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Uptake and Release Effects of Diethylpropion and its Metabolites with Biogenic Amine Transporters

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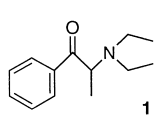
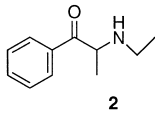
Abstract—Three metabolites of diethylpropion **(1)**, (±)-2-ethylamino-1-phenyl-propan-1-one **(2)**, (1*R*,2*S*)-(−)-*N,N*-diethylnorephedrine **(3a)** and (1*S*,2*R*)-(−)-*N,N*-diethylnorephedrine **(3b)** were synthesized. Their uptake and release effects with biogenic amine transporters were evaluated. A major finding of this study is that the in vivo activity of diethylpropion on biogenic amine transporters is most likely due to metabolite **2** as diethylpropion **(1)** and the metabolites **3a** and **3b** showed little or no effect in the assays studied. These studies also revealed that **2** acted as a substrate at the norepinephrine (IC_{50} = 99 nM) and serotonin transporters (IC_{50} = 2118 nM) and an uptake inhibitor at the dopamine transporter (IC_{50} = 1014 nM). The potent action of **2** at the NE transporter supports the hypothesis that amphetamine-type subjective effects may be mediated in part by brain norepinephrine. © 2000 Published by Elsevier Science Ltd.

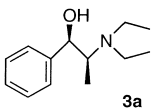
Diethylpropion (**1**) is a clinically available anorectic agent which shares properties with cocaine and amphetamines and has been tested as a possible treatment agent for cocaine dependence.[1–5] When administered either orally or subcutaneously to human volunteers, diethylpropion (**1**) produces amphetamine-like subjective and sympathomimetic effects.[6] However, diethylpropion (**1**) is 10-fold less potent than D-amphetamine in supporting self-administration behavior in baboons.[5]



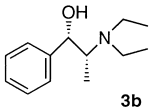
1



2



3a



3b

Diethylpropion (**1**) is readily absorbed from the human gastrointestinal tract and then rapidly and extensively metabolized to 2-ethylamino-1-phenyl-propan-1-one (**2**) and *N,N*-diethylnorephedrine (**3**), which are excreted almost exclusively via the renal pathway.[7] Dangor et al.[7] stated that the metabolites, not the parent drug, mediate the biological effects of diethylpropion (**1**). However, Dangor et al.[7] did not present any data to support that statement and a literature search did not reveal any additional information as to the biological effects of metabolites **2** and **3**. Thus, the major goal of this study was to determine the in vitro biological activity of diethylpropion (**1**) and the two major metabolites at the biogenic amine transporters.

Ligands which bind to transporters and are themselves transported are known as transporter substrates. On the other hand, ligands which bind to transporters and are not transported are termed uptake inhibitors since they block the transport of substrate-type ligands. In previous work[8] we described an in vitro method for determining the potency of a test drug to act as a substrate at the DA, 5-HT and NE (biogenic amine) transporters. Thus, in the present study we determined the activity of diethylpropion and its major metabolites as substrates and uptake inhibitors of the biogenic amine transporters by

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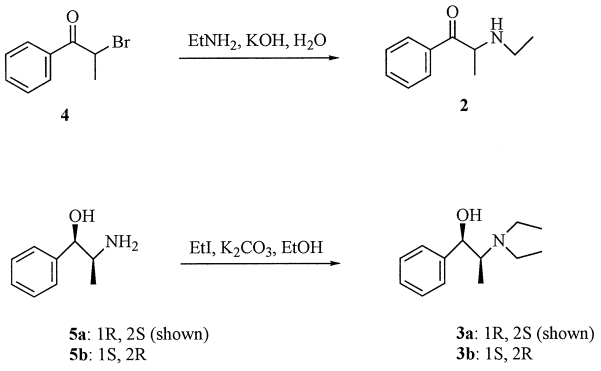
measuring their ability to block the uptake of [^{3}H]DA, [^{3}H]5-HT and [^{3}H]NE in vitro and to release [^{3}H]DA, [^{3}H]5-HT and [^{3}H]NE in vitro.

Compound **2** was synthesized as a racemate from 2-bromopropiophenone.[9] Bromide **4** was converted smoothly to amine **2**[10] by treatment with saturated aqueous potassium hydroxide and ethylamine in water. Metabolite **3** was synthesized as two enantiomers, **3a**[11] and **3b**,[11] from (1*R*,2*S*)-(−)-norephedrine **(5a)** and (1*S*,2*R*)-(+)-norephedrine **(5b)**, respectively (Scheme 1). The transformation was accomplished by heating under reflux a mixture of norephedrine, EtI and K_2CO_3 in EtOH.[12]



Scheme 1.

