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# A-315456: a selective $\alpha_{1D}$-adrenoceptor antagonist with minimal dopamine $D_2$ and 5-$HT_{1A}$ receptor affinity

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**Abstract**

In functional assays, A-315456, *N*-[3-(cyclohexylidene-(1*H*-imidazol-4-ylmethyl))phenyl]ethanesulfonamide, behaved as an $\alpha_{1D}$-adrenoceptor subtype selective antagonist ($pA_2 = 8.34$) in the rat aorta. It was 83-fold less potent at the $\alpha_{1B}$-adrenoceptor subtype expressed in the rat spleen, and was inactive at the $\alpha_{1A}$-adrenoceptor subtype expressed in the rat vas deferens. Radioligand binding assays also revealed high affinity ($pK_i = 8.71$) for the $\alpha_{1D}$-adrenoceptor subtype and weaker affinities at the $\alpha_{1A}$-adrenoceptor ($pK_i = 6.23$) and $\alpha_{1B}$-adrenoceptor ($pK_i = 7.86$). In comparison to its potent affinity at the $\alpha_{1D}$-adrenoceptor subtype, A-315456 was 3020-, 794- and 38-fold weaker at the dopamine $D_2$-, 5-$HT_{1A}$-, and $\alpha_{2a}$-adrenoceptors, respectively. These studies indicate that A-315456 is a potent and selective $\alpha_{1D}$-antagonist that may serve as a useful pharmacological ligand to probe the physiological role of the $\alpha_{1D}$-adrenoceptor subtype in normal and disease states. 

*Keywords:* Adrenoceptor subtype; $\alpha_{1D}$-Adrenoceptor antagonist; A-315456; BMY-7378; SNAP-8719

## 1. Introduction

Three $\alpha_1$-adrenoceptors have been identified, cloned, and pharmacologically characterized (Bylund et al., 1994; Hieble et al., 1995). The identification of the $\alpha_{1A}$-adrenoceptor specific antagonists, WB4101 ((2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) 5-methylurapidil, RS 17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), and REC 15/2739 (8-3-[4-(2-methoxyphenyl)-1-piperazinyl]-propylcarbamoyl-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran dihydrochloride) (Ford et al., 1996; Morrow and Creese, 1986; Leonardi et al., 1997) has improved the understanding of the function of the $\alpha_{1A}$-adrenoceptor subtype expressed in different mammalian tissues such as the prostate.

For the $\alpha_{1B}$-adrenoceptor subtype, the alkylating agent, chloroethylclonidine, was the only $\alpha_{1B}$-adrenoceptor subtype selective antagonist available (Minneman et al., 1988). However, a new quinazoline compound, L-765,314 (4-amino-2-[4-[1-(benzyloxycarbonyl)]-piperazinyl]-6,7-dimethoxyquinazoline, has recently been reported as an $\alpha_{1B}$-adrenoceptor subtype selective antagonist with 10- and 100-fold more selectivity versus the $\alpha_{1D}$- and $\alpha_{1A}$-adrenoceptor subtypes, respectively (Patane et al., 1998). Data derived from mice that were deficient in the $\alpha_{1B}$-adrenoceptor subtype suggest an important role for this subtype in blood pressure regulation (Cavalli et al., 1997).

The $\alpha_{1D}$-adrenoceptor has been identified in the human bladder (Schwinn and Michelotti, 2000) and spinal cord (Smith et al., 1999), where a potential role in modulating the bothersome symptoms associated with bladder filling and storage (such as frequency, urgency, and nocturia) has recently been proposed. The most selective $\alpha_{1D}$-adrenoceptor antagonist reported to date is BMY-7378 ([8-(2-[4-(2-methoxy-phenyl)-1-pierazinyl]ethyl)-8-azaspiro[4.5]decane-7,9-dione dihydrochloride]), as described by Goetz et al. (1995). However, this agent also demonstrates significant affinity for the rat recombinant 5-$HT_{1A}$ and human dopamine $D_2$ binding sites. More recently, Konkel et al. (1998) described SNAP-8719 (8-[(1*R*)-1-methyl-2-[4-(2,4,5-trifluorophenyl)-1-piperazinyl]ethyl]-8-Azaspiro[4.5]decane-7,9-dione hydrochloride), a structural analogue of BMY-7378, as a potent $\alpha_{1D}$-adrenoceptor antagonist with reduced

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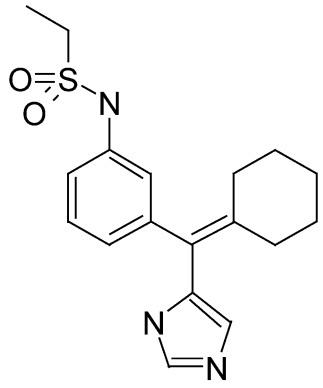

Fig. 1. Chemical structure of A-315456, *N*-[3-(cyclohexylidene-(1*H*-imidazol-4-ylmethyl))phenyl]ethanesulfonamide.

affinities at the $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors, the dopamine $D_2$ and 5-$HT_{1A}$ binding sites. However, no functional data was presented.

