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Lobeline effects on tonic and methamphetamine-induced dopamine release

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ABSTRACT

The mechanisms of interaction between lobeline and the dopamine transporter (DAT) or the vesicular monoamine transporter (VMAT-2) are not clear. The goal of this study was to elucidate the effects of lobeline on these transporters in a cell system co-expressing the DAT and VMAT-2. Lobeline caused release of [^{3}H]dopamine to a similar extent as reserpine (VMAT-2 inhibitor), but was less efficacious than methamphetamine or dopamine. Additionally, lobeline decreased the [^{3}H]dopamine-releasing effects of methamphetamine, unlike reserpine which increased release by methamphetamine. These results suggest that lobeline has unique properties at the DAT and VMAT-2 which may make it useful as a pharmacotherapeutic to treat methamphetamine abuse.

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1. Introduction

Lobeline has been proposed as a potential treatment for psychostimulant abuse [1], and may be a good treatment for methamphetamine abuse for a number of reasons. First, lobeline interacts with both the vesicular monoamine transporter (VMAT-2) [2] and the cell surface dopamine transporter (DAT) [3]. These proteins are key sites of action for methamphetamine and amphetamine [4,5]. Thus, lobeline may be able to directly inhibit the effects of methamphetamine. Secondly, in self-administration paradigms, lobeline is only a weak reinforcer in mice [6], and does not support self-administration in rats [7]. Lobeline also has a long history of use in people (see [1] for review), thus lobeline appears to have a low abuse potential. Further, lobeline decreases self-administration of methamphetamine in rats [8]. Lobeline interacts with nicotinic acetylcholine receptors, and has been used as a smoking cessation aid with mixed results (see [1]). Additionally, lobeline evokes calcium-independent (nonvesicular) release of [^{3}H]dopamine [3,9,10]. Previous studies suggest that lobeline is able to inhibit amphetamine-induced dopamine release [11], however the precise mechanism for this effect is unclear. The purpose of this study was to determine the mechanism(s) of lobeline's action at the DAT and VMAT-2, and its effects on methamphetamine-induced changes in [^{3}H]dopamine release by comparing the effects of lobeline to those of drugs with known activity at the transporters. Therefore, we used HEK-293 cells stably transfected with the DAT and VMAT-2 to minimize the potential confounds of more complicated systems. Primary cultures and synaptosomes contain numerous receptors, and endogenously synthesize and metabolize dopamine, which could interfere with data interpretation.

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2. Materials and methods

2.1. Materials

[^{3}H]dopamine (3,4-[7-^{3}H]dihydroxyphenylethylamine, 5.8–9.7 Ci/mmol) was purchased from Amersham Biosciences (Piscataway, NJ, U.S.A.). RTI-55 was a generous gift from Dr. F. Ivy Carroll at the Research Triangle Institute (Research Triangle Park, NC, U.S.A.), supplied by the National Institute on Drug Abuse drug supply program. Eco-Lume scintillation fluid was purchased from ICN biochemicals, Inc. (Aurora, OH, U.S.A.). All water used in these experiments was purified by a Milli-Q system (Millipore Corp., Bedford, MA, U.S.A.). The human isoform of the VMAT-2 cDNA was generously provided by Dr. Robert Edwards. Methamphetamine, lobeline, nomifensine, pargyline, reserpine, tropolone and most other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.) or from other commercial sources.

2.2. Cell culture

Human embryonic kidney cells expressing the human isoforms of the DAT and VMAT-2 were transfected, selected and grown as previously described [12,13]. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 0.05 U penicillin/streptomycin. Cell stocks were grown on 15 cm diameter tissue culture dishes in 10% CO_2 at 37 °C.

