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Commentary

A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse

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Abstract

Lobeline, an alkaloidal constituent of *Lobelia inflata* LINN., has a long history of therapeutic usage ranging from emetic and respiratory stimulant to tobacco smoking cessation agent. Although classified as both an agonist and an antagonist at nicotinic receptors, lobeline has no structural resemblance to nicotine, and structure–function relationships do not suggest a common pharmacophore. Lobeline inhibits nicotine-evoked dopamine release and [^{3}H]nicotine binding, thus acting as a potent antagonist at both $\alpha3\beta2^*$ and $\alpha4\beta2^*$ neuronal nicotinic receptor subtypes. However, lobeline does not release dopamine from its presynaptic terminal, but appears to induce the metabolism of dopamine intraneuronally. Reevaluation of the mechanism by which lobeline alters dopamine function reveals that its primary mechanism is inhibition of dopamine uptake and promotion of dopamine release from the storage vesicles within the presynaptic terminal, via an interaction with the tetrabenazine-binding site on the vesicular monoamine transporter (VMAT2). Thus, lobeline appears to perturb the fundamental mechanisms of dopamine storage and release. Based on its neurochemical mechanism, the ability of lobeline to functionally antagonize the neurochemical and behavioral effects of the psychostimulants amphetamine and methamphetamine was examined. Lobeline was found to inhibit the amphetamine-induced release of dopamine *in vitro*, and amphetamine-induced hyperactivity, drug discrimination, and self-administration. However, lobeline does not support self-administration in rats, suggesting a lack of addiction liability. Thus, lobeline may reduce the abuse liability of these psychostimulants. The development of lobeline and lobeline analogs with targeted selectivity at VMAT2 represents a novel class of therapeutic agents having good potential as efficacious treatments for methamphetamine abuse. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Lobeline; Psychostimulants; Methamphetamine; Nicotine; Vesicular monoamine transporter; Dopamine transporter

1. Lobeline: history

α-Lobeline (lobeline, Fig. 1) is a lipophilic, nonpyridino, alkaloidal constituent of *Lobelia inflata* LINN., also known as *Rapuntium inflatum* MILL., Indian weed, pukeweed, asthma weed, gagroot, vomitwort, bladderpod, eyebright, and Indian tobacco. The herb was named after Matthias de Lobel (1570–1616), a famous French botanist and physician to the court of King James I. The typically erect biannual or annual plant grows 1–2 ft high, with branched leaves that are sessile and irregularly or obtusely toothed, varying from ovate or oblong below to foliatious above. The root is slender and yellowish-white, and the small, irregular, violet-blue colored flowers are tinted with pale yellow. The plant develops two-celled capsuled fruits, which contain brownish-colored seeds. *L. inflata* is commonly found in dry open fields from Hudson Bay west to Saskatchewan, and south to Georgia and Mississippi, where it flowers from July to October. Historically, *L. inflata* was prepared in compressed oblong packages by the Shakers of New Lebanon for importation to England. After chewing the plant, the taste is similar to tobacco and produces effects like that of *S*-(−)-nicotine (nicotine; Fig. 1), the principal alkaloid in domestic tobacco, *Nicotiana tobaccum*. Thus, the name ''Indian tobacco'' could have been derived from the tobacco-like sensation imparted to the tongue and stomach upon chewing the plant leaves, or from the fact that the American Indians smoked the dried leaves to obtain the CNS effects of the alkaloids in the plant [1].

Linneaus first reported this species of plant in transactions of the Upsala Academy in 1741 [1], and the medicinal properties of *L. inflata* as an emetic and as an application for sore eyes were noted by Schoepf in 1787.

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Abbreviations: DOPAC, dihydroxyphenylacetic acid; nAChR, nicotinic acetylcholine receptor; VMAT2, vesicular monoamine transporter

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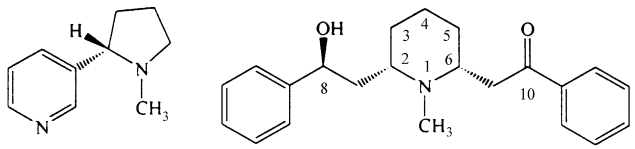

S-(−)-Nicotine 2*S*,6*R*,8*S*-(−)-Lobeline

Fig. 1. Structures of nicotine and lobeline.