The effect of compounds **1**, **2**, **3a** and **3b** on the uptake of [^{3}H]DA, [^{3}H]NE and [^{3}H]5-HT was determined as previously described.[8] Briefly, rat brain synaptosomes were incubated with the radiolabeled neurotransmitter and test drugs in a physiological buffer. Reactions were terminated by rapid filtration over Whatman GF/B filters. Inhibition of uptake was quantitated by measuring the amount of tritium remaining on the filters. In previous work,[8] we also described an in vitro method for determining the potency of a test compound to act as a substrate at the DA, 5-HT and NE (biogenic amine) transporters. Briefly, rat brain synaptosomes were incubated to steady state with radiolabeled neurotransmitter. Compounds were added and the reactions were terminated 5 min later by rapid filtration over Whatman GF/B filters. Compound-induced [^{3}H]neurotransmitter release was quantitated by measuring the

Table 1. Comparison of neurotransmitter uptake inhibition and release by diethylpropion **(1)** and its metabolites **2, 3a** and **3b**[a]

Compounds	Dopamine (DA)		Serotonin (5-HT)		Norepinephrine (NE)	
	Uptake IC_{50}±SD (nM)	Release IC_{50}±SD (nM)	Uptake IC_{50}±SD (nM)	Release IC_{50}±SD (nM)	Uptake IC_{50}±SD (nM)	Release IC_{50}±SD (nM)
(+)-Amphetamine	34±6	24.8±3.5	38,307±170	1765±94	38.9±1.8	7.07±0.95
1	14,990±540	> 10,000	311,000±28,000	> 10,000	18,100±1500	> 10,000
2	1014±80	> 1000	3840±240	2118±98	360±29	99.3±6.6
3a	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
3b	> 10,000	> 10,000	> 10,000	> 10,000	6820±390	> 10,000

[a][^{3}H]DA, [^{3}H]5-HT and [^{3}H]NE uptake and release assays were conducted as described in ref. 8. Values are means of three experiments.

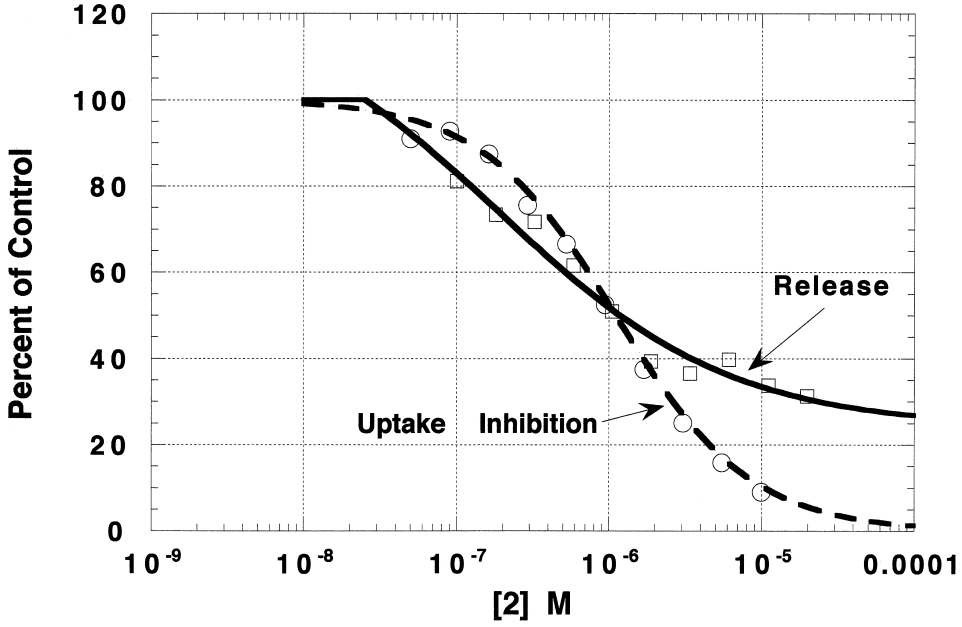


Figure 1. Effects of **2** on [^{3}H]DA uptake and [^{3}H]DA release.

