# DHE Pharmacology Revisited: Does a Broad Receptor Profile Molecule Treat the Whole Migraine?

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## Introduction

- Migraine is a complex and multifaceted disorder with distinct phases, which can include the premonitory, aura, headache, postdrome, and interictal phases; therefore, it is important to consider treating the whole migraine[1,2]
- Most migraine therapies target a very narrow set of receptors focused mainly on headache pain[3]
  - Triptans are 5-hydroxytryptamine (5-HT$_{1B/1D}$) receptor agonists, while some also have affinity at the 5-HT$_{1F}$ receptor in clinical dosing[3]
  - Newer therapies include ditans (5-HT$_{1F}$ receptor agonists), gepants (calcitonin gene-related peptide [CGRP] receptor antagonists), and anti-CGRP monoclonal antibodies[3]
- Dihydroergotamine (DHE) mesylate has a long, established history as an effective migraine therapy and is well regarded by physicians because of its[4]:
  - Rapid onset[4]
  - Efficacy against a full range of acute symptoms of migraine[5,6]
  - Minimal risk of medication overuse[4]
- We have previously proposed a hypothetical model illustrating how DHE mesylate may target the whole migraine, suggesting it may exert a greater influence than single receptor agonists/antagonists over migraine pathophysiology and migraine phases due to its broad pharmacological activity reported in the literature[6]

## Objective

- The aim of this study was to build upon previous work that demonstrated broad receptor coverage of DHE mesylate to update our understanding of DHE receptor activity

## Methods

### *In Vitro* Screening for Functional Receptor Activity of DHE Mesylate and Sumatriptan Succinate

- Functional receptor activity of DHE mesylate was screened against 168 G protein-coupled receptors (GPCRs) using the gpcrMAX Assay Panel (Eurofins DiscoverX), which encompasses 60 distinct receptor families
- The gpcrMAX panel evaluates ß-arrestin recruitment and was carried out in both agonist and antagonist modes
- For agonist activity, cells expressing the various receptors were incubated with DHE mesylate (10 µM) or sumatriptan succinate (10 µM)
  - ß-arrestin associated chemiluminescence was then measured, and the percent activity, relative to a known agonist, for each receptor was calculated
  - Agonist effects were considered significant if receptor activity was >30%
- For antagonist activity, cells were pre-incubated with DHE mesylate (10 µM) or sumatriptan succinate (10 µM), followed by the addition of a known agonist at the specific EC$_{80}$ (0% inhibition) concentration
  - Following the incubation period, chemiluminescence was measured and the percent antagonist activity was calculated
  - Antagonist effects were considered significant if receptor activity was inhibited by >50%

### Radioligand Competition Binding Assays

- Radiolabeled ligand binding assays were performed by Eurofins Cerep (Celle l'Evescault, France) and Eurofins Panlabs (Taipei, Taiwan), in which a range of DHE mesylate concentrations was used to assess binding affinity to select GPCRs: 5-HT$_3$, 5-HT$_{4E}$, 5-HT$_{1B}$, adrenoceptor alpha ($\alpha_{2B}$), and dopaminergic ($D_2$ and $D_5$)
- Membrane fractions of human recombinant cell lines expressing these GPCRs and radiolabeled ligands specific to each receptor were incubated with various concentrations of DHE mesylate encompassing a range that covered the human plasma $C_{max}$ of DHE mesylate (2 nM) after dosing with INP104
- IC$_{50}$ (half maximal inhibitory concentration) determinations were based on the % binding inhibition of the radiolabeled ligand

## Results

### *In Vitro* Screening for Functional Receptor Activity of DHE Mesylate

- DHE mesylate (10 µM) demonstrated agonist activity at $\alpha_{2B}$ CXC chemokine receptor 7 (CXCR7), $D_{2S, 2L, 5}$, and 5-HT$_{1A,1B,2A,2C,5A}$ receptor subtypes (**Table 1**)
- DHE mesylate (10 µM) demonstrated antagonist activity at $\alpha_{1B,2A,2C}$, calcitonin receptor (CALCR)–receptor activity modifying protein 2 (RAMP2), $D_{1,3,4,5}$, and 5-HT$_{1F}$ receptor subtypes (**Table 2**)
- Since DHE mesylate (10 µM) exhibited fairly strong antagonist activity at the 5-HT$_{1F}$ receptor and agonist activity at CXCR7 in the gpcrMAX screening, a more thorough assessment of ß-arrestin recruitment was performed to determine the activity of DHE mesylate at these receptors
  - The IC$_{50}$ for DHE mesylate at the 5-HT$_{1F}$ receptor was 149 nM, and the EC$_{50}$ (half maximal effective concentration) was 6 µM at CXCR7

### *In Vitro* Screening for Functional Receptor Activity of Sumatriptan Succinate

- Sumatriptan succinate (10 µM) demonstrated agonist activity at 5-HT$_{1B,1E,1F,5A}$ receptor subtypes (**Table 1**)
- There was no antagonist activity at any of the receptors screened