The present study characterizes the $\alpha_{1D}$-adrenoceptor selectivity of A-315456 (Fig. 1) using radioligand binding techniques and isolated tissue bath assays to directly compare the results to those published on the current $\alpha_{1D}$-adrenoceptor subtype selective antagonists, BMY-7378, and a related analogue, SNAP-8719. The results reported here suggest that A-315456, like SNAP-8719, could serve as a useful pharmacological agent for further understanding of the role of the $\alpha_{1D}$-adrenoceptor subtype in vivo.

## 2. Materials and methods

### 2.1. Radioligand binding assays

Studies were carried out in accordance with guidelines outlined by the Institutional Animal Care and Use Committee of Abbott Laboratories and the European Community guidelines for the use of experimental animals. Radioligand binding at the adrenergic receptor sites was performed according to Knepper et al. (1995). Briefly, the cDNA encoding bovine $\alpha_{1A}$-, rat $\alpha_{1D}$- and hamster $\alpha_{1B}$- (Lomasney et al., 1991; Cotecchia et al., 1988) adrenoceptors were obtained from Triangle Universities Licensing Consortium (Research Triangle Park, NC), expressed in mouse fibroblast cells ($LTK^-$), and membranes were prepared and frozen at $-70$ °C until the time of assay. Dopamine $D_2$ binding assays were performed using the method described by Vessotskie et al. (1997), using membranes derived from CHO cells expressing recombinant receptors (New England Nuclear, Boston, MA) and the dopamine $D_2/D_3$ selective agonist [$^{125}$I]-PIPAT under high affinity conditions. Radioligand binding assays for the rat 5-$HT_{1A}$-receptor was performed as described by De Vry et al. (1998) using membranes derived from rat cortex expressing recombinant receptors and [$^3$H]-8-hydroxy-DPAT (New England Nuclear).

### 2.2. Functional assays

The epididymal portion of the rat vas deferens $\alpha_{1A}$-adrenoceptors (Burt et al., 1992), the rat spleen $\alpha_{1B}$-adrenoceptors (Han et al., 1987), the rat aorta $\alpha_{1D}$-adrenoceptors (Buckner et al., 1996), and the prostatic rat vas deferens $\alpha_{2A}$-adrenoceptors (Connaughton and Docherty, 1990) were employed as functional subtype specific models. Phenylephrine was used as the reference agonist for all $\alpha_1$-adrenoceptor models. Clonidine was the reference agonist for the electrical field-stimulated $\alpha_{2A}$-adrenoceptor model. A-315456 was allowed a 30-min exposure time prior to generating the second agonist concentration–response curve in the presence of A-315456. Only one concentration of A-315456 was used per tissue.

### 2.3. Drugs and chemicals

A-315456 (*N*-[3-(cyclohexylidene-(1*H*-imidazol-4-ylmethyl))phenyl]ethanesulfonamide), BMY-7378 ([8-(2-[4-(2-methoxy-phenyl)-1-pierazinyl]ethyl)-8-azaspiro[4.5]decane-7,9-dione dihydrochloride]), SNAP-8719 (8-[(1*R*)-1-methyl-2-[4-(2,4,5-trifluorophenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride) and clonidine HCl were synthesized at Abbott Laboratories, USA. L-phenylephrine HCl and (±)-propranolol HCl were purchased from Sigma, St. Louis, MO, USA.

### 2.4. Data analysis

Concentration–response curves were analyzed using a four-parameter curve fitting routine (Zielinski and Buckner, 1998). The maximum peak amplitude response was measured and used for analysis. Data were analyzed as *g* of tension and calculated as a percentage of maximum response, and $EC_{50s}$ were determined. Antagonist potencies were expressed as p$A_2$ values ± S.E.M.; slopes were not different from unity. Differences in p$A_2$ values were compared by analysis of variance (ANOVA), followed by Fisher's probabilistic least significant difference (PLSD) test for significance. For SNAP-8719 in the rat spleen $\alpha_{1B}$-adrenoceptor assay, estimates of antagonism (p$K_B$) were calculated, where $K_B$=[antagonist, M]/[CR-1] (Furchgott, 1972), and expressed as the negative $\log_{10}$(p$K_B$).