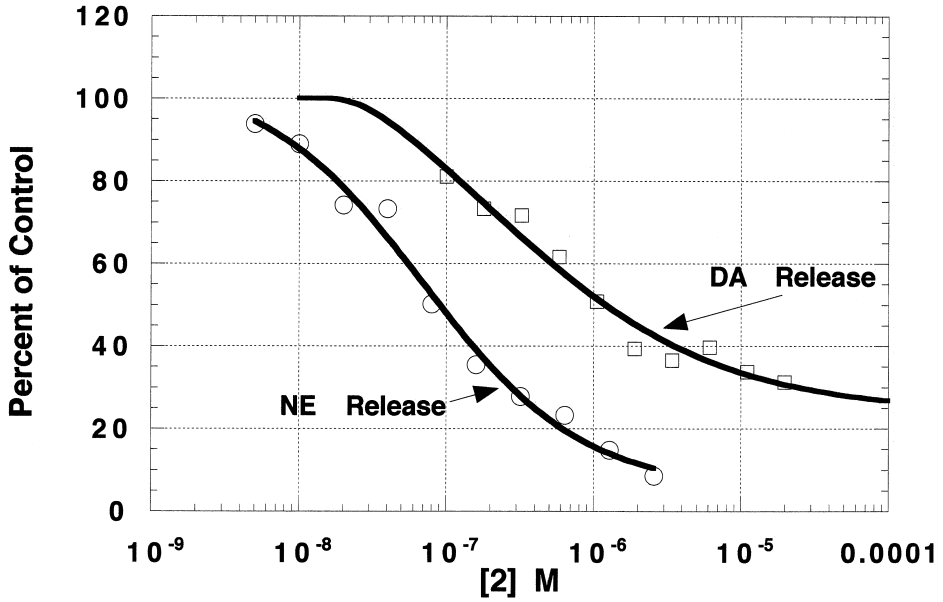


Figure 2. Effects of **2** on [^{3}H]DA release and [^{3}H]NE release.

amount of tritium remaining on the filters. The effects of compounds **1**, **2**, **3a** and **3b** on [^{3}H]DA, [^{3}H]5-HT and [^{3}H]NE uptake and release are shown in Table 1.

The results indicated that diethylpropion (**1**) and its metabolites **3a** and **3b** were inactive (IC_{50} > 6824 nM) in the uptake and release assays. Compound **2**, the *N*-dealkyated metabolite of diethylpropion, was the active component. In both uptake and release assays, compound **2** was most potent at the NE transporter, followed by DA, and then the 5-HT transporter. At the NE transporter, compound **2** was a potent substrate, releasing [^{3}H]NE with an IC_{50} value of 99 nM and inhibiting uptake somewhat less potently (IC_{50} = 360 nM). The effect of compound **2** on [^{3}H]DA uptake and [^{3}H]DA release was quite different (Fig. 1). Compound **2** inhibited [^{3}H]DA uptake with an IC_{50} value of 1014 nM. In the release assay, compound **2** showed the profile observed with uptake inhibitors:[8] a biphasic effect with a plateau (IC_{50} > 1000 nM). Thus, at the DA transporter, compound **2** is an uptake inhibitor, not a substrate. At the 5-HT transporter, compound **2** had the profile of a weak substrate, stimulating [^{3}H]5-HT release (IC_{50} = 2118 nM) with about the same potency as it inhibited [^{3}H]5-HT uptake (IC_{50} = 3840 nM).

An interesting finding to emerge from this study is that diethylpropion is inactive in both the release and uptake inhibition assays. Thus, diethylpropion is a prodrug and, our results indicate, metabolite **2** likely mediates the biological effects of diethylpropion. An interesting finding to emerge from our study is that compound **2** is a substrate at the NE transporter and an uptake inhibitor at the DA transporter. Its most potent action is to release NE (Fig. 2). Indeed, compound **2** is about 10 times more potent at the NE transporter than at the DA transporter. This observation suggests that therapeutic doses of diethylpropion would likely increase brain NE, not DA, perhaps explaining why diethylpropion failed to substitute for cocaine in clinical trials.[4] Finally, the fact that therapeutic doses of diethylpropion produce amphetamine-type subjective effects in humans[6] provides further support for the hypothesis that amphetamine-type subjective effects may be mediated in part by brain NE.[13]

Acknowledgements

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10. Data for **2**: crystallized as **2**·HCl salt from MeOH, mp 181 °C; ^{1}H NMR ($CDCl_3$, 350 MHz) δ 1.13 (t, *J* = 8.4 Hz, 3H), 1.31 (d, *J* = 8.4 Hz, 3H), 2.60 (m, 2H), 4.33 (q, *J* = 8.4 Hz, 2H), 7.47–7.99 (m, 5H).

11. Data for **3a**: crystallized as **3a**·HCl salt from MeOH, mp $>200\,^{\circ}C$; ^{1}H NMR ($CDCl_3$, 350 MHz) δ 0.88 (d, $J=8.1$ Hz, 3H), 0.99 (t, $J=8.4$ Hz, 6H), 2.46 (q, $J=8.4$ Hz, 4H), 3.02 (m, 1H), 5.68 (d, $J=5.6$ Hz, 1H), 7.27 (m, 5H); $[\alpha]_D^{23}-22.9$ (*c* 1.0, MeOH). **3b**: $[\alpha]_D^{23}$ 22.7 (*c* 1.0, MeOH).

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