Subsequently, botanic physicians frequently used the plant. In 1813, *L. inflata* was introduced to the medical profession by Reverend D. Cutler as a valuable remedy for asthma [1]. The first documentation of the pharmacological actions of the plant was obtained from a liquid alkaloid extract, first prepared by Proctor in 1838. Extracts of *L. inflata* were used as an expectorant, emetic, anti-asthmatic, anti-spasmodic, respiratory stimulant, general muscular relaxant, diaphoretic, diuretic, and stimulant and also to treat narcotic overdose.

The seeds of *L. inflata* contain the highest percent of the piperidine alkaloid lobeline, which is the principal alkaloid and pharmacologically active constituent. Lobeline is the most abundant compound in a family of structurally related alkaloids, which include lobelidine, lobelanine, nor-lobelane, lobelanidine, nor-lobelanidine, and isolobenine, as well as 14 other alkaloids. The structural identification of lobeline [2*S*,6*R*,8*S*-(−)-lobeline (Fig. 1)] was reported by Wieland [2], who subsequently carried out its synthesis in 1925 [3], which led to the availability of the pure alkaloidal constituent for a more detailed and comprehensive characterization of its pharmacological properties. During this period of research, the pharmacological similarities between lobeline and nicotine were recognized, leading to the classification of lobeline as a nicotinic agonist. However, no obvious structural resemblance of lobeline to nicotine is apparent (see Fig. 1), and structure–function relationships do not suggest a common pharmacophore [4].

2. General pharmacology and clinical uses of lobeline

Lobeline has many nicotine-like effects, including tachycardia and hypertension [5], bradycardia and hypotension in anesthetized rats [6], hyperalgesia [7], as well as analgesia after intrathecal, but not after subcutaneous, administration [8], anxiolytic activity [9], and improvement of learning and memory [10]. Interestingly, intrathecal administration of lobeline also inhibits the analgesic effect of epibatidine (a potent nAChR agonist) [11,12]. Nicotine has been reported to be avidly self-administered by rats [13–18]. On the other hand, lobeline only marginally supports self-administration in mice [19] and does not support self-administration in rats [20]. Chronic treatment of rats with nicotine increases locomotor activity [21–23] and produces conditioned place preference [24–26], whereas chronic lobeline treatment does not produce these effects [27,28]. Initially, lobeline was found to generalize to nicotine in drug discrimination studies [29]; however, most subsequent studies have failed to replicate this latter finding [30–32]. Thus, differential effects of lobeline and nicotine in behavioral and neurochemical studies suggest that these drugs may not be acting via a common mechanism, even though lobeline has often been considered to be a nicotinic agonist.

Lobeline is also a primary stimulant and secondary depressant of sympathetic and parasympathetic ganglia, the adrenal medulla, the neuromuscular junction, and carotid and aortic body chemoreceptors [33,34]. Lobeline also possesses local anesthetic properties [35], and non-competitively inhibits the neuromuscular junction, without depolarization of the end plate [36,37]. Lobeline blocks the nicotinic acetylcholine receptor (nAChR) ion channel, which explains the secondary nondepolarizing postjunctional blockade at the neuromuscular junction [38].

Lobeline possesses transient and minor cardiovascular effects, resulting from its primary stimulant and secondary depressant effects on sympathetic and parasympathetic ganglia, as well as on the adrenal medulla [6]. Lobeline initially stimulates cardiac vagal ganglion cells to decrease heart rate; this transient bradycardia generally lasts only seconds [6,39]. Lobeline stimulates the cardioaccelerator nerve sympathetic ganglion cells; however, vagal effects predominate, producing transient bradycardia and possible bradyarrhythmia, especially during general anesthesia [39,40]. The stimulatory actions of lobeline on the adrenal medulla result in the release of epinephrine into the circulation and the subsequent stimulation of ganglionic cells, leading to an increase in blood pressure [39]. However, following repeated lobeline administration, a long-term decrease in blood pressure is observed, which results from opposing interactions of the sympathetic and parasympathetic systems.

