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Pharmacological Effects of Ephedrine Alkaloids on Human α_1- and α_2-Adrenergic Receptor Subtypes

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Effects of ephedrine alkaloids on human adrenoceptors

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Abstract

Ephedra species of plants have both beneficial and adverse effects primarily associated with the presence of ephedrine alkaloids. Few reports have appeared that examine the direct actions of ephedrine alkaloids on human subtypes of adrenergic receptors (AR). In the present study, ephedrine alkaloids were evaluated for their binding affinities on human α_{1A}-, α_{1B}-, α_{1D}-, α_{2A}-, α_{2B}-, and α_{2C}-AR subtypes expressed in HEK and CHO cells. Cell-based reporter gene assays were used to establish functional activity of ephedrine alkaloids at α_{1A}-, α_{2A}- and α_{2C}-ARs. The data showed that ephedrine alkaloids did not activate α_1- and α_2-ARs, and that they antagonized the agonist-mediated effects of phenylephrine and medetomidine on α_1- and α_2-ARs, respectively. As in the binding studies, 1R,2R- and 1R,2S–ephedrine showed greater functional antagonist activity than the 1S,2R- and 1S,2S-isomers. The rank order of affinity for the isomers was: 1R,2R > 1R,2S > 1S,2R > 1S,2S. The rank order of potencies of alkaloids containing a 1R,2S-configuration was norephedrine ≥ ephedrine >> N-methylephedrine. These studies have demonstrated that orientation of the β-hydroxyl group on the ethylamino side chain and the state of N-methyl substitution are important for α-AR binding and functional activity of the ephedrine alkaloids. In conclusion, the ephedrine isomers and analogs studied did not exhibit any direct agonist activity and were found to possess moderate antagonist activities on cloned human α-ARs. The blockade of pre-synaptic α_{2A}- and α_{2C}-ARs may have a pharmacological role in the direct actions of *Ephedra* alkaloids.

Introduction

The *Ephedra* or *Ma Huang* species of plants are widely used for their medicinal properties. The principal active constituent in the *Ephedra* species is ephedrine, which possesses two chiral centers, and can exists as four isomers designated as 1R,2S- and 1S,2R-ephedrine; and 1R,2R- and 1S,2S-*pseudo*ephedrine (Griffith and Johnson, 1995). Naturally occurring ephedrine alkaloids include mainly 1R,2S-ephedrine, 1S,2S-*pseudo*ephedrine, 1R,2S-norephedrine, 1R,2S-N-methylephedrine, and 1S,2S-nor*pseudo*ephedrine. These *Ephedra*/ephedrine alkaloids have been used as nasal decongestants, bronchodilators, and CNS stimulants (Kalix et al., 1991, Hoffman, 2001); and *Ephedra* has also been used for the treatment of obesity (Arch et al., 1984; Liu et al., 1995). In recent years, there have been numerous reports of adverse reactions resulting from intake, especially in excessive doses, of herbal products containing extracts of *Ephedra* as weight loss aids (Josefson, 1996). Other effects such as hypertension, tremors, myocardial infarction, seizures, and stroke have resulted in fatalities (Chua et al., 1988; Haller and Benowitz, 2000; White et al., 1997). The US FDA has recently prohibited the sale of dietary supplements containing *Ephedra*. However, herbal products containing *Ephedra* remain in use in other countries.

The beneficial and adverse effects of ephedrine and related analogs are known to be mediated via the α- and β-adrenergic receptors (ARs) and can be elicited by either direct interactions with the receptors as agonists or antagonists, or indirectly by either causing a release of endogenous catecholamines and/or by preventing their neuronal reuptake (Trendelenburg, 1963; Trendelenburg et al., 1963; Vansal and Feller, 1999; Patil et al, 1967; Rothman et al., 2003; Wellman et al., 2003). The relative contribution of

these direct and indirect interactions to the pharmacological effects of the ephedrine isomers *in vivo* on ARs has remained controversial. *Ephedra* alkaloids of the 1R,2S-configuration have generally been shown to exert direct AR effects, whereas isomers of the 1S,2S- and 1S,2R- configuration have indirect actions (Patil et al., 1965, 1967; Patil, 1967; LaPidus et al., 1967; Tye et al., 1967; Waldeck and Widmark, 1985; Kawasuji et al., 1996; Vansal and Feller, 1999; Liles et al., 2006). In studies using ephedrine isomers, indirect actions were predominantly demonstrated by depletion of norepinephrine tissue stores using reserpine, and more recently Rothman et al. (2003), using a battery of *in vitro* tests, reported that the most potent action of ephedrine and norephedrine analogs and isomers, is as substrates for norepinephrine transporters.

Although there are reports of the direct effects of ephedrine isomers on human β-ARs (Vansal and Feller, 1999) no comprehensive information exists on the direct interactions (agonist and antagonist potencies) of the ephedrine isomers and related *Ephedra* alkaloids at human α-ARs**.** Rothman et al. (2003) found that the ephedrine isomers and analogs were not agonists on human α_{1A} – or α_{2A}-AR, but they did not test compounds for either antagonist activity or on other α-AR subtypes. In the present study, the direct effects of the ephedrine isomers and naturally occurring ephedrine alkaloids have been systematically examined as agonists and antagonists on cloned human α_1- (α_{1A}, α_{1B}, α_{1D}) and α_2- (α_{2A}, α_{2B}, α_{2C}) AR subtypes expressed in host cells. A preliminary report of our work has appeared (Ma et al., 2004).

Materials and Methods

Materials

Human embryonic kidney (HEK293) cells stably expressing homogeneous populations of the human α_{1A}-, α_{1B}-, α_{1D}-AR subtypes were obtained from Dr. Kenneth Minneman (Emory University). Human α_{2A}-, α_{2B}- and α_{2C}-AR subtypes stably expressed in CHO cells were obtained from Drs. Marc Caron, Robert Lefkowitz (Duke University, Durham, NC) and Stephen Liggett (University of Cincinnati, Cincinnati, OH). The ephedrine alkaloids used in this study were provided by Dr. Popat N. Patil (The Ohio State University, Columbus, OH), or purchased from Sigma (St. Louis, MO, USA). The compounds were dissolved in water or in a 1:5 mixture of DMSO and water. Stock solutions of 10 mM were prepared fresh daily and diluted in water to appropriate concentrations for the studies. [^{3}H]Rauwolscine and [^{3}H]prazosin were obtained from NEN Life Science Products (Wellesley, MA, USA). The phorbol ester (12-O-tetradecanoylphorbol-13-acetate, TPA) response element-luciferase gene (TRE-LUC) and cyclic-AMP response element-luciferase reporter gene (6 CRE-LUC) were kindly provided by Dr. A. Himmler (Boehringer Ingelheim Research & Development, Vienna, Austria). All cell culture reagents were obtained from Life Technologies (Gaitherburg, MD, USA). Other chemicals were purchased from Sigma Chemical Company.

Cell culture

HEK293 cells stably expressing α_{1A}, α_{1B}, α_{1D}–AR subtypes were grown in 150 cm^2 Corning culture flasks with DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin G (100 U/ml), streptomycin (100 µg/ml) and geneticin (100 µg/ml). Upon confluence, the cells were detached by gentle scraping. CHO cells stably expressing α_{2A}–, α_{2B}–, and α_{2C}–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 G (100 U/ml), streptomycin (100 µg/ml) and geneticin (100 µg/ml). All cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Media were changed every 48 h until the cells were confluent. Upon confluence, the cells were detached by trypsinization (0.25% trypsin EDTA) for 2 min.