## 3. Results

### 3.1. Radioligand binding assays

A-315456 showed high affinity (p$K_i$ = 8.71) for the $\alpha_{1D}$-adrenoceptor subtype expressed in a rat clonal cell line (Table 1). In comparison to its affinity for the $\alpha_{1D}$-adrenoceptor subtype, the agent was weaker at the bovine clonal $\alpha_{1A}$-adrenoceptor subtype (p$K_i$ = 6.23) and at the hamster clonal $\alpha_{1B}$-adrenoceptor subtype (p$K_i$ = 7.86). A-315456

Table 1
Radioligand binding affinities and functional potencies for A-315456, BMY-7378, and SNAP-8719

| | A-315456 | BMY-7378 | SNAP-8719 |
|---|---|---|---|
| *Radioligand binding, $pK_i$* | | | |
| Rat clonal $\alpha_{1D}$ | 8.71 ± 0.12 (1) | 9.05 ± 0.05 (1) | 9.42 ± 0.05 (1) |
| Bovine clonal $\alpha_{1A}$ | 6.23 ± 0.05 (302) | 7.42 ± 0.08 (43) | 5.50 ± 0.07 (8317) |
| Hamster clonal $\alpha_{1B}$ | 7.86 ± 0.66 (7) | 7.33 ± 0.04 (53) | 7.76 ± 0.13 (46) |
| Human clonal $\alpha_{2a}$ | 7.13 ± 0.11 (38) | 5.93 ± 0.09 (1318) | < 5.00 (>25,000) |
| Human clonal $D_2$ | 5.23 ± 0.05 (3020) | 7.62 ± 0.10 (27) | 5.98 ± 0.04 (2754) |
| Rat clonal 5-$HT_{1A}$ | 5.81 ± 0.05 (794) | 8.91 ± 0.04 (1.4) | 6.47 ± 0.02 (891) |
| *Functional antagonism, $pA_2$* | | | |
| Rat aorta $\alpha_{1D}$ | 8.34 ± 0.05 (1) | 8.39 ± 0.07 (1) | 8.72 ± 0.08 (1) |
| Rat vas deferens $\alpha_{1A}$ | < 4 (>10,000) | 5.98 ± 0.15 (257) | < 4.0 (>10,000) |
| Rat spleen $\alpha_{1B}$ | 6.42 ± 0.09 (83) | 7.24 ± 0.10 (14) | 6.58[a] ± 0.16 (138) |
| Rat vas deferens $\alpha_{2A}$ | < 5 (>2000) | < 5 (>2000) | < 5 (>5000) |

Data expressed as radioligand binding, p$K_i$ (mean ± S.E.M., $n = 4–11$) and functional antagonism, p$A_2$ (mean ± S.E.M., $n = 8–24$). Schild slopes were not different from unity. The number in parentheses represents selectivity relative to the $\alpha_{1D}$-adrenoceptor.

[a] p$K_B$ (mean ± S.E.M., $n = 12$, slope < unity).

was also examined for its affinity at 75 ancillary receptors and ion channels, and demonstrated affinity for only three additional receptors, dopamine $D_2$- (p$K_i$ = 5.23), 5-$HT_{1A}$- (p$K_i$ = 5.81), and $\alpha_2$-adrenoceptor (p$K_i$ = 7.13) (Table 1).

BMY-7378 showed greater affinity for all three $\alpha_1$-adrenoceptor subtypes in the current study than was reported by Goetz et al. (1995), Table 1. In our hands, the affinity (p$K_i$) of BMY was 9.05, 7.42, and 7.33 for the $\alpha_{1D}$-, $\alpha_{1A}$-, and $\alpha_{1B}$-adrenoceptor subtypes, respectively. BMY-7378 showed high affinity for the dopamine $D_2$-receptor, p$K_i$ = 7.62, even greater affinity for the 5-$HT_{1A}$-receptor, p$K_i$ = 8.91, and weaker affinity for the human clonal $\alpha_{2a}$-adrenoceptor, p$K_i$ = 5.93.