2.3. Drug-induced [^{3}H]dopamine release

Cells co-expressing the DAT and VMAT-2 were plated on poly-l-lysine-coated 24-well plates and grown until 80–100% confluent. The media was removed and [^{3}H]dopamine uptake (final volume 0.5 ml) was initiated by the addition of 20 nM [^{3}H]dopamine in Krebs-HEPES buffer (25 mM HEPES, 122 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 μM pargyline, 100 μM tropolone, 2 mg glucose/ml, 0.2 mg ascorbic acid/ml, pH 7.4) at 37 °C. [^{3}H]Dopamine uptake continued for 60 min, the time required to reach steady-state, and was terminated by decanting the buffer. After uptake, cells were washed once with 400 μl of buffer. Unlabelled dopamine, lobeline, RTI-55, nomifensine or reserpine was added to the cells, and cells were incubated at 22 °C for 40 min. The assay was terminated by decanting the buffer and addition of 250 μl of 0.1 M HCl to each well. The contents of each well were transferred to scintillation vials containing 4 ml of Ecolume scintillation fluid. Radioactivity was determined using liquid scintillation spectrometry. All experiments were conducted with duplicate determinations, unless otherwise noted.

2.4. Effects of drug pretreatment on methamphetamine-induced [^{3}H]dopamine release

Experiments were carried out as described above for drug-induced [^{3}H]dopamine release with the following alterations. Unlabelled dopamine, lobeline, RTI-55, nomifensine or reserpine was added to the cells, and cells were incubated at 22 °C for 10 min after which 100 μM methamphetamine was added. Cells were incubated for an additional 30 min at 22 °C (total incubation time was 40 min). All other aspects of the experiment were carried out as described above.

2.5. Data analysis

Prism Software (GraphPad Software, San Diego, CA, U.S.A.) was used to analyze all sigmoidal dose–response curves and to generate IC_{50} and EC_{50} values, maximal effect values, and to perform analysis of variance (ANOVA). Data shown are mean ± standard error of the mean (S.E.M.) from at least three independent experiments unless otherwise indicated. Microsoft Excel software (Microsoft Corp., Redmond, WA, U.S.A.) was used to perform *t*-tests (two-tailed, unpaired). The calculation of %Release and [^{3}H]DA release (%) was made as follows:

$$\frac{(\text{dpms recovered in vehicle treated cells} - \text{dpms recovered in drug treated cells})}{\text{dpms recovered in vehicle treated cells}}$$

Prior to drug treatment, both vehicle- and drug-treated cells had comparable levels of [^{3}H]dopamine, as uptake conditions and confluence were identical in both groups. Thus, %Release is a measure of the amount of [^{3}H]dopamine no longer retained within the cells as a result of drug treatment.

3. Results

To examine the specific mechanistic interactions of lobeline, we used DAT antagonists (nomifensine and RTI-55), a VMAT-2 antagonist (reserpine) and a DAT and VMAT-2 substrate (dopamine). Characterization of these cells, as determined by radioligand binding, suggests that the ratio of DAT to VMAT-2 (DAT:VMAT-2; 1.35 – 5.22) is comparable to that found in human brain (DAT:VMAT-2; 0.76 – 2.31) [12–14]. The ability of the drugs to induce [^{3}H]dopamine release was compared. In addition, the ability of these drugs to inhibit, or in some cases enhance, methamphetamine-induced [^{3}H]dopamine efflux was also examined. Previous experiments demonstrated that methamphetamine was not an effective releaser of [^{3}H]dopamine from cells expressing only the DAT under the conditions used here [13]. Therefore, experiments were performed using DAT and VMAT-2 co-expressing cells exclusively. The concentration of methamphetamine (100 μM) was chosen because it is approximately the EC_{70} for methamphetamine-induced [^{3}H]dopamine release from these cells and reliably induces release of ~40% of preloaded [^{3}H]dopamine (Table 1) [13]. A sub-maximal concentration of methamphetamine was chosen so we could monitor the effects of compounds that enhanced or attenuated methamphetamine-induced release of [^{3}H]dopamine.

An ANOVA was used to compare the [^{3}H]dopamine-releasing effect of lobeline to that of other drugs (Fig. 1, open symbols, Table 1). *Post hoc* comparisons were performed using Bonferroni corrections. Lobeline elicited less [^{3}H]dopamine release than the DAT and VMAT-2 substrate dopamine ($p < 0.001$), but more than the DAT antagonists nomifensine and RTI-55 ($p < 0.001$). In contrast, reserpine and lobeline induced similar levels of [^{3}H]dopamine release ($p > 0.05$). It is

Table 1 – Potentiation or attenuation of basal and methamphetamine-induced [^{3}H]dopamine release