Competitive radioligand binding assays

Radioligand binding assays were carried out on intact HEK293 cells stably expressing α_{1A-}, α_{1B-}, α_{1D} –AR subtypes and intact CHO cells stably expressing α_{2A}-, α_{2B}-, and α_{2C} -AR subtypes as described by Lalchandani et al. (2002). The detached cells were washed, centrifuged with Tris-EDTA buffer, pH 7.4; containing 50 mM Tris, 20 mM di-sodium EDTA and 154 mM NaCl, in which they were finally suspended. The radioligand was used at a fixed concentration of 0.1 µCi in the absence and presence of various concentrations (the range was 10^{-10} - 10^{-3} M or 10^{-11} -10^{-4} M) of competing drugs. The drugs were added to the cells (50,000) in 50 mM Tris-EDTA buffer to a total volume of 2.0 ml and allowed to incubate at 37 °C for 1 hr. Nonspecific binding was determined in the presence of 10 µM phentolamine. Reactions were terminated by rapid filtration through Whatman GF/C filters using a Brandel 12 R cell harvester followed by washes with ice-cold buffer twice. Radioactivity on the dried filter discs was measured using a liquid scintillation analyzer (Packard, TRI-CARB 2900TR, Meriden, CT). The displacement curves were plotted and the Ki values of the test ligands for the receptor subtypes were determined using Graph Pad Prism (Graph Pad Software Inc. San Diego, CA, USA). The percent specific binding was determined by dividing the difference between total bound (DPM) and non-specific bound (DPM) by the total bound (DPM).

Functional assays

A recently developed sensitive reporter gene assay (Lalchandani et al., 2002) was used to elucidate the effects of the ephedrine alkaloids on human α_2-AR subtypes. In α_1-AR studies, a phorbol ester response element (TRE)-LUC plasmid provided by Dr. A. Himmler (Stratowa et al., 1995) was used to study the functional effects of the ephedrine alkaloids. HEK293 cells stably expressing α_{1A}–AR were transfected with the TRE-LUC plasmid (40 µg/ml) using electroporation (70 msec, single pulse, 150 volts). The transfected cells were seeded at a density of 50,000 cells/well in microtiter plates (Cultureplate®, Packard) in 200 µl media and allowed to grow for 24 hours with incubation at 37 °C (5% CO_2). After 24 hr, the cells were treated with varying drug concentrations for a period of 20 hours, which was found to be optimum during time-course analyses performed earlier (data not presented). When antagonist studies were performed, the compounds were added 15 min prior to the addition of agonist, L-phenylephrine. Following drug exposure, the cells were lysed, and luciferase activity was measured using the Luclite assay kit (Packard, Gronigen, the Netherlands).

In α_2-AR studies, the cell-based cyclic-AMP response element-luciferase reporter gene assay (CRE-LUC) was conducted as described previously by Lalchandani et al. (2002). The CHO cells stably expressing α_{2A}- and α_{2C}-ARs were transfected with the 6 CRE-LUC plasmid (40 µg/ml) using electroporation, (70 msec, single pulse, 150 volts). The transfected cells were seeded at a density of 50,000 cells/well in microtiter plates (Cultureplate®, Packard, Meriden, CT) in 200 µl media and allowed to grow for 24 hours with incubation at 37°C (5% CO_2). After 24 hr, the cells were treated with varying drug

concentrations for 4 hr. When antagonist studies were performed, the compounds were added 15 min prior to the addition of the direct adenylate cyclase activator forskolin (3 μM). Following drug exposure, the cells were lysed, and luciferase activity was measured using the Luclite assay kit (Packard, Gronigen, the Netherlands).

For both, α_{1A}-, and α_{2A}- and α_{2C}-AR cells, the luciferase activity (cps, changes in light production) was determined using a TopCount^{TM} Microplate Scintillation & Luminescence Counter (Packard, Model B9904, Meriden, CT). Data were normalized relative to luciferase changes of L-phenylephrine (3×10^{-4} M = 100%) and forskolin (3×10^{-6} M = 100%) in α_{1A}- and α_{2A}-/α_{2C}-AR expressed in HEK293 and CHO cells, respectively.

Data accumulation and analysis

For binding studies in cell lines, varying concentrations of each drug were added in duplicate within each experiment, and the individual molar inhibitory concentration−50 (IC_{50}) values were determined using Graph Pad Prism software. The Ki value of each ligand was determined according to the equation described by Cheng and Prusoff (1973), and final data presented as pKi ± SEM of n = 6 experiments. The concentration-dependent reversal of forskolin-induced (3 μM) luciferase activity changes in CHO cells by medetomidine and selected ephedrine analogs was used to assess agonist activity, and data for medetomidine were expressed as molar effective concentration-50 (EC_{50}) values ± SEM of n = 6 experiments. Antagonist activities of the ephedrine analogs were determined by their addition prior to incubation with a fixed concentration of the α_2-AR agonist, medetomidine (10 μM). Data were expressed as molar inhibitory

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concentration-50 (IC_{50}) values ± SEM of at least $n = 6$ experiments. Differences between means of binding affinities and functional responses of individual drugs were analyzed using a paired *t* test. Values were considered to be statistically significant when $P < 0.05$.

Results

Receptor binding

In order to obtain a better understanding of the direct effect of ephedrine isomers on α–ARs, the binding affinity of ephedrine isomers and closely related analogs were compared on human α_1- and α_2 –AR subtypes. The structures of all the ephedrine isomers are provided in Fig. 1. With selected *Ephedra* alkaloids of the 1R,2S-configuration, the rank order of competitive displacement of radioligands on both α_{1A}- and α_{2A}-AR subtypes was norephedrine ≥ ephedrine > N-methylephedrine (see Fig. 2). The comparative affinities of these *Ephedra* alkaloids as displacing ligands were much lower than the standard agonist analogs, L-phenylephrine and medetomidine. Calculated affinity values of the standard agonists and all of the ephedrine alkaloids on α_{1A}- and α_{2A}-AR subtypes are given in Tables 1 and 2. 1R,2S-Norephedrine, lacking an N-methyl group as in 1R,2S-ephedrine, showed increased binding affinities at the subtypes tested. The presence of an additional N-methyl group, as in 1R,2S-N-methylephedrine, showed decreased binding affinities as compared to 1R,2S-ephedrine at the α_{1A}-, α_{2A}-, and α_{2C}-AR. The rank order of affinity (Ki values) of ephedrine isomers on human α_1-AR in HEK293 cells was: 1R,2R-*pseudo*ephedrine > 1R,2S-ephedrine > 1S,2R-ephedrine > 1S,2S-*pseudo*ephedrine at α_{1A}-AR; 1S,2R-ephedrine > 1R,2R-*pseudo*ephedrine >

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1R,2S-ephedrine > 1S,2S-*pseudo*ephedrine at α_{1B}-AR; and 1R,2R-*pseudo*ephedrine > 1R,2S-ephedrine >1S,2R-ephedrine >1S,2S-*pseudo*ephedrine at α_{1D}-AR. The affinities of 1R,2S-norephedrine and 1S,2S-nor*pseudo*ephedrine were similar to 1R,2S-ephedrine at the α_{1A}-AR. The rank order of affinity (Ki values) of ephedrine isomers on human α_2-AR in CHO cells was: 1R,2R-*pseudo*ephedrine > 1R,2S-ephedrine > 1S,2R-ephedrine > 1S,2S-*pseudo*ephedrine at α_{2A}-AR; 1R,2R-*pseudo*ephedrine > 1R,2S-ephedrine = 1S,2R-ephedrine > 1S,2S-*pseudo*ephedrine at α_{2B}-AR; and 1R,2R-*pseudo*ephedrine > 1R,2S-ephedrine > 1S,2R-ephedrine > 1S,2S-*pseudo*ephedrine at α_{2C}-AR. As shown in Tables 1 and 2, the 1R,2R- and the 1R,2S-ephedrine isomers had greater affinities than the 1S,2R- and 1S,2S-isomers at all α-ARs, except at the α_{1B}-AR. 1S,2S-*Pseudo*ephedrine displayed the lowest binding affinity at all α-ARs. Furthermore, the binding affinities of 1R,2S-norephedrine and 1S,2S-nor*pseudo*ephedrine were similar to that of 1R,2S-ephedrine on these subtypes.