Reflex effects on respiration, circulation, and gastrointestinal motility can result from the ability of lobeline to stimulate chemoreceptors of the carotid body [33], aortic body [41], carotid sinus [42], and pulmonary circulation [43]. Lobeline-induced nausea and emesis result from stimulation of the emetic center in the CNS, as well as from a direct irritant action upon the gastrointestinal tract. Lobeline stimulates sensory nerve endings to inhibit gastric contraction and intestinal motility, an action that is

blocked by subdiaphramatic vagotomy, indicating vagal control [40,44].

One of the earliest known uses of lobeline was as a safe, short-acting respiratory stimulant [45]. Stimulation of nicotinic receptors in the pleura or respiratory tract supplied by the pulmonary circulation results in a transient, sharp substernal pain, which can be blocked by the tetraethylammonium ion [46]. This respiratory stimulation has been shown to be due to direct stimulation of the carotid body chemoreceptors. The powerful respiratory stimulant action of lobeline has been advantageous for stimulation of respiration in fever cases, cases of paralysis of respiratory centers due to narcotic poisoning, alcohol, soporifics, morphine, narcosis, or spinal anesthesia. Lobeline has also been used in treating accident victims who have been buried, nearly drowned, hit by lightning or after electric shock, and following poisoning by asphyxiating gases. In addition, lobeline has been reported to afford good results in treating asphyxia in newborn infants. Lobeline has also been used to treat bronchitic asthma, bronchitis, spasmodic asthma, whooping cough, spasmodic croup, membranous croup, infantile convulsions, puerperal eclampsia, tetanus, epilepsy, hysterical convulsions, diphtheria, and pneumonia [1,47]. However, due to the availability of more effective agents and procedures, lobeline is not currently used to treat the above conditions. Furthermore, lobeline is noted to have untoward effects including dizziness, nausea, hypertension, vomiting, stupor, tremors, paralysis, convulsions, coma, and death.

Lobeline may have some clinical benefit as a smoking cessation agent, as is indicated by a plethora of human studies and reports in the literature. The first use of lobeline as a smoking cessation agent was reported in 1936 by Dorsey, who concluded that lobeline alleviates the symptoms of nicotine withdrawal; however, a number of controlled, short-term trials have determined that lobeline has no effect on smoking [48]. Davison and Rosen [49] reported that poor methodological quality in previous studies has prevented any conclusions on the efficacy of lobeline in smoking cessation to be definitively made. More recently, there has been a renewed interest in investigating the efficacy of lobeline in smoking cessation. Schneider and Olsson [50] have suggested that development of a lobeline formulation with improved bioavailability may increase efficacy in smoking cessation. A clinical trial comparing 7.5 mg sublingual lobeline with a placebo for 6 weeks afforded abstinence from tobacco use during the last 4 weeks of the treatment period in 10 of 34 treated subjects compared with 8 of 47 subjects receiving the placebo [50]. However, a subsequent multi-center study of sublingual lobeline use in a group of 750 subjects [51] reported no statistical differences between placebo and lobeline sublingual tablets at a 6-week follow-up. Nevertheless, one of the three sites in the latter study demonstrated significant efficacy. Thus, the utility of lobeline as a smoking cessation agent remains controversial.

3. Classical mechanism of action of lobeline in the CNS

Lobeline has been categorized as a nicotinic receptor agonist, and is purported to exert its effects on the CNS via a mechanism similar to nicotine [52]. Lobeline was considered until only recently to be an agonist at nAChRs, but with a unique pharmacological profile. Thus, lobeline displaces [^{3}H]nicotine binding from native nAChRs in the CNS with high affinity ($K_i = 4$–30 nM) [8,53–57]. However, although chronic nicotine treatment results in nAChR up-regulation in many brain regions [58–60], chronic lobeline treatment does not produce up-regulation [59,61]. The nAChR subtype(s) to which lobeline binds is currently being elucidated. Similar to nicotine, lobeline displaces [^{3}H]cytisine binding to rat cortical membranes. *In vivo*, lobeline has been shown to displace several $\alpha4\beta2$ nAChR ligands using positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging of mouse brain [62–64]. Also, experiments performed in oocyte expression systems reveal that $\beta2$-containing nAChRs had an 85-fold higher affinity for lobeline than did $\beta4$-containing nAChRs, independent of the α-subtype included in the pairwise combination [65]. Lobeline also inhibits ($K_i = 6.6$ μM) [^{3}H]methyllycaconitine (an $\alpha7$ selective ligand) binding to rat brain membranes (our unpublished observations), supporting an interaction with the $\alpha7$ subtype.