### Table 1. gpcrMAX Agonist Mode Results

| Receptor/Receptor Subtype | % Activity | |
|---|---|---|
| | DHE Mesylate | Sumatriptan Succinate |
| $\alpha_{2B}$ | 88 | - |
| **CXCR7** | 83 | - |
| $D_{2L}$ | 70 | - |
| $D_{2S}$ | 60 | - |
| $D_5$ | 57 | - |
| **5-HT$_{1A}$** | 100 | - |
| **5-HT$_{1B}$** | 52 | 115 |
| **5-HT$_{1E}$** | - | 51 |
| **5-HT$_{1F}$** | - | 83 |
| **5-HT$_{2A}$** | 56 | - |
| **5-HT$_{2C}$** | 76 | - |
| **5-HT$_{5A}$** | 66 | 48 |

Note: A dash (-) indicates activity did not meet cutoff criteria to demonstrate an effect at a specific receptor. 5-HT$_{1D}$ activity was not available in this screen.
$\alpha_{2B}$ = adrenoceptor alpha 2B; CXCR7 = CXC chemokine receptor 7; $D_{2L}$= dopaminergic receptor 2L; $D_{2S}$ = dopaminergic receptor 2S; $D_5$ = dopaminergic receptor 5. 5-HT$_{1A}$ = 5-hydroxytryptamine receptor 1A; 5-HT$_{1B}$ = 5-hydroxytryptamine receptor 1B; 5-HT$_{1E}$ = 5-hydroxytryptamine receptor 1E; 5-HT$_{1F}$ = 5-hydroxytryptamine receptor 1F; 5-HT$_{2A}$ = 5-hydroxytryptamine receptor 2A; 5-HT$_{2C}$ = 5-hydroxytryptamine receptor 2C; 5-HT$_{5A}$ = 5-hydroxytryptamine receptor 5A.

### Table 2. gpcrMAX Antagonist Mode Results

| Receptor/Receptor Subtype | % Inhibition |
|---|---|
| $\alpha_{1B}$ | 95 |
| $\alpha_{2A}$ | 115 |
| $\alpha_{2C}$ | 124 |
| **CALCR-RAMP2** | 57 |
| $D_1$ | 71 |
| $D_3$ | 91 |
| $D_4$ | 83 |
| $D_5$ | 54 |
| **5-HT$_{1F}$** | 92 |

5-HT$_{1F}$ = 5-hydroxytryptamine receptor 1F; $\alpha_{1B}$ = adrenoceptor alpha 1B; $\alpha_{2A}$ = adrenoceptor alpha 2A; $\alpha_{2C}$ = adrenoceptor alpha 2C; CALCR-RAMP2 = calcitonin receptor–receptor activity modifying protein 2; $D_1$ = dopaminergic receptor 1; $D_3$ = dopaminergic receptor 3; $D_4$ = dopaminergic receptor 4; $D_5$ = dopaminergic receptor 5.

### Radioligand Competition Binding Assays

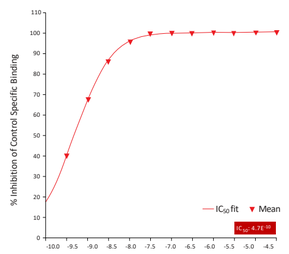
- Radioligand competition binding assays revealed that DHE mesylate did not bind to the 5-HT$_3$ receptor at concentrations up to 300 nM and bound with limited affinity to the 5-HT$_{4E}$ and $D_5$ receptors demonstrating IC$_{50}$ values of 230 and 370 nM, respectively (**Table 3**)
- DHE mesylate bound with higher affinity to the $D_2$, 5-HT$_{1B}$, and $\alpha_{2B}$ receptors with IC$_{50}$ values of 0.47, 0.58, and 2.8 nM, respectively (**Figures 1–3, Table 3**)

### Table 3. Radiolabeled Ligand Binding Assay Results

| Receptor/Receptor Subtype | IC$_{50}$ (nM) |
|---|---|
| **5-HT$_{1B}$** | 0.58 |
| **5-HT$_3$** | >300 |
| **5-HT$_{4E}$** | 230 |
| $\alpha_{2B}$ | 2.8 |
| $D_2$ | 0.47 |
| $D_5$ | 370 |

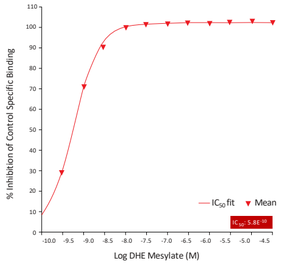
5-HT$_{1B}$ = 5-hydroxytryptamine receptor 1B; 5-HT$_3$ = 5-hydroxytryptamine receptor 3; 5-HT$_{4E}$ = 5-hydroxytryptamine receptor 4E; $\alpha_{2B}$ =adrenoceptor alpha 2B; $D_2$ = dopaminergic receptor 2; $D_5$ = dopaminergic receptor 5; IC$_{50}$ = half maximal inhibitory concentration.

