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# Cabergoline: Pharmacology, ocular hypotensive studies in multiple species, and aqueous humor dynamic modulation in the Cynomolgus monkey eyes

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ABSTRACT

The aims of the current studies were to determine the *in vitro* and *in vivo* ocular and non-ocular pharmacological properties of cabergoline using well documented receptor binding, cell-based functional assays, and *in vivo* models. Cabergoline bound to native and/or human cloned serotonin-2A/B/C ($5HT_{2A/B/C}$), $5HT_{1A}$, $5HT_7$, $\alpha_{2B}$, and dopamine-2/3 ($D_{2/3}$) receptor subtypes with nanomolar affinity. Cabergoline was an agonist at human recombinant $5HT_2$, $5HT_{1A}$ and $D_{2/3}$ receptors but an antagonist at $5HT_7$ and $\alpha_2$ receptors. In primary human ciliary muscle (h-CM) and trabecular meshwork (h-TM) cells, cabergoline stimulated phosphoinositide (PI) hydrolysis ($EC_{50} = 19 \pm 7$ nM in TM; 76 nM in h-CM) and intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) mobilization ($EC_{50} = 570 \pm 83$ nM in h-TM; $EC_{50} = 900 \pm 320$ nM in h-CM). Cabergoline-induced $[Ca^{2+}]_i$ mobilization in h-TM and h-CM cells was potently antagonized by a $5HT_{2A}$-selective antagonist (M-100907, $K_i = 0.29$–$0.53$ nM). Cabergoline also stimulated $[Ca^{2+}]_i$ mobilization more potently via human cloned $5HT_{2A}$ ($EC_{50} = 63.4 \pm 10.3$ nM) than via $5HT_{2B}$ and $5HT_{2C}$ receptors. In h-CM cells, cabergoline (1 μM) stimulated production of pro-matrix metalloproteinases-1 and -3 and synergized with forskolin to enhance cAMP production. Cabergoline (1 μM) perfused through anterior segments of porcine eyes caused a significant (27%) increase in outflow facility. Topically administered cabergoline (300–500 μg) in Dutch-belted rabbit eyes yielded 4.5 μM and 1.97 μM levels in the aqueous humor 30 min and 90 min post-dose but failed to modulate intraocular pressure (IOP). However, cabergoline was an efficacious IOP-lowering agent in normotensive Brown Norway rats (25% IOP decrease with 6 μg at 4 h post-dose) and in conscious ocular hypertensive cynomolgus monkeys (peak reduction of $30.6 \pm 3.6$% with 50 μg at 3 h post-dose; $30.4 \pm 4.5$% with 500 μg at 7 h post-dose). In ketamine-sedated monkeys, IOP was significantly lowered at 2.5 h after the second topical ocular dose (300 μg) of cabergoline by 23% ($p < 0.02$) and 35% ($p < 0.004$) in normotensive and ocular hypertensive eyes, respectively. In normotensive eyes, cabergoline increased uveoscleral outflow ($0.69 \pm 0.7$ μL/min–$1.61 \pm 0.97$ μL/min, $n = 13$; $p < 0.01$). However, only seven of the eleven ocular hypertensive monkeys showed significantly increased uveoscleral outflow. These data indicate that cabergoline's most prominent agonist activity involves activation of $5HT_2$, $5HT_{1A}$, and $D_{2/3}$ receptors. Since $5HT_{1A}$ agonists, $5HT_7$ antagonists, and $\alpha_2$ antagonists do not lower IOP in conscious ocular hypertensive monkeys, the $5HT_2$ and dopaminergic agonist activities of cabergoline probably mediated the IOP reduction observed with this compound in this species.