Although it has been generally accepted that lobeline acts as an agonist at nAChRs, lobeline has been reported to exhibit antagonist effects ($IC_{50} = 8.5$ μM) at wild-type human $\alpha7$ receptors expressed in *Xenopus* oocytes [66]. Another functional assay used to probe nAChRs on presynaptic dopaminergic terminals is the dopamine release assay. In this assay, both nicotine and lobeline have been shown to evoke [^{3}H] overflow from striatal synaptosomes [67–70] and striatal slices [71–76] preloaded with [^{3}H]dopamine. The nicotine-evoked release was via stimulation of the $\alpha3\beta2^*$ subtype of nAChRs; however, the effect of lobeline was not nicotinic-receptor mediated. Recently, lobeline was reported to evoke endogenous dopamine release during application via microdialysis, and this effect appeared to be nicotinic-receptor mediated [77]. Lobeline released [^{3}H]norepinephrine from superfused hippocampal slices; however, it was the least potent in comparison with other nAChR agonists such as epibatidine, cytisine, and nicotine [78]. Thus, lobeline also appears to be capable of altering catecholaminergic neurotransmission.

4. Reevaluation of the neurochemical mechanism of action of lobeline

In contrast to nicotine, lobeline evokes $^{86}Rb^+$ efflux from striatal synaptosomes with low efficacy; this effect is

mecamylamine-, dihydro-β-erythroidine (DHβE)-, α-bungarotoxin- and atropine-insensitive [79]. Thus, although lobeline binds to nAChRs, it does not appear to act like nicotine in an $\alpha4\beta2^*$ subtype functional assay ($^{86}Rb^+$ efflux). Unfortunately, the latter study by Terry *et al.* [79] did not determine whether lobeline inhibited nicotine-evoked $^{86}Rb^+$ efflux. Thus, a disconnect appears between the ability of lobeline to bind to nAChRs and its effects in functional assays probing these nAChR subtypes, making it an intriguing nicotinic receptor ligand for further study.

Lobeline evokes [^{3}H]dopamine overflow from rat striatal slices superfused in the presence of nomifensine and pargyline (compounds that inhibit [^{3}H]dopamine reuptake and metabolism, respectively); however, in the absence of nomifensine and pargyline, lobeline evokes dihydroxyphenylacetic acid (DOPAC) overflow, rather than dopamine overflow [75,76]. More importantly, the lobeline-evoked [^{3}H]dopamine release is not dependent upon extracellular calcium, and is not sensitive to mecamylamine, suggesting that this dopamine release is not mediated by nAChRs in striatum, and that lobeline does not act as an agonist at these nAChRs [75,76,80]. Interestingly, lobeline also does not release either dopamine or DOPAC in microdialysate from rat nucleus accumbens *in vivo*, but inhibits nicotine-evoked dopamine and DOPAC overflow when administered 10 min, but not 60 min, prior to nicotine [81]. In this respect, we have determined recently that lobeline inhibits nicotine-evoked [^{3}H]dopamine overflow from rat striatal slices [82]. Additionally, in microdialysis studies, lobeline was found not to evoke the release of acetylcholine from the hippocampus, nor did it inhibit nicotine-evoked acetylcholine release [83]. Although lobeline released [^{3}H]norepinephrine from superfused hippocampal slices, this effect was shown not to be mediated by nAChRs [78]. Moreover, lobeline has been shown to inhibit nicotine-evoked [^{3}H]norepinephrine release from rat locus coeruleus cells in culture [84]. Also consistent with an antagonist effect of lobeline at nAChRs is the observation that lobeline does not activate, but rather, in a concentration-dependent manner, antagonizes the effect of nicotine in voltage-clamped *Xenopus* oocytes expressing α4β2 nAChRs [8]. Taken together, the above studies clearly indicate that lobeline acts as a nAChR antagonist, inhibiting nicotinic agonist-evoked catecholamine release, and although relatively high concentrations of lobeline release catecholamines from their presynaptic terminals, this release occurs via a mechanism that does not involve nAChRs.