Recently, Konkel et al. (1998) presented a series of BMY-7378 analogues that identified SNAP-8719 as a potent $\alpha_{1D}$-adrenoceptor subtype selective agent (p$K_i$ = 8.89), potency confirmed here. SNAP-8719 was tested in these same assays and generated affinities p$K_i$ = 9.42, 5.50, and 7.76 for the $\alpha_{1D}$-, $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors, respectively (Table 1); results slightly higher than those reported by Konkel et al. (1998). SNAP-8719 showed affinity for the dopamine $D_2$-, 5-$HT_{1A}$-, and the human clonal $\alpha_{2a}$-adrenoceptor, with potencies (p$K_i$) of 5.98, 6.47, and < 5.0, respectively; results weaker than those observed at the $\alpha_{1D}$-adrenoceptor.

### 3.2. Functional assays

In isolated tissue bath studies, A-315456 was a potent antagonist of the $\alpha_{1D}$-adrenoceptor subtype (p$A_2$ = 8.34), as assessed by the competitive blockade of phenylephrine-induced contractions of the rat aorta (Table 1). At a maximum concentration of 100 μM, A-315456 showed no antagonist activity at the $\alpha_{1A}$- or $\alpha_{2A}$-adrenoceptor examined in the phenylephrine-stimulated epididymal rat vas deferens or the field-stimulated prostatic rat vas deferens, respectively. As an antagonist of the $\alpha_{1B}$-adrenoceptor subtype (p$A_2$ = 6.42), A-315456 was 83-fold weaker than that observed at the rat aorta $\alpha_{1D}$-adrenoceptor, as assessed in the phenylephrine-stimulated rat spleen. With the exception of the field-stimulated prostatic rat vas deferens, A-315456 showed no intrinsic stimulatory activity at any of the functional receptor sites examined up to a maximum concentration of 100 μM. However, in the field-stimulated vas deferens assay ($\alpha_{2A}$-adrenoceptor assay), A-315456, BMY-7378, and SNAP-8719 caused a concentration-dependent increase in the twitch response, thus limiting the analysis to concentrations < 100 μM.

BMY-7378 was also a potent antagonist at the $\alpha_{1D}$-adrenoceptor subtype with a p$A_2$ = 8.39 (Table 1). Goetz et al. (1995) reported a p$A_2$ = 8.9. BMY-7378 showed weak activity at the rat epididymal vas deferens $\alpha_{1A}$-adrenoceptor (p$A_2$ = 5.98), modest potency at the rat spleen $\alpha_{1B}$-adrenoceptor (p$A_2$ = 7.24), and no activity at the prostatic vas deferens $\alpha_{2A}$-adrenoceptor (p$A_2$ < 5) (Table 1). The potency of SNAP-8719 was determined in these same assays and was found to be the most potent antagonist at the $\alpha_{1D}$-adrenoceptor (p$A_2$ = 8.72), demonstrated no activity at the $\alpha_{1A}$-adrenoceptor (p$K_B$ < 4), and demonstrated modest noncompetitive inhibition at the $\alpha_{1B}$-adrenoceptor (p$K_B$ = 6.58) (Table 1).

## 4. Discussion

In radioligand binding studies, the relative affinities of A-315456 for the $\alpha_1$-adrenoceptor subtypes were $\alpha_{1D}$>$\alpha_{1B}$ (7-fold)>$\alpha_{1A}$ (302-fold), Table 1. A more complete biochemical study in 75 additional receptors showed that A-315456 was inactive at all receptors examined except for the dopamine $D_2$, 5-$HT_{1A}$, and $\alpha_{2a}$-adrenoceptor where it was 3020-, 794- and 38-fold weaker, respectively, compared to the $\alpha_{1D}$-adrenoceptor. BMY-7378 was equipotent at the $\alpha_{1D}$-adrenoceptor and the 5-$HT_{1A}$-receptor, and 27-fold selective against the dopamine $D_2$ binding site. The $\alpha_1$-adrenoceptor subtype

selectivity for BMY-7378 was $\alpha_{1D}$>[$\alpha_{1B}$ (53-fold) and $\alpha_{1A}$ (43-fold)]. Compared to its activity at the $\alpha_{1D}$-adrenoceptor, SNAP-8719 showed greater than 8000-fold weaker affinity at the $\alpha_{1A}$-receptor (p$K_i$ = 5.50) and 46-fold weaker affinity at the $\alpha_{1B}$-adrenoceptor (p$K_i$ = 7.76). Radioligand binding affinities at the dopamine $D_2$-receptor (p$K_i$ = 5.98) and 5-$HT_{1A}$-receptor (p$K_i$ = 6.47) were less than seen with BMY-7873, but were greater than seen with A-315456 (Table 1). However, the greater $\alpha_{1D}$-adrenoceptor affinity of SNAP-8719 compared to A-315456 created similar selectivity ratios. The weaker affinity of A-315456 and SNAP-8719 for the dopamine $D_2$ and 5$HT_{1A}$-receptor sites makes the two compounds the most selective $\alpha_{1D}$-adrenoceptor antagonists published, to date, with respect to ancillary binding activity.