Direct agonist effects of ephedrine alkaloids on α-AR subtypes

Cell-based reporter gene assays were used to establish functional activity at the α_{1A}-, α_{2A}-, and α_{2C}-ARs. The direct agonist effects of ephedrine isomers on α_1-ARs were studied. As shown in Figure 3, luciferase assay studies using the TRE-LUC plasmid showed that 1R,2S-ephedrine, 1S,2R-ephedrine, 1R,2R-*pseudo*ephedrine, 1S,2S-*pseudo*ephedrine, 1R,2S-norephedrine, 1R,2S-N-methylephedrine and 1S,2S- nor*pseudo*-ephedrine had little effects on α_{1A}- AR at the highest concentration tested 0.3 mM. These isomers gave a response that was <12% of the L-phenylephrine maximum. L-phenylephrine activated α_{1A}-AR, giving an EC_{50} value of 2.01 ± 0.39 μM.

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The agonistic effect of the ephedrine isomers on α_{2A}- and α_{2C}-AR subtypes were examined for their abilities to reverse forskolin-induced cAMP elevation measured using the 6 CRE-luciferase (CRE-LUC) reporter gene assay. The results in Fig. 3 show that none of the ephedrine alkaloids (1R,2S-ephedrine, 1S,2R-ephedrine, 1R,2R-*pseudo*-ephedrine, 1S,2S-*pseudo*ephedrine, 1R,2S-norephedrine, 1R,2S-N-methylephedrine and 1S,2S-nor*pseudo*ephedrine) reversed forskolin-induced cAMP elevations in the α_{2A}- and α_{2C}-AR subtypes. However, the agonist medetomidine significantly reversed forskolin-induced cAMP elevations in the α_{2A}- and α_{2C}-AR subtypes with EC_{50} values of 79.8 ±3.5 and 78.3 ± 7.3 nM (see Fig. 3). These data indicate that the ephedrine alkaloids do not act as direct agonists on these AR subtypes.

Antagonistic effects of ephedrine alkaloids on α-AR subtypes

Studies were undertaken to examine the antagonistic effects of ephedrine alkaloids on α_{1A}-, α_{2A}- and α_{2C}-AR subtypes in HEK293 and CHO cells (see Table 3, Fig. 4). The results showed that ephedrine isomers and analogs antagonized the effects of the agonists, L-phenylephrine on α_1-ARs (Fig. 4a) and medetomidine on α_2-ARs (Fig. 4b). In Table 3, it was noteworthy that the antagonistic potencies of ephedrine isomers on the α_{2A}- and α_{2C}-AR subtypes were considerably higher (5 to 24 fold) than for the inhibition of the α_1-AR. Similar to the rank order found in binding studies, 1R,2S- and 1R,2R–*pseudo*ephedrine showed greater antagonist activity than 1S,2R- and 1S,2S-*pseudo*ephedrine (compare data in Tables 1 and 3). No difference was noted in the antagonist potencies of the primary and secondary amines of 1R,2S-norephedrine and ephedrine, respectively. However, 1S,2S-nor*pseudo*ephedrine (the primary amine analog)

was more potent than 1S,2S-*pseudo*ephedrine as an antagonist on the three α-AR subtypes and its potency was the same as the 1R,2S-isomers of ephedrine and norephedrine on these ARs. It appears that the orientation of the 1R hydroxyl substitutent and the methylation state (primary versus secondary amine) in the ephedrine alkaloids are important for α-AR activity. The antagonistic activities of all ephedrine isomers were consistent with their binding affinities.

Discussion

Chemically, ephedrine possesses two chiral centers, and 1R,2S-ephedrine is a long-studied stimulant available both as a prescription and over-the-counter medication, and is a major ingredient in widely marketed herbal preparations. Another isomer, 1S,2S-*pseudo*ephedrine is used as a nasal decongestant and precursor for the illicit synthesis of methamphetamine. Standard pharmacology textbooks emphasize that ephedrine is both a direct and indirect acting drug for the activation of adrenergic receptors (AR) (Hoffman, 2001). Previous reports attributed the effects of ephedrine alkaloids to direct agonist activity and by release of NE from presynaptic nerve terminal via a carrier-mediated exchange mechanism (Trendelenburg, 1963; Trendelenburg, et al., 1963; Vansal and Feller, 1999; Patil et al., 1967; Rothman et al., 2003). In the present study, we characterized the direct receptor-mediated effects of the stereoisomers of ephedrine and closely related naturally occurring compounds (1R,2S-isomers of ephedrine, norephedrine and N-methylephedrine, and 1S,2S isomers of *pseudo*ephedrine and nor*pseudo*ephedrine).

Radioligand binding studies of the ephedrine alkaloids showed that the 1R,2R- and the 1R,2S-isomers generally had greater affinities than their corresponding 1S,2R- and 1S,2S- isomers on all of the α-AR subtypes. An exception was at the α_{1B}-AR. 1S,2S-*Pseudo*ephedrine displayed the lowest binding affinity at all α-ARs. 1R,2S-Norephedrine, lacking an N-methyl group as in 1R,2S-ephedrine, showed increased binding affinities at the subtypes tested. The presence of an additional N-methyl group, as in 1R,2S-N-methylephedrine, showed decreased binding affinities as compared to 1R,2S-ephedrine at the α_{1A}-, α_{2A}-, and α_{2C}-AR. In summary, the steric orientation of the hydroxyl group in the 1R configuration and the presence of a primary or secondary amine on the side chain were associated with the highest binding affinities to α-AR subtypes.

Rothman et al. (2003) reported that the ephedrine isomers, at 10 μM, lacked agonist activity in functional studies of intracellular calcium changes in cells expressing human α_{1A}- and α_{2A-} -ARs. No further studies were done to determine if ephedrine analogs were antagonists in these cells. In our study, using **c**ell-based reporter gene assays to measure functional effects at the α_{1A}-, α_{2A}- and α_{2C}-ARs, we demonstrated that ephedrine isomers and related analogs were also inactive as direct agonists, and showed that the ephedrine alkaloids antagonized the effects of the agonists, L-phenylephrine on α_1- and medetomidine on α_2-ARs. Similar to the rank order found in binding studies, 1R,2S- and 1R,2R–ephedrine showed greater antagonist activity than 1S,2R- and 1S,2S-*pseudo*ephedrine, which indicated that β-hydroxyl substituent on the side chain in the R-configuration is important for α-AR antagonist activity. The antagonistic potencies of the isomers were consistent with their binding affinities, both of which were in the μM range.

Taken collectively, these findings suggest that the ephedrine isomers possess only direct antagonist activity in cloned human α-AR systems.

Early reports by Patil and colleagues (Patil et al., 1967; Tye et al., 1967; Lapidus et al., 1967) have demonstrated that the 1R,2S- and 1R,2R-isomers of ephedrine possess agonist or antagonist activity (dependent upon the tissue examined), whereas the remaining ephedrine isomers exhibit antagonist activities or possessed an indirect mechanism of action on isolated tissues (lung, ileum, vas deferens and vascular preparations) that contained α- and/or β-ARs. In another study by Lee et al. (1974) using rat epididymal fat tissue and measurement of lipolysis, the ephedrine isomers were devoid of β-AR activity on lipolysis, and they reported that 1R, 2S-ephedrine is more potent as an antagonist than 1S,2R-ephedrine. In this regard, Wellman et al. (2003) recently reported that 1R,2S-ephedrine-induced hypophagia in rats was attenuated by prazosin, which suggested that this *in vivo* ephedrine action was mediated via the α_1-AR. The α_1-AR subtype that mediated this CNS effect of ephedrine on hypophagia was not established. It is apparent that the mechanism of action for *Ephedra* alkaloids is dependent upon the stereochemistry of the ephedrine analog(s) used, the abundance and distribution of AR subtypes present, and whether nerves remain intact in the target tissue under study.

To date, only a few studies have been completed using human AR**s**. On the three human β-AR subtypes expressed in CHO cells, the ephedrine isomers showed weak agonist activities (Vansal and Feller, 1999), and the 1R,2S-ephedrine isomer was the most active agonist on the three subtypes, exhibiting the most potent activity on the β_2-AR and weakest agonist activity on the β_3-AR subtype. In contrast, Rothman and co-

workers (2003) reported that 1R,2S-ephedrine was not active as an agonist on the human β-AR subtypes (data not presented) and suggested that the results of the earlier study by Vansal and Feller (1999) may have been due to the use of cells with higher receptor densities. Other work using fluorescent labeling techniques to evaluate direct β_2-AR interactions with 1R,2S-ephedrine (Gether et al., 1995) have yielded more insight into conformational changes. In this study, only full agonists but not weaker partial agonists like ephedrine, produced significant reductions in fluorescence providing an interesting approach to investigate the direct AR effects (agonist or antagonist changes). Additional studies with the isomers of *Ephedra* alkaloids and related analogs may be useful in probing ligand-specific sites of interaction on AR subtypes.

