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SHORT COMMUNICATION

Direct Effects of Ephedrine Isomers on Human β-Adrenergic Receptor Subtypes

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ABSTRACT. Ephedrine and its alkaloids are used for the treatment of asthma, nasal congestion, and obesity. Ephedrine, with two chiral centers, exists as four isomers that exhibit direct and indirect effects on both α- and β-adrenergic receptors (AR). Our main goal was to study the direct effects of the ephedrine isomers on human β_1-, β_2-, and β_3-AR expressed in Chinese hamster ovary cells. Previous work indicated that the ephedrine isomers are inactive as agonists and that 1*R*,2*S*-ephedrine is more potent than the 1*S*,2*R*-isomer as an antagonist of catecholamine-induced lipolysis in rat adipose tissue (Lee *et al.*, *J Pharmacol Exp Ther* **190**: 249–259, 1974). Stimulation of adenylyl cyclase, associated with cyclic AMP accumulations, was measured by a luciferase reporter gene assay. On human β_1-AR, the rank order of potency (EC_{50} values, maximal response relative to isoproterenol = 100%) was 1*R*,2*S*-ephedrine (0.5 μM, 68%) > 1*S*,2*R*-ephedrine (72 μM, 66%) > 1*S*,2*S*-*pseudo*ephedrine (309 μM, 53%) = 1*R*,2*R*-*pseudo*ephedrine (1122 μM, 53%). On human β_2-AR, the rank order of potency was 1*R*,2*S*-ephedrine (0.36 μM, 78%) > 1*R*,2*R*-*pseudo*ephedrine (7 μM, 50%) ≥ 1*S*,2*S*-*pseudo*ephedrine (10 μM, 47%) > 1*S*,2*R*-ephedrine (106 μM, 22%). Only 1*R*,2*S*-ephedrine showed significant agonist activity on human β_3-AR with an EC_{50} = 45 μM and a maximal response of 31%. Our studies demonstrated that (a) stereoselective and rank order differences exist among the direct effects of ephedrine isomers; (b) 1*R*,2*S*-ephedrine is the most potent of the four ephedrine isomers on all three human β-AR; and (c) 1*R*,2*S*- ephedrine was nearly equipotent as a β_1-/β_2-AR agonist and the only isomer possessing weak partial agonist activity on β_3-AR. BIOCHEM PHARMACOL **58**;5:807–810, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. ephedrine; human β-adrenergic receptors; chirality; reporter gene; cyclic AMP; luciferase

The *Ephedra* species of plants are among the oldest known plants used for their medicinal properties. The major active constituent in the *Ephedra* species is the alkaloid ephedrine, which was isolated about 100 years ago. Ephedrine possesses two asymmetrical carbon atoms and exists as four isomers designated as 1*R*,2*S*- and 1*S*,2*R*-ephedrine and 1*R*,2*R*- and 1*S*,2*S*-*pseudo*ephedrine [1]. These absolute configurations will be used throughout this paper. The naturally occurring isomers are 1*R*,2*S*-ephedrine and 1*S*,2*S*-*pseudo*ephedrine. 1*R*,2*S*-Ephedrine and 1*R*,2*R*-*pseudo*ephedrine have been used as nasal decongestants, bronchodilators, and CNS stimulants. Recently, there has been a resurgence in the use of herbal products containing extracts of *Ephedra*, especially for the attempted treatment of obesity. There have been reports of the adverse effects of herbal products containing *Ephedra* alkaloids. Some of these side-effects include hypertension, tremors, myocardial infarction, seizures, and stroke, and intakes have also resulted in fatalities [2–4].