Initial work from our laboratories reevaluating the mechanism by which lobeline alters dopamine function indicates that the primary mechanism of action of lobeline is to inhibit dopamine uptake and promote dopamine release from synaptic vesicles within dopaminergic terminals [75,76,82,85], thereby perturbing the fundamental mechanisms of presynaptic dopamine storage and release. Specifically, in the presence of nomifensine and pargyline in the superfusion buffer, lobeline increases [^{3}H]dopamine overflow from superfused rat striatal slices in a concentration-dependent manner. However, in the absence of nomifensine and pargyline, endogenous dopamine was detected in superfusate from striatal slices *only* after exposure to the highest concentration (100 μM) of lobeline. Moreover, lobeline evoked an increase in endogenous DOPAC in superfusate in a concentration-dependent manner, suggesting that lobeline exposure results in increased cytosolic dopamine, which is rapidly metabolized to DOPAC and subsequently released into superfusate. Furthermore, the latter results suggested that lobeline does not inhibit monoamine oxidase, since DOPAC overflow resulted following superfusion with lobeline. Lobeline inhibited ($IC_{50} = 80$ μM) [^{3}H]dopamine uptake into rat striatal synaptosomes, and moreover, potently inhibited ($IC_{50} = 0.88$ μM) [^{3}H]dopamine uptake into rat striatal synaptic vesicles. Thus, lobeline alters presynaptic dopamine storage and utilization by potent inhibition of dopamine uptake into synaptic vesicles, and stimulates the release of dopamine from the vesicles into the cytosol, which subsequently is metabolized to DOPAC by monoamine oxidase (see Fig. 2).

Further elucidation of the mechanism of action of lobeline was revealed by the finding that lobeline inhibited vesicular membrane [^{3}H]dihydrotetrabenazine binding with an IC_{50} value of 0.90 μM, which is consistent with the value obtained for lobeline's inhibition of [^{3}H]dopamine uptake into vesicles [75,76]. [^{3}H]Dihydrotetrabenazine, a structural analog of tetrabenazine, binds to a single class of high-affinity sites on the vesicular monoamine transporter (VMAT2) to inhibit vesicular dopamine uptake [86–89]. Interestingly, lobeline completely inhibited [^{3}H]dopamine uptake into vesicles, while not completely inhibiting [^{3}H]dihydrotetrabenazine binding to VMAT2, suggesting that another site of action on VMAT2 may be involved (perhaps the substrate recognition site probed by [^{3}H]reserpine). Worthy of note is the observation that tetrabenazine does not alter spontaneous efflux of [^{3}H]dopamine from rat brain vesicles [90]. Thus, tetrabenazine appears to block [^{3}H]dopamine uptake into vesicles, but does not promote [^{3}H]dopamine release from vesicles. Taken together, these results indicate that lobeline specifically interacts with dihydrotetrabenazine sites on the synaptic VMAT2 to inhibit dopamine uptake into synaptic vesicles (see Fig. 2). Consistent with these findings, Vizi's research group has reported recently that lobeline inhibits ($IC_{50} = 1.19$ μM) [^{3}H]norepinephrine uptake by VMAT2 in hippocampal vesicular preparations, suggesting that lobeline increases cytoplasmic norepinephrine accumulation and subsequently releases norepinephrine from the terminal via reversal of the norepinephrine transporter [91]. Thus, lobeline reverses the norepinephrine transporter to release norepinephrine, but does not reverse the dopamine transporter to release dopamine into the synaptic