Taken collectively, our present studies show only a weak partial agonist activity (at $> 10^{-4}$ M) by the 1R,2S-isomers of ephedrine and norephedrine; and that all isomers of tested *Ephedra*/ephedrine alkaloids produce only moderate antagonist activities on α-AR subtypes. Based upon our findings, it is likely that the *in vivo* actions of these *Ephedra* alkaloids are mediated principally by an indirect action on the subtypes of human α-ARs. However, a mechanism of direct action for 1R,2S-ephedrine on ARs has been proposed to explain the observed *in vivo* effects for the pharmacological treatment of asthma (Griffith and Johnson, 1995, Hoffman, 2001) and for its CNS stimulatory and/or cardiovascular (heart, stroke) adverse effects observed with its abuse and misuse in humans (Haller and Benowitz, 2000). Interestingly, our findings contrast with recent *in vivo* reports on pressor effects (Liles et al., 2006) and hypophagic activity in rats (Wellman et al., 2003), in which they demonstrated that 1R,2S-ephedrine exhibits its agonist activity via direct effects on the α_1-AR receptors. In this regard, an *in vitro* study

involving mutational changes (Waugh et al., 2000) in the α_{1A}-AR demonstrated a 4-fold increase in functional potency for the 1R,2S-ephedrine isomer, whereas potency for epinephrine was decreased by 36-fold. Thus, the presence of the aromatic catechol group in epinephrine was more affected by the mutation than a molecule lacking the catechol functionality (ephedrine). Waugh et al. (2000) demonstrated partial agonist activity for only this ephedrine isomer on wild-type and mutated cloned rat and hamster α_{1A}-ARs. In summary, it is apparent that the 1R,2S ephedrine isomer has direct agonist activity, *in vitro* and *in vivo*. Our studies and those of Rothman et al. (2003) failed to demonstrate significant agonist activity for 1R,2S-ephedrine on human α_1-ARs. The observed agonist responses to 1R,2S-ephedrine, as reported by Waugh et al. (2000); may be explained in part by an increased density of receptors in the cells used in their experiments. Overall, these results provide evidence in support of both *in vivo* and *in vitro* agonist activities for 1R,2S-ephedrine on α-ARs.

Ephedra alkaloids are expected to produce their pharmacological actions by mixed mechanisms, involving indirect and direct actions on α-AR subtypes. In conclusion, our studies have shown that the ephedrine isomers and analogs studied did not exhibit any significant direct agonist activity on humaṇ α_1- and α_2-AR subtypes, and can be classified as antagonists. We propose that the observed *in vivo* functional effects of *Ephedra*/ephedrine alkaloids may be produced, in part, by blocking the action of norepinephrine at α_{1A}- and /or α_{2A}-AR. The results of our studies, particularly with the findings on human α_2-AR, suggest that an inhibition of AR subtypes by ephedrine analogs may be related to their *in vivo* effects. In particular, it is plausible to suggest that naturally occurring ephedrine alkaloids may act as antagonists of pre-synaptic $\alpha_{2A/2C}$-ARs

present in nerve terminals. As such, ephedrine analogs may interfere with pre-synaptic norepinephrine uptake and enhance synaptic concentrations and response to this neurotransmitter. In this regard, Rothman et al. (2003) used *in vitro* assays to measure the effect of phenylpropanolamines on the release or re-uptake of norepinephrine in rat whole brain (minus caudate and cerebellum) preparations, and proposed that ephedrine analogs act via an indirect mechanism leading to an enhanced release of norepinephrine. They also showed that ephedrine isomers and related analogs produce a similar pattern, but with less potency, for dopamine release; and that ephedrine analogs also have affinity for interaction with serotonergic receptors. The study of *Ephedra* alkaloid effects on dopamine and serotonin receptor subtypes may also be worthwhile but is beyond the scope of the present work. Thus, it is also clear that interpretation of the *in vivo* and *in vitro* results of the pharmacology of ephedrine alkaloids may be further complicated by combination and relative abundance of α- and/or β-AR subtypes, and other pharmacological receptors, and their signaling pathways that are present in the target tissue. More studies will be required to establish the importance of these and other receptors to assess the overall actions of these sympathomimetic amines, *in vivo*. Irrespective of the underlying differences between *in vitro* and *in vivo* actions of *Ephedra* or its alkaloids, it is apparent that their pharmacological effects on ARs will be dependent upon the dose; and may involve both direct and/or indirect mechanisms on noradrenergic-dependent pathways.

References

Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennittt MV, Thody VE, Wilson C, and Wilson S (1984) Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature* **309**:163-165.

Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (Ki) and the concentration of the inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochemical Pharmacol* **22**:3099-3108.

Chua SS and Benrimoj SI (1988) Non-prescription sympathomimetic agents and hypertension. *Med Toxicol Adverse Drug Exp* **3**:387-414.

Gether U, Lin S and Kobilka BK (1995). Fluorescent labeling of purified beta 2 adrenergic receptor. Evidence for ligand-specific conformational changes. *J Biol Chem*. **270**:28268-75.

Griffith RK and Johnson EA (1995) Adrenergic drugs. In: *Principles of Medicinal chemistry* (Eds. Foye WO, Lemke TL and Williams DA), Williams & Wilkins, Baltimore, MD. p. 345-365.

Haller CA and Benowitz NL (2000) Adverse cardiovascular and central nervous system events associated with dietary supplements containing *ephedra* alkaloids. *N Engl J Med* **343**:1833-1838.

Hoffman BB (2001) Catecholamines, sympathomimetic drugs and adrenergic receptor antagonists, Chapter 10, p. 237. In: Goodman and Gilman's Pharmacological Basis of Therapeutics, Eds. J G Hardman and LE Limbird, McGraw Hill Publishers, NYC, NY.

Josefson D (1996) Herbal stimulant causes US deaths. *BMJ* **312**:1378-1379.

Kalix P (1991) The pharmacology of psychoactive alkaloids from *ephedra* and *catha*. *J Ethnopharmacol* **32**:201-208.

Kawasuji T, Koike K, and Saito H (1996). Chronotropic effects of optical isomers of ephedrine and methylephedrine in the isolated rat right atria and in vitro assessment of direct and indirect actions on beta 1-adrenoceptors. *Biol Pharm Bull*. **19**:1423-8.

Lalchandani SG, Lei L, Zhang W, Suin MM, Moore BM, Liggett SB, Miller DD, and Feller DR (2002) Yohimbine dimmers exhibiting selectivity for the human α2c-adrenoceptor subtype. *J Pharmacol Exp Ther* **303**:979-984.

Lapidus JB, Tye A, and Patil PN. Steric aspects of adrenergic drugs VII (1967) Certain pharmacological actions of D (-)-*pseudo*ephedrine. *J Pharmaceutical Sci*. **56**:1125-1130.

Lee OS, Bescak G, Miller DD, and Feller DR (1974) Influence of substituted phenethylamines on lipolysis, in vitro. III. Stereoselectivity. *J Pharmacol Exp Ther* **190**:249-259.

Liles JT, Dabisch PA, Hude KE, Pradhan L, Varner KJ, Porter JR, Hicks AR, Corll C, Baber SR, and Kadowitz PJ (2006). Pressor responses to ephedrine are mediated by a direct mechanism in the rat. *J Pharmacol Exp Ther*. **316**:95-105.

Liu YL, Toubro S, Astrup A, and Stock MJ (1995) Contribution of β_3-adrenergic activation to ephedrine-induced thermogenesis in humans. *Int J Obes relat metab Disord* **19**:678-685.