The pharmacological and toxicological effects of the ephedrine isomers are mediated through the α- and β-AR† and can be elicited by either direct interactions with the receptors or indirectly by causing a release of endogenous catecholamines and/or preventing their reuptake [5, 6]. The contribution of these direct and indirect interactions to the final effects of the ephedrine isomers *in vivo* has remained controversial, since it is difficult to resolve these actions independently. Moreover, data remain scarce on the direct effects of these ephedrine isomers on human β-AR, and especially on the human β_3-AR that are involved in lipolysis and non-shivering thermogenesis. In this study, we have examined the direct effects of the four ephedrine isomers on human β_1-, β_2-, and β_3-AR subtypes expressed in CHO cells.

MATERIALS AND METHODS

Cell Culture

CHO cells stably expressing β_1-, β_2-, or β_3-AR were grown in 150 cm^2 Corning culture flasks with Ham's F12 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (100 U/mL), and streptomycin (100 μg/

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† *Abbreviations*: AR, adrenergic receptor(s); cAMP, adenosine-3′,5′-cyclophosphate; CHO, Chinese hamster ovary; CRE, cAMP response element; and LUC, luciferase.

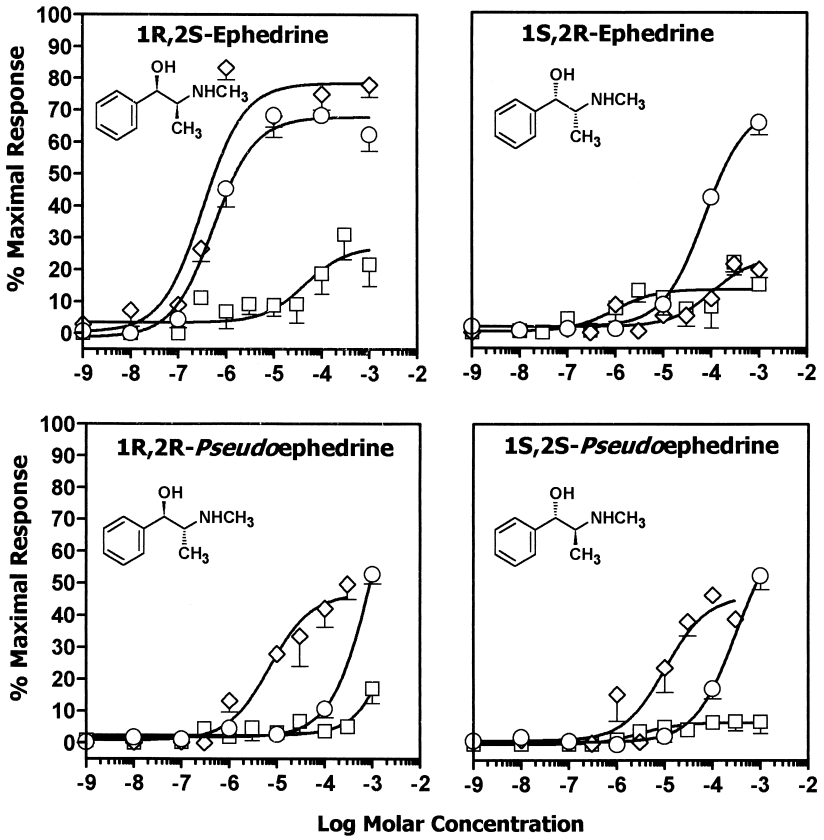

FIG. 1. Cyclic AMP-mediated effects of ephedrine isomers on CHO cells expressing the human β_1- (○), β_2- (◇), and β_3- (□) AR. Data are presented as percentages of the maximal response obtained with (-)-isoproterenol (10^{-7} M). The basal levels for LUC induction, as measured in arbitrary light units were 2600 ± 740 for the cells expressing the human β_1-, β_2-, and β_3-AR. Maximal levels in response to 10^{-7} M (-)-isoproterenol were at least 15-fold greater than basal levels. Individual points are expressed as mean ± SEM, N = 4–6.

mL). Medium was changed every 48 hr until the cells were confluent. Upon confluency, the cells were detached by trypsinization (0.05% trypsin EDTA, 10 min). All cell culture reagents were obtained from Life Technologies. Human β_1- and β_2-AR expressing CHO cells were obtained from Dr. L. J. Emorine (Institut Cochin de Genetique Moleculaire), and the human β_3-AR expressing CHO cells from Dr. Stephen Liggett (University of Cincinnati).