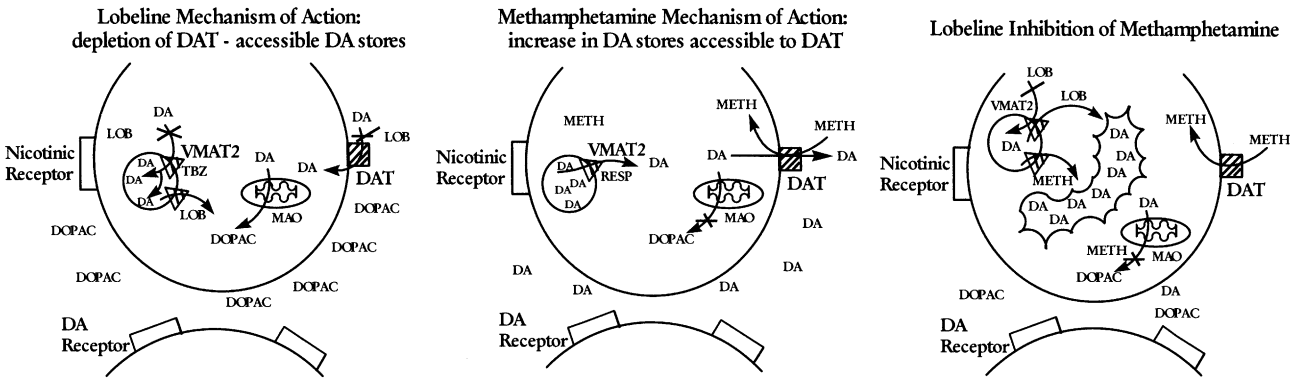

Fig. 2. Current understanding of the primary mechanism of action of lobeline in comparison to methamphetamine. The left panel illustrates the proposed mechanism of action of lobeline to inhibit dopamine uptake into synaptic vesicles via the VMAT2, which results in a corresponding redistribution of presynaptic dopamine storage and an increase in the cytosolic dopamine pool. The resulting increase in cytosolic dopamine leads to an increase in DOPAC as a result of metabolism of the cytosolic dopamine pool by monoamine oxidase. This redistribution ultimately leads to a decrease in the cytosolic dopamine pool available for reverse transport by the dopamine transporter. The middle panel illustrates methamphetamine-induced dopamine release from the vesicle via the VMAT2, resulting in a corresponding redistribution of presynaptic dopamine storage with an increase in the cytosolic dopamine pool (due to methamphetamine inhibition of monoamine oxidase). The cytsolic dopamine is subsequently available for release into the synaptic cleft via methamphetamine-induced reversal of the dopamine transporter, increasing the synaptic dopamine concentration available for stimulation of postsynaptic dopamine receptors. The right panel illustrates that lobeline redistributes dopamine storage into a presynaptic terminal pool that is not available to undergo reverse transport via the dopamine transporter. Thus, methamphetamine does not release dopamine from the presynaptic terminal in the presence of lobeline. Abbreviations: DA, dopamine; DAT, dopamine transporter; DOPAC, dihydroxyphenylacetic acid; LOB, lobeline; MAO, monoamine transporter; METH, methamphetamine; RESP, reserpine; TBZ, tetrabenazine; VMAT2, vesicular monoamine transporter.

cleft. Therefore, the previously accepted mechanism of action of lobeline as a nicotinic receptor agonist is clearly not consistent with its more recently reported neuropharmacological properties.

5. Potential of lobeline as a pharmacotherapy for psychostimulant abuse

Drugs of abuse (e.g. amphetamine and methamphetamine) are thought to produce their reinforcing effects, at least in part, by activating the mesolimbic dopaminergic system [92–97], although other neurotransmitter systems are certainly involved as well. The dopamine projection from the ventral tegmental area to the nucleus accumbens is thought to be involved in reward and in the regulation of cognitive and emotional behaviors [98], and is particularly sensitive to the acute effects of intrinsically reinforcing drugs [99–101]. Direct injection of amphetamine into the nucleus accumbens produces reward [102,103]. The rewarding effect of amphetamine is attenuated by injecting either dopamine antagonists or the neurotoxin 6-hydroxydopamine directly into the nucleus accumbens [99,104,105]. Superfusion of striatal slices with amphetamine evokes endogenous dopamine overflow [106,107], as a result of an increase in cytosolic dopamine via augmentation of vesicular dopamine release, inhibition of vesicular dopamine uptake, and promotion of reverse transport of the dopamine transporter (see Fig. 2) [108–110]. More recently, amphetamine has been reported to release dopamine from synaptic vesicles of the *Planorbis corneus* giant dopamine cell, increasing dopamine concentrations in the cytosol and promoting reverse transport of dopamine via the dopamine transporter [111,112]. Additionally, amphetamine inhibits monoamine uptake into CV-1 cells expressing the human VMAT2 [113]. Moreover, since amphetamine inhibits monoamine oxidase [114,115], the increased extravesicular, cytosolic dopamine is available for release from the terminal by amphetamine-induced reversal of the dopamine transporter. Furthermore, amphetamine exhibits low potency inhibition of [^{3}H]dihydrotetrabenazine binding to rat striatal homogenates [116] and to the human VMAT2 expressed in COS cells [117]. Intriguing data have been reported which indicate that normal or intact synaptic vesicle function may be necessary for full amphetamine-induced conditioned reward, as revealed by diminished amphetamine-induced conditioned place preference in VMAT2 knockout mice, when compared with wild-type mice [118].