Drug	EC_{50} (μM)[a]	%Release[a]	IC_{50} (μM) METH (100 μM) + drug[b]	%Release METH (100 μM) + drug[b]
Lobeline	50 ± 28	33 ± 3	135 ± 29	33 ± 1
Nomifensine	0.83 ± 0.82	14 ± 1*	13 ± 1	15 ± 3*
RTI-55	0.36 ± 0.33	14 ± 2*	0.36 ± 0.1	16 ± 2*
Drug	EC_{50} (μM)[a]	%Release[a]	EC_{50} (μM) METH (100 μM) + drug[b]	%Release METH (100 μM) + drug[b]
Dopamine	45 ± 6	73 ± 1*	96 ± 2	76 ± 3*
Reserpine	0.008 ± 0.004	37 ± 1	0.006 ± 0.002	57 ± 2*

Methamphetamine (100 μM) alone caused 41.0 ± 0.7% release of [^{3}H]dopamine. Data shown are the mean ± S.E.M. of at least three independent experiments, conducted as described in the text. %Release is the amount of [^{3}H]dopamine efflux in the presence of the most efficacious concentration of drug.

* $p < 0.001$, one-way ANOVA (with *post hoc* comparisons using Bonferroni corrections) comparing the %Release of lobeline to each of the other drugs.

[a] EC_{50} (the concentration of drug required to elicit 50% of the maximal [^{3}H]dopamine released) and maximal effect (%Release) of drug (Dopamine, lobeline, nomifensine, reserpine and RTI-55) alone.

[b] IC_{50} or EC_{50} (the concentration of drug required to inhibit or enhance 50% of the maximal [^{3}H]dopamine released following pretreatment with drug and treatment with methamphetamine) and maximal effect (% of release) of drug with methamphetamine (100 μM).

possible that the apparent release of [^{3}H]dopamine by nomifensine and RTI-55 (DAT antagonists) is inhibition of reuptake as opposed to drug-induced release. The inability to distinguish between these two types of release is a limitation of attached cell release assays such as these.

The effect of drugs on methamphetamine-induced [^{3}H]dopamine release was analyzed using paired *t*-tests, comparing the effect of methamphetamine alone with that of drug with methamphetamine. Nomifensine and RTI-55 were more effective at blocking methamphetamine-induced [^{3}H]dopamine release than lobeline ($p < 0.001$), although lobeline did block some of the methamphetamine-induced [^{3}H]dopamine release. Pretreatment with very low concentrations of lobeline caused a slight, but significant increase in methamphetamine-induced [^{3}H]dopamine release (4–5%). This increase is probably not meaningful. Dopamine and reserpine greatly enhanced release of [^{3}H]dopamine when combined with methamphetamine ($p < 0.001$). The combined effect of dopamine and methamphetamine was not larger than the effect of dopamine alone, suggesting that the mechanism by which dopamine and methamphetamine elicit release of [^{3}H]dopamine is similar. In contrast, lobeline appears to have a unique pharmacological profile when interacting with the DAT and VMAT-2, behaving unlike a DAT antagonist, VMAT-2 antagonist, or a DAT/VMAT-2 substrate.

4. Discussion

Lobeline inhibits binding of [^{3}H]dihydrotetrabenazine (a VMAT-2 antagonist) to the VMAT-2 with relatively high affinity (900 nM) [2], and also causes release of [^{3}H]dopamine from rat striatal slices [2,3]. Our results demonstrate that lobeline induces some release of [^{3}H]dopamine from mammalian cells expressing the DAT and VMAT-2. These results are corroborated by the observation that lobeline induces much greater [^{3}H]dopamine release than the DAT antagonists nomifensine and RTI-55 (Fig. 1). The effect of nomifensine or RTI-55 was likely due to inhibition of reuptake and not drug-induced release. That lobeline induced [^{3}H]dopamine release beyond the release caused by these DAT antagonists suggests that this effect is not due solely to inhibition of reuptake. The releasing effect may be due to an interaction with the DAT, VMAT-2, or both proteins. Previous work found that pretreatment of DAT and VMAT-2 expressing cells with dihydrotetrabenazine blocks the dopamine-releasing effects of lobeline [13]. Furthermore, the [^{3}H]dopamine release profile of lobeline, which has a much greater maximal effect than RTI-55 or nomifensine, mirrored that of the VMAT-2 inhibitor reserpine and elicited the same maximal amount of [^{3}H]dopamine release. The finding that reserpine alone caused release of [^{3}H]dopamine was somewhat surprising considering that others have found VMAT-2 inhibitors had no effect on dopamine efflux [15]. In contrast, Chantry et al. found that reserpine treatment alone was capable of releasing catecholamines from the adrenal medulla [16]. Although in some ways, the effects of lobeline mirror those of amphetamine and methamphetamine (interacting with the DAT and VMAT-2 and increasing cytosolic and extracellular levels of dopamine), lobeline is not a DAT substrate like amphetamine or methamphetamine. Our findings suggest that the dopamine-releasing effects of lobeline are mediated primarily by its interaction with the VMAT-2.