Transfection

A recently developed sensitive reporter gene assay [7, 8] was used to elucidate the direct effects of the ephedrine isomers on human β-AR subtypes. CHO cells stably expressing β_1-, β_2-, or β_3-AR were transfected with a 6 CRE-LUC plasmid (20 μg/400 μL volume; 1×10^7 to 2×10^7 cells) using electroporation with a single 70 msec, 150 V pulse [7]. The CRE-LUC plasmid was provided by Dr. A. Himmler (Ernst Boehringer Institut) [8]. The transfected CHO cells were seeded at a density of 40,000/well in 96-well microtiter plates (Culturplate®, Packard) and allowed to grow for 20 hr.

Assay

After 20 hr, the cells were treated with various drug concentrations for 4 hr, which was found to be optimum during time–course analyses performed earlier [7]. When antagonist studies were performed, the compounds were added 15 min prior to the addition of isoproterenol (100 nM). Following drug exposures, the cells were lysed, and luciferase activity was measured using the LucLite® assay kit (Packard). Changes in light production were measured by a Topcount® luminometer (Packard). Plasmid isolation was conducted using the DH5α strain of *Escherichia coli* cells and a Qiagen® plasmid purification kit. 1*R*,2*S*-Ephedrine hydrochloride was obtained from Mallinckrodt. 1*S*,2*R*-Ephedrine hydrochloride, 1*S*,2*S*-*pseudo*ephedrine hydrochloride, and 1*R*,2*R*-*pseudo*ephedrine base were obtained from the Sigma Chemical Co.

RESULTS AND DISCUSSION

Recombinant cell lines expressing homogeneous populations of receptors provide invaluable tools for characterizing the direct effects of drugs on specific receptor subtypes. Our

TABLE 1. Cyclic AMP effects of ephedrine isomers on human β-adrenoceptor subtypes expressed in CHO cells

	β_1-Adrenoceptors*			β_2-Adrenoceptors*			β_3-Adrenoceptors*		
Compound	pK_{act} (*a*)	I.A. (%)	R.P.	pK_{act} (*n*)	I.A. (%)	R.P.	pK_{act} (*n*)	I.A. (%)	R.P.
(-)-Isoproterenol	8.13 ± 0.10 (10)	100	1	9.46 ± 0.09 (16)	100	1	7.74 ± 0.07 (16)	100	1
1*S*,2*R*-Ephedrine	4.14 ± 0.07 (6)	66	9,773	ND† (4)	21	ND	ND (4)	22	ND
1*R*,2*S*-Ephedrine	6.26 ± 0.12 (6)	68	74	6.48 ± 0.24 (4)	78	955	4.35 ± 0.35 (4)	31	2,454
1*S*,2*S*-*Pseudo*ephedrine	3.51 ± 0.05 (6)	53	41,688	5.00 ± 0.25 (4)	47	28,843	ND (4)	7	ND
1*R*,2*R*-*Pseudo*ephedrine	2.95 ± 0.19 (6)	53	151,358	5.15 ± 0.16 (4)	50	20,420	ND (4)	17	ND

* Data for I.A. (intrinsic activity) are expressed as percent responses to that of (-)-isoproterenol (3×10^{-8} M = 100%).
pK_{act} values are expressed as means ± SEM. R.P. = relative potency, calculated as EC_{50} of drug/EC_{50} of (-)-isoproterenol.
† ND = not determined due to low intrinsic activity.

studies used human β-AR subtypes expressed in CHO cells and the four ephedrine isomers (Fig. 1). In this regard, the rank order of potency of the ephedrine isomers on human β-AR expressed in CHO cells was:

