et al. *(30)* has suggested that there is little if any direct effect on adrenergic receptors and that the most likely mechanism for ephedrine's effects is through its ability to stimulate the release of NE.

A number of small trials examining ephedrine alone, ephedrine plus caffeine, or herbal combinations containing ephedrine and caffeine have shown efficacy in reducing weight *(20,59)*. Typically, these studies have been for short durations in the range of 8–20 wk; so, durability of the effect on weight loss has not been demonstrated. Overall, there appears to be no evidence that the combination of ephedrine and caffeine produces weight loss efficacy that is different from the other anorectic agents that act by releasing NE.

Caffeine is a plant-derived chemical that is consumed broadly in tea and coffee, as well as in a host of other beverages to which it is added. Caffeine, which is generally classed as a CNS stimulant, appears to produce its effects by acting as an antagonist at the adenosine A_1 and A_{2A} receptors. Caffeine, which by itself appears to have little effect on body weight, has been alleged to facilitate the weight-reducing effects of ephedrine. However, this hypothesis does not appear to have been rigorously tested.

Nonprescription preparations of plant materials containing ephedrine with or without caffeine were very popular as weight-loss supplements, but generated significant controversy regarding their safety. In 2004, the FDA issued a final ruling prohibiting the sale of dietary supplements containing ephedrine alkaloids (ephedra). In issuing this ruling the FDA stated that "dietary supplements containing ephedrine alkaloids pose a risk of serious adverse events, including heart attack, stroke, and death, and that these risks are unreasonable in light of any benefits that may result from the use of these products" (http://www.fda.gov/oc/initiatives/ephedra/february2004/finalsummary.html). Although the sale of dietary supplements containing ephedrine alkaloids was banned, ephedrine itself continues to be approved as a prescription medication for certain indications. These include use as a vasopressor in shock, as a bronchodilator to treat asthma/bronchospasm, and as a decongestant for relief of nasal congestion due to colds, hay fever, rhinitis, or sinusitis. Ephedrine is not approved for the treatment of obesity.

Ephedrine, either alone or in combination with caffeine, is often discussed as producing thermogenesis, i.e., increasing energy expenditure. While it is clear that the combination of ephedrine and caffeine can produce small increases in energy expenditure, it is not clear that this is significantly different from other sympathomimetic compounds or this contributes significantly to the overall effects of these compounds on weight reduction.

Histaminergic System Targets

There is a substantial literature suggesting the involvement of central histaminergic systems in feeding behavior (for reviews see refs. *60* and *61*). Specifically, activation of the histamine H_1 receptor has been shown to decrease feeding, while antagonism of the H_1 receptor increases feeding and weight gain. In humans the ability of antipsychotic drugs to induce weight gain is highly correlated with their antagonist potency at the histamine H_1 receptor *(62)*. Unfortunately, central H_1 receptors are not a good drug target for antiobesity agents, because a peripherally administered histamine H_1 receptor agonist would activate the H_1 receptors that are responsible for the allergic/inflammatory responses that are seen when peripheral mast cells release histamine. These include symptoms such as itching, edema, hives, and bronchoconstriction.

An alternative approach to administering a histamine H_1 receptor agonist is to increase synaptic levels of histamine in the brain to stimulate central H_1 receptors. One way to do this is to block the histamine H_3 receptor, an autoreceptor on histaminergic neurons that regulates the release of histamine. While it is clear that H_3 receptor antagonists can

increase synaptic levels of histamine, their effects on feeding and weight gain are less clear. There seems to be a mix of findings with some groups reporting decreased feeding and weight loss and others being unable to produce such findings (for a comprehensive review see ref. *63*). However, it appears that the preponderance of studies find that histamine H_3 receptor antagonists decrease food consumption and weight gain *(63,64)*. Interpretation of the different findings with H_3 receptor antagonists is complicated by the fact that there are multiple splice variants of the H_3 receptor and species differences in the pharmacology of the receptor (for a review see ref. *65*). In addition, the histamine H_3 receptor appears to have a high level of constitutive activity *(66,67)*, and many of the H_3 receptor antagonists are actually inverse agonists. Thus, the degree of inverse agonist efficacy could also potentially influence the apparent activity of compounds in vivo.

To date, no reports have surfaced of clinical trials of histamine H_3 receptor antagonists. Ultimately, this will be necessary to understand whether this mechanism has any potential in the treatment of human obesity.

Summary

Overall, the monoaminergic neuronal systems have clearly been shown to be involved in eating behavior and energy balance. Less clear is the extent and specifics of the involvement of individual monoamines and of individual receptor subtypes in regulating the various components of eating behavior and energy balance. Ultimately, the interest in these systems is driven by the desire to understand and modify human eating behavior to aid in managing a healthy weight. Over the years a number of antiobesity medications, based on altering monoaminergic activity, have appeared on the market and/or in clinical trials. The first examples of these agents used to treat human obesity were the so-called CNS stimulants, e.g., the amphetamines, phentermine, diethylpropion, etc. It now seems likely that the primary common action of these molecules to affect obesity is through the release of NE. As a group, these agents have limited usefulness in the treatment of obesity because of the tendency for reduced efficacy with chronic treatment, and indeed those agents that are still in use are only approved for the short-term treatment of obesity. Only a single compound from the group affecting monoaminergic neurotransmission, i.e., sibutramine, is currently approved for the long-term treatment of obesity. Sibutramine's pharmacology is complex, as it produces metabolites that inhibit uptake through NET, DAT, and SERT to varying degrees. For serotonergic systems, the success of fenfluramine (before it was pulled from the market) has led to a focus on developing selective 5-HT_{2C} receptor agonists to test in the clinic. The histaminergic system has also generated some interest as an antiobesity target, and a number of laboratories are pursuing H_3 receptor antagonists as possible antiobesity agents. Given the generally modest antiobesity effects of the drugs affecting monoaminergic systems, future exploration of these systems will likely involve understanding the effects of combinations compounds affecting different components of central monoaminergic systems to see if larger antiobesity effects can be achieved. Likewise it is also likely that drugs targeting monoaminergic systems will be examined in combination with other antiobesity strategies to facilitate greater control of human body weight.

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