These results suggest that although both lobeline and amphetamine redistribute presynaptic dopamine storage by increasing the cytosolic pool of dopamine, the cytosolic dopamine is metabolized to DOPAC in the presence of lobeline, as a consequence of the inability of lobeline to inhibit monoamine oxidase. However, in the presence of amphetamine, cytosolic dopamine is not metabolized to DOPAC, since amphetamine inhibits monoamine oxidase. Thus, amphetamine releases the cytosolic dopamine into the synaptic cleft via reversal of the dopamine transporter at the plasma membrane. In the endogenous dopamine release assay, amphetamine evokes dopamine overflow, rather than DOPAC overflow, whereas lobeline evokes DOPAC overflow from striatal slices (only high lobeline concentrations of 100 μM resulted in dopamine in the superfusate). The amphetamine-induced increase in

dopamine overflow is generally believed to be responsible for its abuse liability.

In very recent studies [85], rat striatal slices were superfused in the absence or presence of pargyline (10 μM), and the ability of lobeline (0.1–1.0 μM) to inhibit amphetamine (1.0 μM)-evoked endogenous dopamine and DOPAC overflow was determined. In the presence of pargyline, DOPAC was not detected in superfusate during the 30-min exposure to amphetamine. In the absence of pargyline, DOPAC was detected in the superfusate; however, neither amphetamine nor lobeline at the concentrations examined altered DOPAC overflow. Thus, under these conditions neither lobeline nor amphetamine significantly inhibited monoamine oxidase activity. In the presence of pargyline, amphetamine (1.0 μM) increased dopamine overflow 2-fold above basal dopamine concentrations across the 30-min superfusion period. Most importantly, prior superfusion for 30 min with 0.1 or 0.3 μM lobeline resulted in a 90% decrease in amphetamine-evoked endogenous dopamine overflow, thus supporting the proposed mechanism of action of lobeline. In the absence of pargyline, 0.3 and 1.0 μM lobeline also significantly inhibited amphetamine-evoked dopamine overflow, lending further support to this mechanism. A low concentration (0.1 μM) of lobeline did not inhibit amphetamine-evoked dopamine overflow in the absence of pargyline, which was a surprising result considering that this concentration significantly inhibited amphetamine-induced dopamine release in the presence of pargyline. Thus, lobeline inhibited the effect of amphetamine whether or not pargyline was included in the buffer, suggesting that at least at this low concentration of amphetamine, the activity of monoamine oxidase was not a significant factor in the lobeline-induced inhibition. It is clear that the ability of lobeline to inhibit amphetamine-evoked endogenous dopamine release is not simply via an action at the dopamine transporter, since the IC_{50} for lobeline to inhibit the dopamine transporter is ~80 μM, whereas lobeline (within a concentration range of 0.1 to 0.3 μM) maximally inhibited amphetamine-evoked dopamine release. These low lobeline concentrations are more consistent with lobeline-induced inhibition of dopamine uptake at VMAT2 ($IC_{50} = 0.88$ μM) rather than at the dopamine transporter. Thus, the results obtained support the contention that VMAT2 may be the more important site of action of lobeline to reduce amphetamine-evoked dopamine release.