Patil PN, Tye A, and Lapidus JB (1965). A pharmacological study of the ephedrine isomers. *J Pharmacol Exp Ther*. **148**:158-68.

Ma G, Bavadekar, SA, Davis, MY, Lalchandani, SG, Nagmani, R , Khan I and Feller DR (2004). Pharmacological effects of *Ephedra* alkaloids on human alpha-adrenergic receptor subtypes. Abstract presented at 25th Southeastern Pharmacology Society Meeting, The University of Mississippi, University, MS, November 4-5, 2004.

Patil PN (1967) Steric aspects of adrenergic drugs. VIII. Optical isomers of beta adrenergic receptor antagonists. *J Pharmacol Exp Ther* **160**:308-14.

Patil PN, Lapidus JB, Molinari S, and Tye A (1967) Steric aspects of adrenergic drugs IV. Antagonism of norepinephrine effects by ephedrine isomers on the isolated rabbit ileum. *J Pharmaceutical Sci* **56**:287-288.

Rothman RB, Vu N, Partilla JS, Roth BL, Hufeisen SJ, Compton-Toth BA, Birkes J,Young R, and Glennon RA (2003) In vitro characterization of ephedrine-related

stereoisomers at biogenic amine transporters and the receptorome reveals selective action as norepinephrine transporter substrates. *J Pharmacol Exp Ther* **307**:138-145.

Stratowa C, Machat H, Burger E, Himmler A, Schafer R, Spevak W, Weyer U, and Wiche-Castanon M (1995) Functional characterization of the human neurokinin receptors NK1, NK2, and NK3 based on a cellular assay system. *J Recept Res* **15**:617-630.

Trendelenburg U (1963) Supersensitivity and subsensitivity to sympathominetic amines. *Pharmacol Rev **15***:225-276.

Trendelenburg U, Delasierra BG, and Muskus A (1963). Modification by reserpine of the response of atrial pacemaker to sympathomimetic amines. *J Pharmacol Exp Ther* **141**:301-9.

Tye A, Baldeserger R, LaPidus JB, and Patil PN (1967) Steric aspects of adrenergic drugs VI. Beta adrenergic effects of ephedrine isomers. *J Pharmacol Exp Ther* **157**:56-362.

Vansal SS and Feller DR (1999). Direct effects of ephedrine isomers on human beta-adrenergic receptor subtypes. *Biochem Pharmacol **58***:807-10.

Waldeck B and Widmark E (1985). The interaction of ephedrine with beta-adrenoceptors in tracheal, cardiac and skeletal muscles. *Clin Exp Pharmacol Physiol*. **12**:439-42.

Waugh DJ, Zhao MM, Zuscik MJ, and Perez DM (2000). Novel aromatic residues in transmembrane domains IV and V involved in agonist binding at alpha(1a)-adrenergic receptors. *J Biol Chem.* **275**:11698-705.

Wellman PJ, Miller DK, and Ho DH (2003). Noradrenergic modulation of ephedrine-induced hypophagia. *Synapse* **48**:18-24.

White LM, Gardner SF, Curley BJ, Narx MA, Wang P and Estes M (1997). Pharmacokinetics and cardiovascular effects of Ma Huang (*Ephedra sinica)* in normotensive adults. *J Clin Pharmacol* **37**:116-122.

Footnotes

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Figures and legends

Figure 1. The chemical structures of ephedrine isomers and naturally occurring alkaloids.

Figure 2. Binding displacement curves of ephedrine isomers for α_{1A}-(A), α_{2A}-(B), and α_{2C}-(C), ARs expressed in HEK293 or CHO cells. Data are expressed as mean ± SEM (*n* = 6 experiments). Key: L-phenylephrine, (■); medetomidine**, (▲),** 1R,2S-ephedrine, (□**);** 1R,2S-norephedrine, **(●);** 1R, 2S-N-methylephedrine, **(○).**

Figure 3a. Direct effects of L-phenylephrine and ephedrine alkaloids on α_{1A}-AR in HEK 293 cells. Control and L-phenylephrine (300 μM) luciferase activities (cps, mean ± SEM, *n* = 4-6) were 1154 and 7560, respectively. Normalized data (300 μM L-phenylephrine = 100%) are expressed as the mean ± SEM of *n* = 4-6 experiments. Key: A, 1R,2S-ephedrine (300 μM); B, 1S,2R-ephedrine (300 μM); C, 1R,2R-*pseudo*ephedrine (300 μM); D, 1S,2S-*pseudo*ephedrine (300 μM); E, 1R,2S-norephedrine (300 μM); G, 1R,2S-N-methylephedrine (300 μM); H, 1S,2S- nor*pseudo*-ephedrine (300 μM). Figure 3b, Concentration-dependent effects of medetomidine and ephedrine alkaloids for the reversal of effects on forskloin–induced cyclic-AMP elevation on the α_{2A}- and α_{2C}-ARs expressed in CHO cells. Control and forskolin-induced luciferase activities (cps, mean ± SEM, *n* = 4-6) were 1560 and 12200 for α_{2A}, and 3069 and 34576 for α_{2C} respectively. Normalized data (3 μM forskolin = 100%) are expressed as the mean ± SEM of *n* = 4-6 experiments. Key: A, 1R,2S-ephedrine (300 μM); B, 1S,2R-ephedrine (300 μM); C, 1R,2R-*pseudo*ephedrine (300 μM); D, 1S,2S-*pseudo*ephedrine (300 μM); E, 1R,2S-

norephedrine (300 μM); G, 1R,2S-N-methylephedrine (300 μM); H, 1S,2S- nor*pseudo*-ephedrine (300 μM); F, forskolin (3 μM).

Figure 4a. Displacement curves of antagonist effects of ephedrine alkaloids on L-phenylephrine-mediated agonistic effects on α_{1A}-AR expressing in HEK 293 cells. Data are expressed as the mean ± SEM of *n* = 4-6 experiments. Key: L-phenylephrine (0.1-300 μM), **(■)**; 1R,2S-ephedrine (300 μM) + L-phenylephrine, (□); 1R,2S-norephedrine (300 μM) + L-phenylephrine, **(●)** ; 1R, 2S-N-methylephedrine (300 μM) + L-phenylephrine, **(○)**. Control and L-phenylephrine (300 μM) luciferase activities (dps, mean ± SEM, *n* = 4-6) were 1269 and 7980, respectively. Normalized data (300 μM L-phenylephrine = 100%) are expressed as the mean ± SEM of *n* = 4-6 experiments. Figure 4b. Reversal of medetomidine inhibition of forskolin-induced cAMP elevation by ephedrine alkaloids on human α_{2A}- versus (B) α_{2C}-AR. Cyclic AMP changes were assessed by measurement of luciferase activity. Control and forskolin induced measurements of luciferase activity (cps, mean ± SEM, *n* = 4-6) were 1483 and 12780 for α_{2A}, and 3069 and 32576 for α_{2c} respectively. pIC_{50} and IC_{50} values (Table 3) were determined as the concentration of ephedrine isomers that reversed the inhibition effect of medetomidine on the luciferase response to forskolin). Keys: A, 1R,2S-ephedrine (1, 10, 100 μM); B**,** 1R,2S-norephedrine (1, 10, 100 μM); C, 1R, 2S-N-methylephedrine (1, 10, 100 μM); M, medetomidine (0.01 μM); F, forskolin (3 μM).