### Figure 1. Percent Inhibition of Radioligand Binding to $D_2$ in the Presence of DHE Mesylate



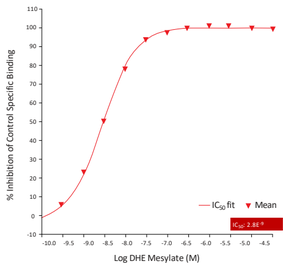

Note: Analysis was for $D_{2S}$.
DHE = dihydroergotamine; IC$_{50}$ = half maximal inhibitory concentration.

### Figure 2. Percent Inhibition of Radioligand Binding to 5-HT$_{1B}$ in the Presence of DHE Mesylate




DHE = dihydroergotamine; IC$_{50}$= half maximal inhibitory concentration.

### Figure 3. Percent Inhibition of Radioligand Binding to $\alpha_{2B}$ in the Presence of DHE Mesylate




DHE = dihydroergotamine; IC$_{50}$= half maximal inhibitory concentration.

## Discussion

### Clinical Relevance:

- Previous reports in the literature have measured either affinity, binding, kinetics, or activity at different concentrations of different DHE forms (DHE or DHE salts), offering a fragmented picture of DHE pharmacology[7,8]
- A functional readout of the ligand interaction at therapeutic concentrations provides a more clinically meaningful understanding of the mechanism of action of DHE
- Findings of agonist activity at the 5-HT$_{1B}$ receptor
  - Activation of 5-HT$_{1B}$ produces vasoconstriction of intracranial extracerebral blood vessels, which may be involved in alleviation of headache pain symptoms[7-9]
  - Agonist activity may be involved in the inhibition of CGRP release and result in pain relief[9]
- Findings of agonist activity at the $D_2$ and $\alpha_{2B}$ receptor subtypes contrasts with previous findings of antagonist activity[7,8]
  - Transient hypertension has been associated with agonist activity at peripheral $\alpha_{2B}$ receptors[10]; however, increased blood pressure has not been associated with some newer DHE mesylate products that are currently in development[11]
  - Possibly, discrepancies in results may be the outcome of different methodologies or higher concentrations of DHE mesylate used in the present study[7]
- It is unlikely that DHE mesylate is active at CXCR7 or 5-HT$_{1F}$ receptors under physiologically relevant conditions
  - Activity was only observed with >1.0 µM DHE mesylate at CXCR7
  - IC$_{50}$ of 149 nM at 5-HT$_{1F}$ suggests limited efficacy
- A limitation of this study is that 5-HT$_{1D}$ was not screened because a cell line with human 5-HT$_{1D}$ expression was not available for the assay

## Conclusion

- Unlike other migraine therapeutics, which only target single receptor subtypes,[3] DHE mesylate has a broad receptor pharmacology and may exhibit a greater impact on the migraine cycle
- DHE mesylate (10 µM) was screened for functional activity at 168 GPCRs, and demonstrated:
  - Agonist activity at 10 receptors including 5-HT$_{1A,1B,2A,2C,5A}$, $D_{2S,2L,5}$, $\alpha_{2B}$, and CXCR7
  - Antagonist activity at 9 receptors including $D_{1,3,4,5}$, $\alpha_{1B,2A,2C}$, 5-HT$_{1F}$, and CALCR-RAMP2
  - A broader receptor profile than sumatriptan succinate
- Further investigation demonstrated high binding affinity at $D_2$, 5-HT$_{1B}$, and $\alpha_{2B}$ receptor subtypes using clinically relevant doses of DHE mesylate
- Data reported here may explain the high consistency and sustained effect of DHE mesylate when used to acutely treat migraine[4,12]

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### Disclosures and Acknowledgments

Sheena K. Aurora, Sutapa Ray, Kelsey Satterly, Lisa McConnachie, Stephen B. Shrewsbury and John Hoekman are full-time employees and stockholders of Impel NeuroPharma. Peter Goadsby consults for Impel NeuroPharma, Aeon Biopharma, Alder Biopharmaceuticals, Amgen, Allergan, Biohaven Pharmaceuticals Inc, Clexio, Eli Lilly and Company, eNeura Inc, EpaleX, Electrocore LLC, GlaxoSmithKline, Lundbeck, MundiPharma, Novartis, Pfizer, Praxis, Santara Therapeutics, Sanofi, Satsuma, Teva Pharmaceuticals, WL Gore, and Dr. Reddy's. Peter Goadsby has received research grants from Amgen, Celgene, and Eli Lilly and Company and stock options from Trigemina Inc. This research was sponsored by Impel NeuroPharma. Editorial support was provided by IMPRINT Science and funded by Impel NeuroPharma.