## 1. Introduction

Glaucoma is a major sight threatening optic neuropathy that is the second leading cause of blindness in the developed world (Quigley, 1996). Therapeutic agents currently used for the treatment of glaucoma reduce elevated intraocular pressure (IOP), one of the most important causative risk factors associated with this disease (Sugrue, 1997; Clark and Yorio, 2003; Sharif and Klimko, 2007). Although many classes of drugs are known to reduce IOP and some are used clinically (e.g. FP-class prostaglandins, β-blockers, alpha-2 agonists, carbonic anhydrase inhibitors) (Sugrue, 1997; Clark and Yorio, 2003; Sharif and Klimko, 2007), there continues to be a need for more efficacious agents that provide long duration of IOP reduction and agents with fewer undesirable ocular and systemic side-effects.

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The biogenic amine serotonin (5-hydroxytryptamine, 5HT) has been identified in the aqueous humor of humans (Martin et al., 1988; Martin et al., 1992; Veglio et al., 1998), and numerous classes of 5HT receptors have been identified in relevant ocular tissues, such as the rabbit and human iris–ciliary body (Tobin et al., 1988; Chidlow et al., 1995; Harris et al., 2001, 2002), bovine ciliary epithelium (Inoue-Matsuhisa et al., 2003), human ciliary muscle (Lograno and Romano, 2003; Sharif et al., 2006a,b), and human trabecular meshwork (Sharif and Senchyna, 2006; Sharif et al., 2006a,b). These findings have generated interest regarding the role that serotonin might have in modulating and controlling IOP. Indeed, a number of $5HT_2$ receptor agonists, such as (R)-DOI (May et al., 2003; Gabelt et al., 2005), α-methyl 5HT (May et al., 2003), and AL-34662 (May et al., 2006; Sharif et al., 2007) have been shown to be effective in lowering IOP in the lasered monkey model of ocular hypertension and represent a potential new class of topical ocular hypotensive agents. Another amine, dopamine (DA), has also been found in the aqueous humor (Cooper et al., 1984; Trope et al., 1987) and various DA receptor subtypes are present in relevant ocular cells/tissues (Mancino et al., 1992). In addition, dopaminergic agonists have been shown to elicit ocular hypotension in a number of species, mainly rabbits, but under specific conditions (Chu et al., 1999; Ogidigben et al., 1993; Chiou and Li, 1992; Crosson et al., 1987; Potter and Burke, 1982; Ohia et al., 2005) and by apparently suppressing aqueous humor production (Lograno et al., 1990; Chu et al., 2000). Two dopaminergic agonists, bromocriptine and SDZ-GLC-756, administered topical ocular, were shown to lower IOP in some human subjects (Geyer et al., 1987; Mekki et al., 1983; Prunte et al., 1997), while intravenous fenoldopam raised IOP (Piltz et al., 1998). Interestingly, a $D_1$ receptor agonist, ibopamine, increased aqueous humor production in glaucomatous and normotensive patients and increased IOP (Virno et al., 1996).

Cabergoline (Fig. 1) is an agent that has been used to treat Parkinsonian symptoms (Grondin et al., 1996) and to reduce prolactin release by virtue of its DA agonist activity (Colao et al., 2006). It also has shown promise as a potential drug to treat restless leg syndrome (Nardone et al., 2006). Since a number of other ergot derivatives (bromocriptine, lergotrile, lisuride and pergolide) have been shown to reduce IOP in some animals (Potter et al., 1998; Mekki et al., 1983), we decided to evaluate the pharmacological properties of cabergoline in ocular and non-ocular cells, to determine its IOP-lowering activity in a number of animal species, and to determine its ability to modulate aqueous humor dynamics in cynomolgus monkey eyes.

## 2. Material and methods

### 2.1. Receptor binding assays

[$^{125}$I]-(1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane) ([$^{125}$I]-DOI; 80 pM final) binding to $5HT_2$ receptors in washed rat and human cerebral cortex homogenates was performed in the absence or presence of 10 μM methiothepin to define total and non-specific binding, respectively, as previously described (May et al., 2003). Additional studies to determine the relative affinity and selectivity of $5HT_2$ antagonists using [$^{125}$I]-DOI binding to CHO cells expressing human cloned $5HT_{2A,}$ $5HT_{2B,}$ $5HT_{2C}$ receptors were conducted at Cerep Inc. (Poitiers, France).