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Table 1. Binding affinities (p*Ki* values) of L-phenylephrine and ephedrine alkaloids on human α_{1A}-, α_{1B}-, and α_{1D}- ARs in HEK293 cells[a]

Compounds	α_{1A}-AR pKi ± SEM	α_{1B}-AR pKi ± SEM	α_{1D}-AR pKi ± SEM
L-Phenylephrine (PE)	6.32 ± 0.30	nd[b]	nd
Oxymetazoline	8.11 ± 0.05	6.32 ± 0.001	6.07 ± 0.03
1R,2S-Ephedrine	4.95 ± 0.04[d f,]	<3.00 (37.4% [c]) [d]	4.28 ± 0.09 [d]
1S,2R-Ephedrine	4.41 ± 0.05[d]	4.11 ± 0.06 [d]	3.95 ± 0.11[d]
1R,2R-*Pseudo*ephedrine	5.01 ± 0.11[e]	4.04 ± 0.05[e]	4.54 ± 0.21[e]
1S,2S-*Pseudo*ephedrine	4.16 ± 0.08[e]	<3.00 (12.5% [c])[e]	<3.00 (27.3% [c])[e]
1R,2S-Norephedrine	5.14 ± 0.14[f]	nd[b]	nd
1S,2S-Nor*pseudo*ephedrine	4.57 ± 0.20	nd	nd
1R,2S-N-methylephedrine	4.20 ± 0.19[g]	nd	nd

[a][^{3}H]Prazosin was used as the radioligand in equilibrium competition radioligand binding assays for the α_{1A}-, α_{1B}-, and α_{1D}-ARs, and non-specific binding was measured in the presence of 10 μM of phentolamine. pKi value = -log Ki (Ki was caculated according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973) and the data are mean ± SEM of n = 6.

[b]Not determined.

[c]Maximum percent inhibition of specific binding observed at 1 mM.

[d]The mean pKi values for 1R,2S- and 1S,2R-ephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's t test).

[e] The mean pKi values for 1R,2R- and 1S,2S-*pseudo*ephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's t test).

[f]The mean pKi values for 1R,2S-ephendrine and 1R,2S-norephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's t test).

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[g]The mean pKi value for 1R,2S-N-methylephedrine was significantly different from the mean values of 1R,2S-ephedrine and 1R,2S-norephedrine on the α_{1A}-AR subtype ($P < 0.05$ using the paired Student's *t* test).

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Table 2. Binding affinities (p*Ki* values) of medetomidine and ephedrine alkaloids on human α_{2A}-, α_{2B}-, and α_{2C}-ARs in CHO cells[a]

Compounds	α_{2A}-AR pKi ± SEM	α_{2B}-AR pKi ± SEM	α_{2C}-AR pKi ± SEM
Medetomidine	6.15 ± 0.05	7.50 ± 0.03	7.65 ± 0.03
1R,2S-Ephedrine	4.83 ± 0.16[b]	4.78 ± 0.02	4.75 ± 0.12
1S,2R-Ephedrine	4.44 ± 0.19[b,d]	4.77 ± 0.05	4.65 ± 0.02
1R,2R-*Pseudo*ephedrine	4.95 ± 0.20[c]	5.26 ± 0.08[c]	4.88 ± 0.10[c]
1S,2S-*Pseudo*ephedrine	4.19± 0.10[c]	4.36 ± 0.10[c]	4.18 ± 0.11[c]
1R,2S-Norephedrine	5.16 ± 0.05[d]	nd[f]	4.96 ± 0.20
1S,2S-Nor*pseudo*ephedrine	4.32 ± 0.05	nd	4.50 ± 0.13
1R,2S-N-methylephedrine	4.20 ± 0.30[e]	4.29 ± 0.09[e]	4.10 ± 0.30[e]

[a][^{3}H]Rauwolscine was used as the radioligand in equilibrium competition radioligand binding assays for the α_{2A}-, α_{2B}-, and α_{2C}-ARs, and non-specific binding was measured in the presence of 10 μM of phentolamine. pKi value = -log Ki (Ki was calculated according to the Cheng-prusoff equation (Cheng and Prusoff, 1973) and the data are mean ± SEM of n = 6.

[b]The mean pKi values for 1R,2S- and 1S,2R-ephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

[c]The mean pKi values for 1R,2R- and 1S,2S-*pseudo*ephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

[d]The mean pK_i values for 1R,2S-ephedrine and 1R,2S-norephedrine were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

[e]The mean pKi value for 1R,2S-N-methylephedrine was significantly different from the mean values of 1R,2S-ephedrine and 1R,2S-norephedrine on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

[f]Not determined

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Table 3. The pK_B or pIC_{50} values of antagonist effects of ephedrine alkaloids on human α_{1A}-, α_{2A}-, and α_{2C}-ARs in HEK293 and CHO cells. Each value is the mean ± standard error of data from three experiments performed in duplicate[a]

Compounds	α_{1A}-adrenoceptors		α_{2A}-adrenoceptors		α_{2C}-adrenoceptors	
	pK_B	K_B (μM)	pIC_{50}	IC_{50} (μM)	pIC_{50}	IC_{50} (μM)
1R,2S-Ephedrine	4.78 ± 0.05[d]	16.6	5.08 ± 0.10[b]	8.3	4.97 ± 0.18[b]	10.7
1S,2R-Ephedrine	4.70 ± 0.08	19.9	4.81 ± 0.30[b]	15.5	4.65 ± 0.29[b]	22.4
1R,2R-*Pseudo*ephedrine	5.05 ± 0.29[c]	10.2	5.12 ± 0.10[c]	7.6	5.11 ± 0.11[c]	7.8
1S,2S-*Pseudo*ephedrine	3.91 ± 0.07[c]	22.0	4.32 ± 0.10[c]	47.9	4.25±0.23[c]	56.2
1R,2S-Norephedrine	5.02 ± 0.20[d]	10.5	5.08 ± 0.10	8.3	4.84 ± 0.31	14.5
1S,2S-Nor*pseudo*ephedrine	4.79 ± 0.13	16.9	5.10 ± 0.10	7.9	4.76 ± 019	17.4
1R,2S-N-methylephedrine	4.39 ± 0.10[e]	42.7	4.60 ± 0.00[e]	25.1	4.26 ± 0.21[e]	55.0

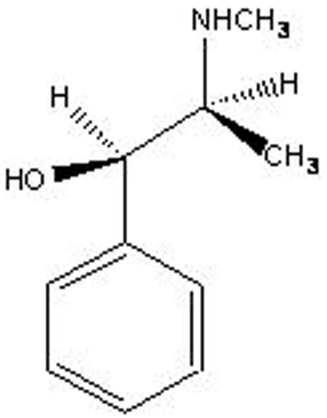
[a]Values were determined as the effective concentration (IC_{50}) and negative log (pIC_{50}) of each ephedrine analog that reversed the effect of medetomidine on the maximum cAMP response of forskolin.

[b]The mean pK_B or pIC_{50} values for 1R,2S- and 1S,2R-ephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

[c] The mean pK_B or pIC_{50} values for 1R,2R- and 1S,2S-*pseudo*ephedrine isomers were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

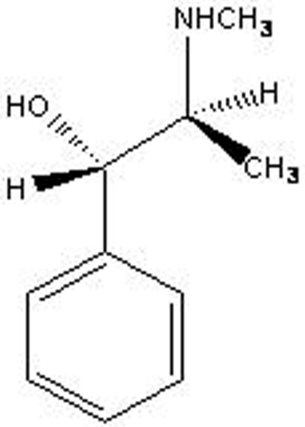
[d]The mean pK_B or pIC_{50} values for 1R,2S-ephedrine and 1R,2S-norephedrine were significantly different on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).

[e]The mean pK_B or pIC_{50} value for 1R,2S-N-methylephedrine was significantly different from the mean values of 1R,2S-ephedrine and 1R,2S-norephedrine on the receptor subtype ($P < 0.05$ using the paired Student's *t* test).