$\alpha_1$-Adrenoceptor subtype selectivity was observed for A-315456 in functional tissue bath assays with $\alpha_{1D}$>$\alpha_{1B}$ (83-fold)≫[$\alpha_{2A}$ and $\alpha_{1A}$>(2000- to 10,000-fold)]. The observation that the functional antagonism at the $\alpha_{1A}$-adrenoceptor subtype expressed in the rat vas deferens was less than that predicted by the radioligand binding affinity is difficult to explain. However, A-315456 (100 μM) was also inactive at the functional $\alpha_{1A}$-adrenoceptor subtype expressed in both the rabbit urethra and canine prostate gland (data not shown). Furthermore, A-315456 showed comparable radioligand binding affinity on another $\alpha_{1A}$-adrenoceptor model, the rat submandibular gland with a p$K_i$ = 6.64 ± 0.03. A-315456 did not demonstrate any solubility problems, and increasing the exposure time to 60 min did not alter the data. Since A-315456 did not demonstrate any intrinsic agonist activity in the functional $\alpha_{1A}$-adrenoceptor models, one could eliminate interference associated with partial agonism as a contributing factor. One might speculate a role for inverse agonism where an "agonist may behave as a positive and inverse agonist on the same receptor and differ in the stimulus pattern they produce in physiological systems" (Kenakin, 2001; Rossier et al., 1999).

BMY-7378 was also a potent antagonist at the functional $\alpha_{1D}$-adrenoceptor subtype, however, it was less selective than A-315456 with $\alpha_{1D}$>$\alpha_{1B}$ (14-fold)≫$_{1A}$ (>257-fold). Although Konkel et al. (1998) did not report functional data for SNAP-8719, we were able to demonstrate potent $\alpha_{1D}$-, no $\alpha_{1A}$-, and only moderate $\alpha_{1B}$-adrenoceptor antagonism using the same assays described above. The potency of SNAP-8719 at the spleen $\alpha_{1B}$-adrenoceptor was expressed as a p$K_B$ since the slope of the Schild plot was significantly less than unity. This may be due to a solubility issue at the higher concentrations. SNAP-8719 tends to adhere to glass, a problem solved by dissolving in dimethyl sulfoxide or methanol; however, precipitation is seen when the solution is then added to the tissue bath.

SNAP-8719 was more potent than A-315456 at the $\alpha_{1D}$-adrenoceptor but also more potent at the $\alpha_{1B}$-adrenoceptor subtype. The $\alpha_{1D}$ to $\alpha_{1B}$ selectivity of A-315456 and SNAP-8719 were similar, 83- and 138-fold, respectively. None of the three agents that were tested showed any functional antagonism against clonidine attenuation of field-stimulated twitch in the rat vas deferens $\alpha_{2A}$-subtype. However, they all enhanced the stimulated twitch at concentrations >100 μM, thus limiting the analysis to concentrations lower than 100 μM. The mechanism for this effect is not known; we have only seen this type of response when an uptake inhibitor like cocaine is given in the presence of an $\alpha_2$-adrenoceptor antagonist like rauwolscine.

The importance of the $\alpha_{1D}$-subtype in the functional control of micturition has not been established, and agents like A-315456 and SNAP-8719 may play an important role in exploring this issue. It has been demonstrated that the $\alpha_{1D}$-adrenoceptor present in the rat bladder is up-regulated secondary to bladder outlet obstruction (Hampel et al., 2000), a paradigm like benign prostatic hyperplasia. The $\alpha_{1D}$-adrenoceptor may serve a role in irritative and filling disorders (Schwinn and Michelotti, 2000). Spinal and supraspinal $\alpha_{1D}$-adrenoceptors may also modulate bladder responses (Smith et al., 1999).

In summary, A-315456 and SNAP-8719 are potent and selective antagonists at the functional $\alpha_{1D}$-adrenoceptor subtype in vitro. Additionally, both agents demonstrate weaker affinity for the dopamine $D_2$ and 5-$HT_{1A}$ radioligand binding sites than BMY-7378. Therefore, A-315456, as well as SNAP-8719, may serve as useful pharmacological tools for further defining the role of the $\alpha_{1D}$-adrenoceptor subtype in mammalian tissues in vivo.

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