Although lobeline and reserpine each increase the release of [^{3}H]dopamine, they have different effects when administered prior to methamphetamine. Pretreatment with lobeline decreased methamphetamine-induced [^{3}H]dopamine release, while pretreatment with reserpine increased methamphetamine-induced [^{3}H]dopamine release. Lobeline also binds to the DAT (unlike reserpine), but with a lower affinity (29 μM) [11]. At low concentrations, lobeline may act through a mechanism similar to reserpine (co-administration of low concentrations of lobeline with methamphetamine slightly, but significantly increased [^{3}H]dopamine release (Fig. 1)). However, this small increase in released [^{3}H]dopamine may not be physiologically meaningful, as previous studies found that similarly low concentrations of lobeline actually inhibit

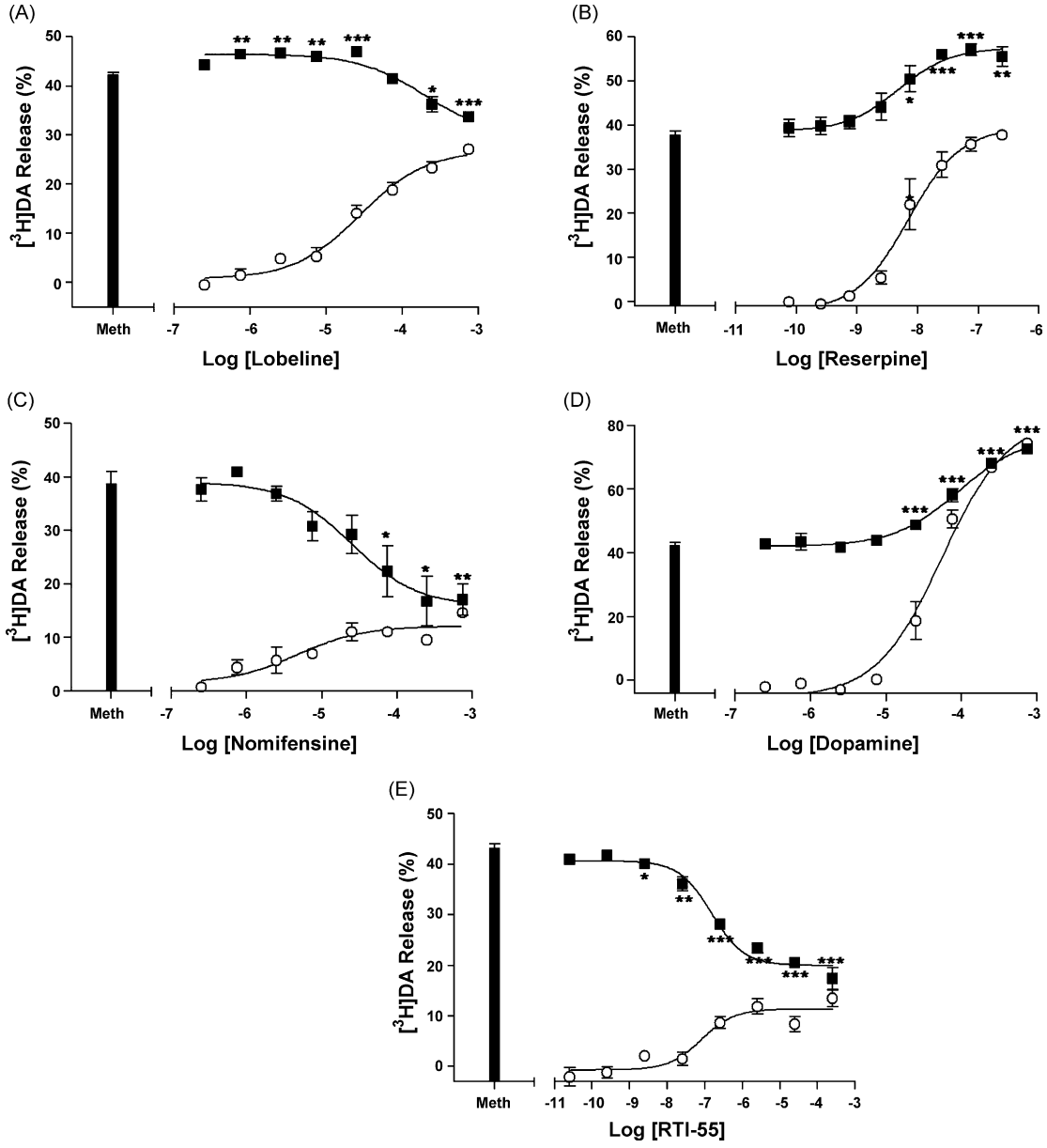

Fig. 1 – Modulation of methamphetamine-induced [^{3}H]dopamine. DAT and VMAT-2 expressing cells were preloaded with [^{3}H]dopamine. Various drugs were administered solely, or in combination with methamphetamine (METH) to examine their effects on [^{3}H]dopamine release. Bars represent [^{3}H]dopamine efflux in the presence of 100 μM methamphetamine. Solid squares (■) represent the combined effect on efflux of 100 μM methamphetamine and the concentration of drug as indicated on the *x*-axis. Open circles (○) represent [^{3}H]dopamine efflux in the absence of methamphetamine, and at the drug concentration indicated on the *x*-axis. Data shown are the mean ± S.E.M. of at least three independent experiments. An ANOVA was carried out comparing the [^{3}H]dopamine release induced by methamphetamine, and pretreatment with drug followed by methamphetamine. The effect of 100 μM METH was assessed on each plate to control for any differences in cell cycle or confluence. *Post hoc* comparisons were carried out with Bonferroni corrections. (*) Denotes $p < 0.05$; (**) denotes $p < 0.01$; (***) denotes $p < 0.001$ compared to 100 μM methamphetamine alone.