1R,2S

(-)-Ephedrine



1S,2S

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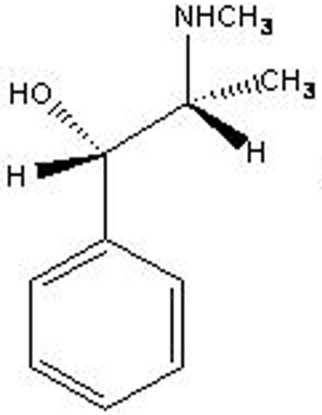
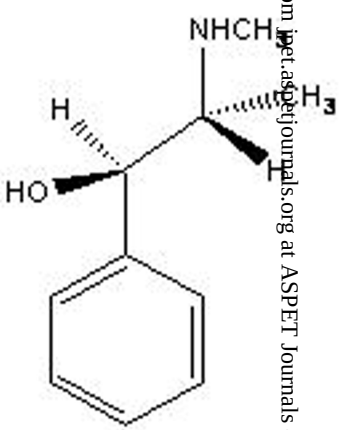


1S,2R

(+)-Ephedrine



1R,2R

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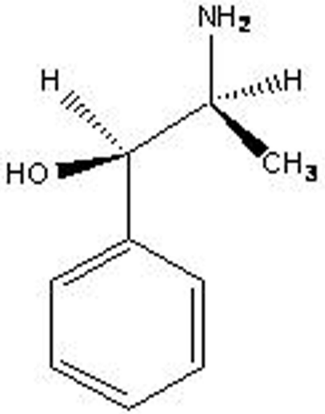
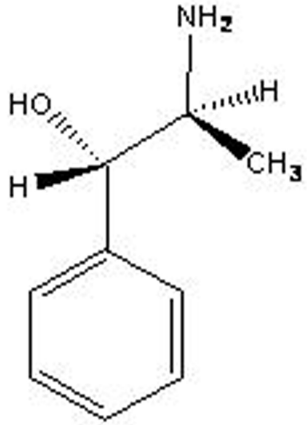


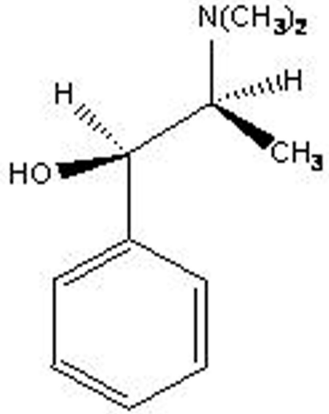
1R,2S

(-)-Norephedrine



1S,2S

(-)-Nor*pseudo*ephedrine



1R,2S

(-)-N-methylephedrine

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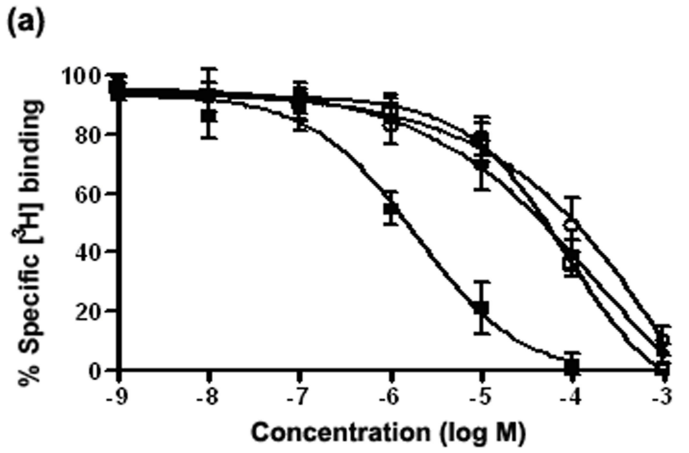
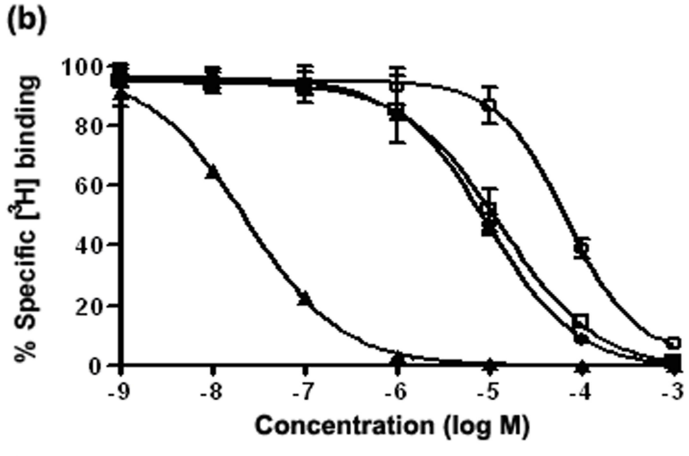
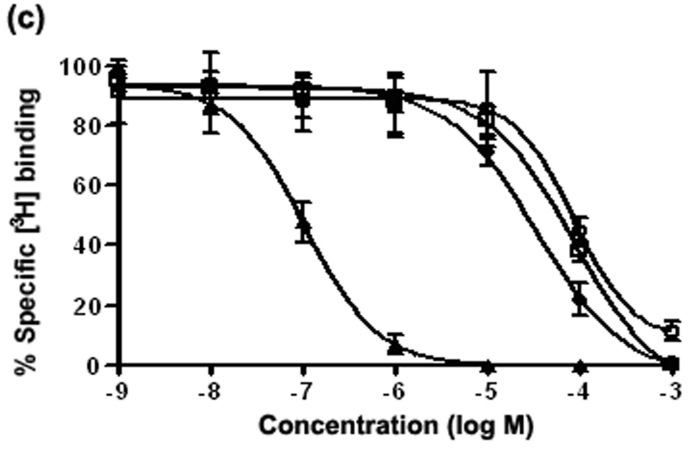
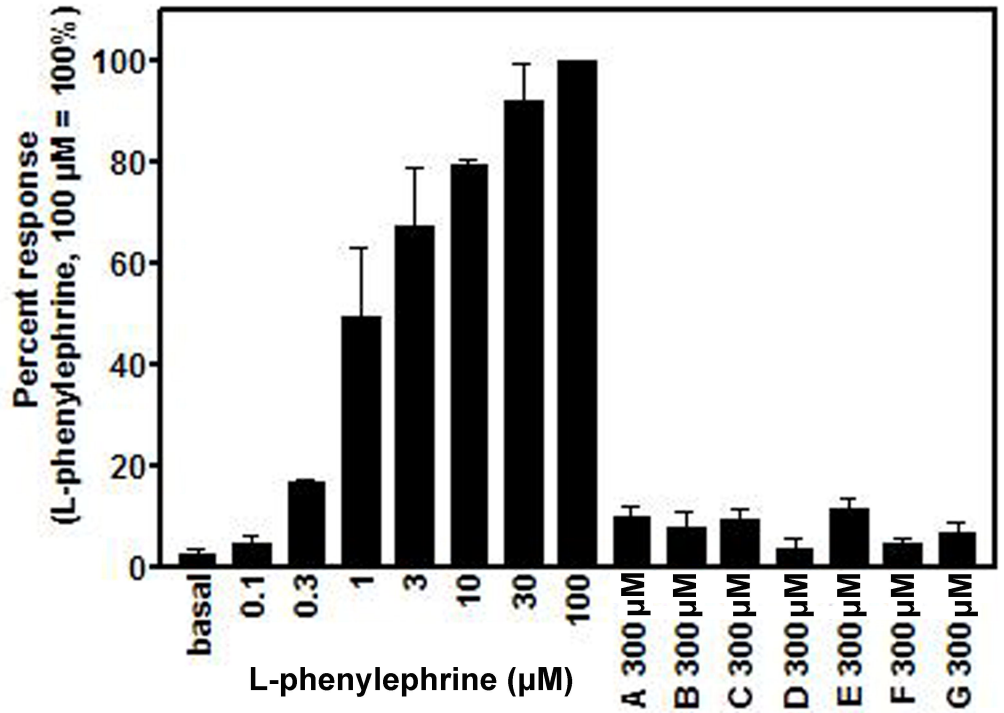