β_1-AR: 1*R*,2*S*-ephedrine > 1*S*,2*R*-ephedrine > 1*S*,2*S*-*pseudo*ephedrine = 1*R*,2*R*-*pseudo*ephedrine

β_2-AR: 1*R*,2*S*-ephedrine > 1*R*,2*R*-*pseudo*ephedrine = 1*S*,2*S*-*pseudo*ephedrine > 1*S*,2*R*-ephedrine

β_3-AR: 1*R*,2*S*-ephedrine > 1*S*,2*R*-ephedrine > 1*S*,2*S*-*pseudo*ephedrine = 1*R*,2*R*-*pseudo*ephedrine

1*R*,2*S*-Ephedrine was the most potent of the four ephedrine isomers on all three human β-AR subtypes (Fig. 1). This result is in agreement with previously reported results using isolated tissues and *in vivo* measurements [6, 9]. Additionally, 1*R*,2*S*-ephedrine showed the highest intrinsic activity (I.A. = 78%) on human β_2-AR and was most potent on the human β_1-AR when comparing relative potency values (Table 1). This has been shown in other studies and also corresponds to the major side-effects of ephedrine ingestion such as increased heart rate [10]. The relative potency values normalized to (-)-isoproterenol have been reported in order to correct for differences, if any, in the receptor expression levels in the CHO cells. All the other isomers of ephedrine are partial agonists on the human β_1- and β_2-AR subtypes. Both *pseudo*ephedrine isomers (1*S*,2*S*-*pseudo*ephedrine and 1*R*,2*R*-*pseudo*ephedrine) exhibited similar activity profiles within the same human β-AR subtype (Table 1). Of the ephedrine isomers (1*S*,2*R*-ephedrine and 1*R*,2*S*-ephedrine), the 1*R*,2*S*-isomer was more than 100 times more potent on both human β_1- and β_2-AR (Table 1).

The β_3-AR is known to mediate lipolysis and brown adipocyte thermogenesis [11]. Since many herbal products claiming anti-obesity properties have *Ephedra* alkaloids as major constituents, we wanted to investigate if any of these properties were mediated by any direct action on the human β_3-AR. Of the ephedrine isomers, only 1*R*,2*S*-ephedrine showed any appreciable direct activity on the human β_3-AR (Fig. 1). However, this effect was at very high concentrations and appeared inconsequential since the therapeutic plasma levels of ephedrine in humans following ingestion of ephedrine in the form of *Ephedra sinica* capsules (19.4 mg ephedrine), an ephedrine tablet (20 mg ephedrine), or an ephedrine solution (22 mg) are 81 ng/mL [10], 73.9 ng/mL, and 79.4 ng/mL [12], respectively. This translates into a plasma concentration of 360–400 nM. A comparison of this concentration range to those observed in our *in vitro* studies would suggest that ephedrine elicits only β_1-/β_2-AR mediated effects. However, at these plasma concentrations, it is unlikely that ephedrine elicits any effects on the human β_3-AR even though studies done in rodents [13, 14] show brown adipocyte thermogenesis by ephedrine isomers. Liu *et al.* [15] have reported that ephedrine-induced thermogenesis in humans is mediated partly by the β_3-AR due to lack of total blockade by nadolol (a non-selective β-AR antagonist). It is possible that these effects on thermogenesis may be mediated through the putative β_4-adrenoceptor [16]. The inactive isomers of ephedrine on the human β_3-AR were also tested for their inhibition of isoproterenol-mediated cAMP responses. The isomers of ephedrine did not show any antagonist activity on the human β_3-AR in the presence of 100 nM isoproterenol (data not shown).

In conclusion, our studies demonstrated that the rank order potencies of the four ephedrine isomers on the human β-AR subtypes are different. 1*R*,2*S*-(-)-Ephedrine was the most potent of the four ephedrine isomers on all three human β-AR subtypes and the only ephedrine isomer to possess some agonist activity on the human β_3-AR.

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