amphetamine-induced dopamine efflux from rat striatal slices [17]. At higher concentrations, lobeline may block [^{3}H]dopamine efflux through the DAT. Although the relatively high concentrations of lobeline required to achieve this effect may argue against its therapeutic use, results from *in vivo* experiments suggest that the concentration of lobeline required to decrease methamphetamine self-administration may be in the low micromolar range [7]. Another possibility is that lobeline blocks the interaction of methamphetamine with the VMAT-2 to decrease release of [^{3}H]dopamine. This latter possibility is unlikely, however, as pretreatment with reserpine enhanced methamphetamine-induced release of [^{3}H]dopamine, and pretreatment with dihydrotetrabenazine led to a similar increase in methamphetamine-induced

[^{3}H]dopamine release [13]. Thus, lobeline is unique in that it inhibits methamphetamine-induced [^{3}H]dopamine release, and also increases non-stimulated release of [^{3}H]dopamine. The latter mechanism involves its interaction with the VMAT-2, while the former effect is not a result of lobeline's interaction with the VMAT-2, but perhaps is also due to interaction with the DAT. The low level of lobeline-induced [^{3}H]dopamine release may be sufficient to reduce methamphetamine craving. A similar mechanism is hypothesized to play a role in the effects of drugs like buproprion that are under consideration as anti-cocaine and anti-methamphetamine treatment medications [18]. The [^{3}H]dopamine release caused by lobeline alone, contrasted with lobeline's ability to inhibit methamphetamine-induced [^{3}H]dopamine release (Fig. 1A) suggests that lobeline's actions are a combination of indirect releasing and blockade mechanisms. This pharmacological profile makes lobeline a potential and unique treatment for methamphetamine abuse.

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