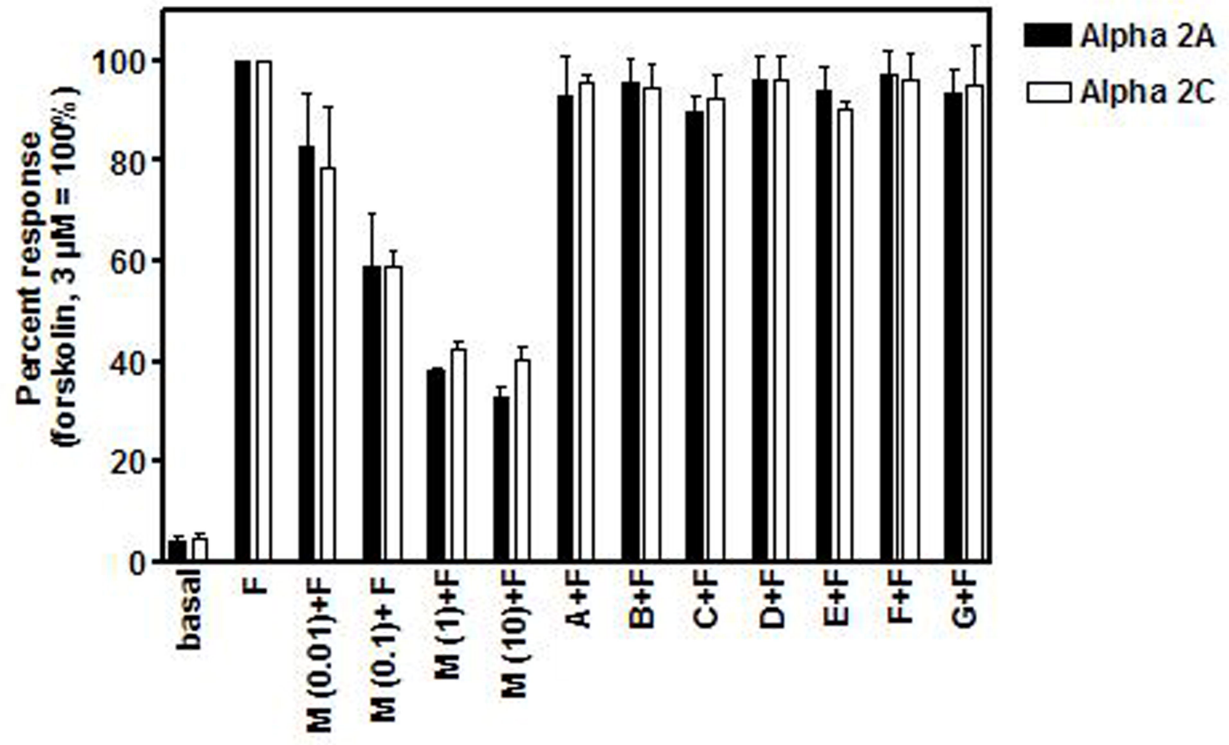




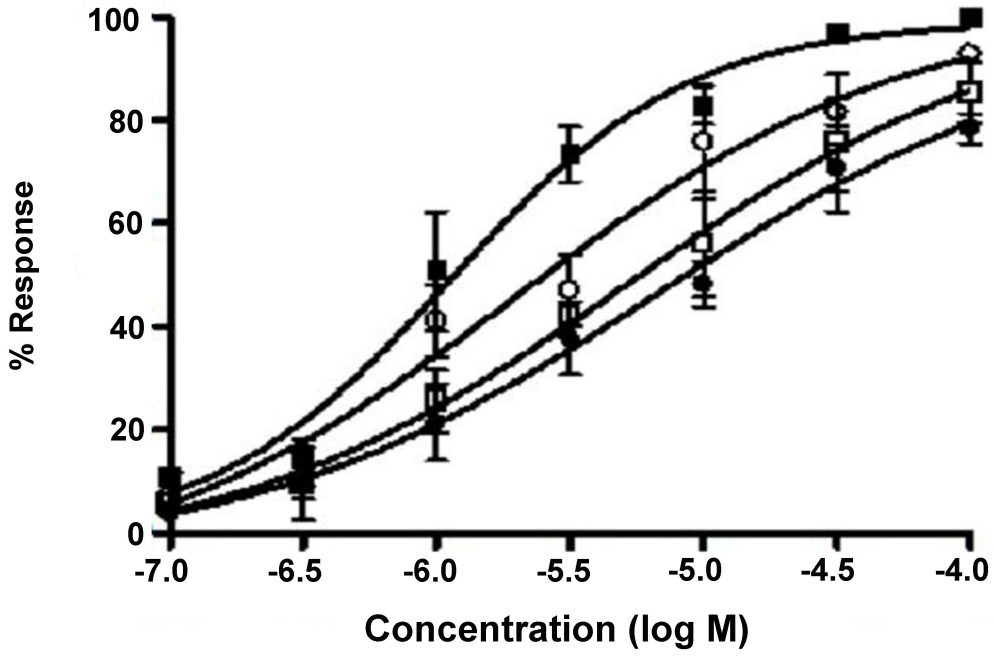
(a)


Percent response
(L-phenylephrine, 100 µM = 100%)
0
20
40
60
80
100
basal
0.1
0.3
1
3
10
30
100
A 300 µM
B 300 µM
C 300 µM
D 300 µM
E 300 µM
F 300 µM
G 300 µM
L-phenylephrine (µM)

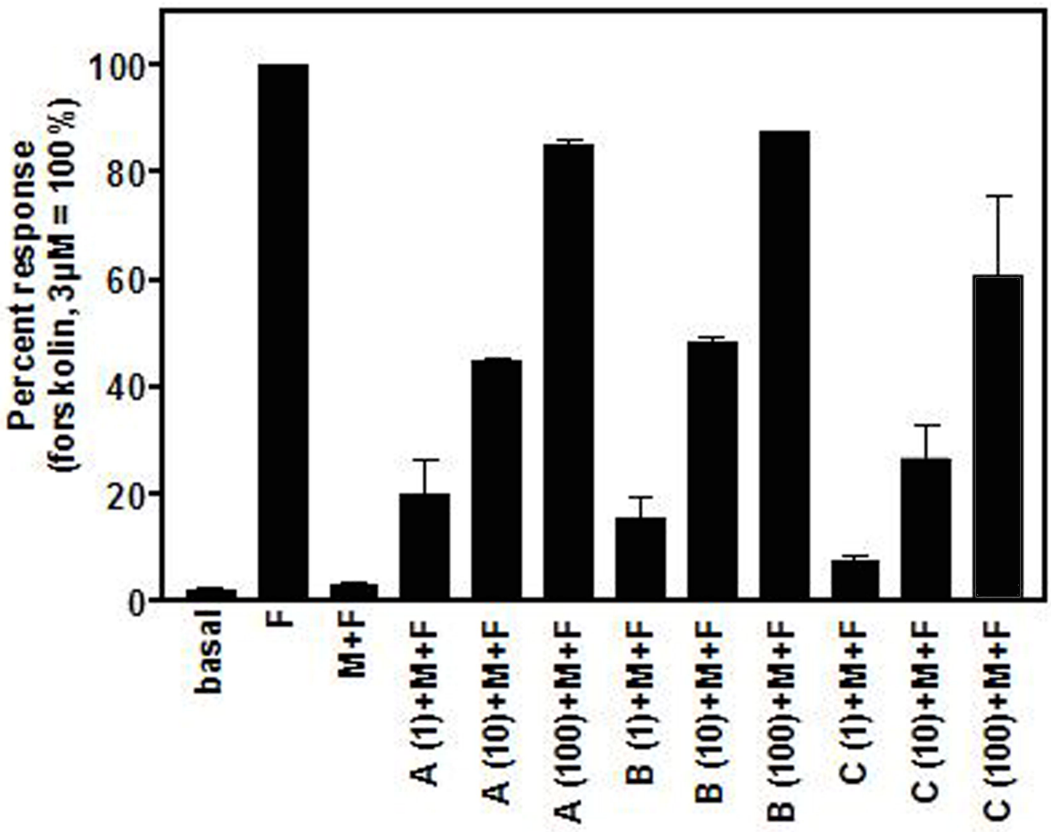
(b)


Percent response
(forskolin, 3 µM = 100%)
0
20
40
60
80
100
basal
F
M (0.01)+F
M (0.1)+ F
M (1)+F
M (10)+F
A+F
B+F
C+F
D+F
E+F
F+F
G+F
Alpha 2A
Alpha 2C

(a)


100
80
60
40
20
0
% Response
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
Concentration (log M)

(b)


Percent response
(forskolin, 3μM = 100 %)
100
80
60
40
20
0
basal
F
M+F
A (1)+M+F
A (10)+M+F
A (100)+M+F
B (1)+M+F
B (10)+M+F
B (100)+M+F
C (1)+M+F
C (10)+M+F
C (100)+M+F