Since both lobeline and amphetamine alter synaptic vesicle function, it is important to determine the behavioral profile of lobeline, particularly with regard to its potential reinforcing effects. In this respect, lobeline does not produce conditioned place preference [27,28] and only marginally supports self-administration in mice [19]. Recent results also demonstrate that lobeline pretreatment inhibits amphetamine- and methamphetamine-induced locomotor hyperactivity [85], inhibits methamphetamine-induced drug discrimination [85], and decreases methamphetamine self-administration in rats [119]. The latter study also demonstrated that the lobeline-induced decrease in methamphetamine self-administration was not surmountable when the methamphetamine unit dose was increased, suggesting that the mechanism of lobeline action is non-competitive. Furthermore, lobeline does not support self-administration in rats [20], suggesting a lack of addiction liability. Thus, lobeline diminishes the rewarding effect of methamphetamine, and may reduce the abuse liability of this drug.

In summary, lobeline acts both as a nAChR antagonist at the presynaptic dopamine terminal to inhibit dopamine release into the synaptic cleft and/or as an inhibitor at the tetrabenazine site on VMAT2 on synaptic vesicle membranes. This most likely leads to a decrease in the vesicular dopamine pool and an increase in the cytosolic dopamine pool, ultimately exposing the cytosolic dopamine to monoamine oxidase and facilitating its metabolism to DOPAC. Thus, this lobeline-induced redistribution of dopamine storage reduces the cytoplasmic pool available for amphetamine-induced reverse transport by the dopamine transporter. The net effect of both of these mechanisms of action for lobeline is predicted to result in a diminished concentration of dopamine in the synaptic cleft and diminished activation of postsynaptic dopamine receptors. Thus, lobeline inhibits the action of amphetamine and functionally acts as an indirect dopamine receptor antagonist.

6. Future directions

The current findings are consistent with the proposed mechanism that lobeline reduces the cytosolic pool of dopamine available for reverse transport via the dopamine transporter, diminishing methamphetamine-evoked dopamine release. Thus, VMAT2 appears to be a novel target in the development of drug therapies for the treatment of methamphetamine abuse. Due to the multiple pharmacological actions of lobeline, it will be important to examine structural analogs of lobeline in future studies to begin to define the receptor pharmacophores involved in the inhibitory action of lobeline. In this respect, Terry *et al.* [79] have reported results from a structure–activity relationship (SAR) study of partial lobeline analogs to ascertain whether the whole lobeline molecule or just a fragment is required for interaction with nicotinic receptors, as assessed by the [^{3}H]cytisine binding and $^{86}Rb^+$ efflux assays. In the latter study, analogs were designed to represent different parts of the lobeline structure, i.e. one analog without the C6-piperidino substituent [i.e. 2-(2-phenyl-2-hydroxyethyl)-*N*-methylpiperidine] and the other analog without the C2-piperidino substituent [i.e. 2-(2-phenyl-2-oxoethyl)-*N*-methylpiperidine]. Results demonstrated that these analogs were 200- to 300-fold less potent than lobeline in the nicotine receptor binding assay, and were 10-fold less potent and less efficacious than lobeline in

the $^{86}Rb^{+}$ efflux assay. Unfortunately, these synthetic products were isolated as heterogeneous mixtures of isomers, thus compromising the interpretation of their SAR. Our preliminary results have shown that structural changes to the lobeline molecule, such as removal of one or both of the oxygen functionalities, result in a large decrease in nicotinic receptor affinity [57,120,121]. Flammia *et al.* [122] have corroborated our findings, and, in addition, have shown that stereochemically defined, partial lobeline analogs similar to those reported by Terry *et al.* [79] lack affinity for nicotinic receptors. Thus, it is possible that analogs of lobeline can be developed that will have selectivity for VMAT2. Such agents constitute a novel class of therapeutics with good potential as efficacious treatments for methamphetamine abuse. Lobeline analogs may have distinct therapeutic advantages over other agents that interact with VMAT2. For example, reserpine, which binds to the substrate site of VMAT2, is a well-known antihypertensive agent, which depletes CNS catecholamines causing sedation and depression as side-effects [123]. Tetrabenazine acts at a distinct site on VMAT2 and has less peripheral side-effects compared to reserpine, but it is not selective. Tetrabenazine has been reported to be an antagonist at dopamine receptors [124] and to inhibit calcium channels, preventing catecholamine release [125]. Lobeline acts at the tetrabenazine binding site but, as discussed previously, is not selective for this site. Drug discovery targeting VMAT2 may provide a unique opportunity to further probe the underlying neurochemical mechanism responsible for psychostimulant abuse and should provide a novel approach for its treatment.

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