Ligand binding to $5HT_{1A}$ receptors was performed as previously described (Sharif et al., 2004) using membranes from CHO cells expressing the cloned human $5HT_{1A}$ receptor. Other radioligand binding assays for various receptors, ion-channels, transporters, etc. were performed at NovaScreen Biosciences Corp. (Hanover, MD) using 1 nM, 100 nM and 10 μM of the test agent using standard published procedures (Sweetnam et al., 1993,1995).

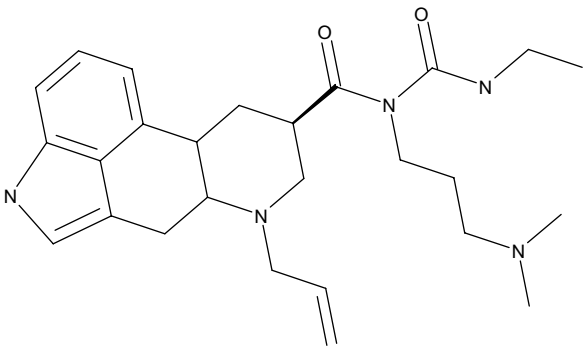


**Fig. 1.** The chemical structure of cabergoline is shown. The chemical name of cabergoline is: 1-[(6-allylergolin-8b-yl)carbonyl]-1-[3-(dimethyl-amino)propyl]-3-ethylurea.

### 2.2. Cyclic AMP production in cultured cells

Direct agonist-dependent inhibition or stimulation of forskolin-stimulated cyclic AMP formation was measured by a method described previously (Crider et al., 2003; Sharif et al., 2004). Immortalized human corneal epithelial (CEPI-17-CL4) cells expressing endogenous $5HT_7$ receptors (Crider et al., 2003), human cloned $5HT_{1A}$ receptors expressed in Chinese hamster ovary cells (Sharif et al., 2004), HT-29 human colonic epithelial cells expressing endogenous alpha-2A receptors (Bylund, 1992) and h-CM cells were used for cAMP response studies with cabergoline.

### 2.3. cAMP measurements

cAMP production was measured using an EIA kit purchased from Amersham Pharmacia Biotech (Piscataway, NJ). This assay was conducted according to the package insert in an automated manner using the Biomek® 2000 robot (Beckman Instruments; Fullerton, CA) (Crider et al., 2003; Sharif et al., 2004).

### 2.4. Cell culture conditions

Primary cultures of normal human ciliary muscle (h-CM) cells were established from CM tissue isolated from five different human donors' eyes with no reported ocular diseases (ages 33–75 years; three males and two females) (Zhan et al., 1998; Husain et al., 2002; Sharif et al., 2006). h-CM cells were prepared from normal human cadaver eyes using the procedure previously described (Zhan et al., 1998; Husain et al., 2002). The human eyes were obtained from Life-Point Ocular Tissue Division (Storm Eye Institute, MUSC, Charleston, SC). Briefly, ciliary muscles were dissected with the aid of a dissecting microscope under sterile conditions, cleaned, and cut into 1–2 mm pieces. The explants were placed in DMEM containing 2 mg/mL collagenase type IA, 10% fetal bovine serum (FBS), and 50 μg/mL gentamicin and then incubated for 1–2 h at 37 °C with occasional shaking. When a major part of the explant was dispersed into single cells or groups of cells, the cell suspension was centrifuged at 200 *g* for 10 min and resuspended in DMEM supplemented with 10% FBS, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B and maintained in a 5% $CO_2$ humidified atmosphere. The confluent cells were sub-cultured at a split ratio of 1:4 using 0.05% trypsin and 0.02% EDTA. Cultures of primary human trabecular meshwork (h-TM) cells were established from TM tissue derived from up to 20 human donors' eyes (ages 48 days–95 years) as previously described (Sharif and Senchyna, 2006; Sharif et al., 2006a,b).

### 2.5. Phosphoinositide (PI) turnover assays

The relative agonist activity of serotonergic compounds at the 5-$HT_2$ receptor was determined *in vitro* using the ability of the