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Functional characterization of α-adrenoceptors mediating pupillary dilation in rats

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Abstract

Previously, we reported that the α_{1A}-adrenoceptor, but not the α_{1D}-adrenoceptor, mediates pupillary dilation elicited by sympathetic nerve stimulation in rats. This study was undertaken to further characterize the α-adrenoceptor subtypes mediating pupillary dilation in response to both neural and agonist activation. Pupillary dilator response curves were generated by intravenous injection of norepinephrine in pentobarbital-anesthetized rats. Involvement of α_1-adrenoceptors was established as mydriatic responses were inhibited by systemic administration of nonselective α-adrenoceptor antagonists, phentolamine (0.3–3 mg/kg) and phenoxybenzamine (0.03–0.3 mg/kg), as well as by the selective α_1-adrenoceptor antagonist, prazosin (0.3 mg/kg). The α_2-adrenoceptor antagonist, rauwolscine (0.5 mg/kg), was without antagonistic effects. α_{1A}-Adrenoceptor selective antagonists, 2-([2,6-dimethoxyphenoxyethyl]aminomethyl)-1,4-benzodioxane (WB-4101; 0.1–1 mg/kg) and 5-methylurapidil (0.1–1 mg/kg), the α_{1B}-adrenoceptor selective antagonist, 4-amino-2-[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino]carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline (L-765314; 0.3–1 mg/kg), as well as the α_{1D}-adrenoceptor selective antagonist, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY-7378; 1 mg/kg), were used to delineate the adrenoceptor subtypes involved. Mydriatic responses to norepinephrine were significantly antagonized by intravenous administration of both WB-4101 and 5-methylurapidil, but neither by L-765314 nor by BMY-7378. L-765314 (0.3–3 mg/kg, i.v.) was also ineffective in inhibiting the mydriasis evoked by cervical sympathetic nerve stimulation. These results suggest that α_{1B}-adrenoceptors do not mediate sympathetic mydriasis in rats, and that the α_{1A}-adrenoceptor is the exclusive subtype mediating mydriatic responses in this species.

Keywords: Adrenoceptor subtype; Mydriasis; (Rat); Norepinephrine; 5-Methylurapidil; L-765314

1. Introduction

Current pharmacological classification recognizes three subtypes of α_1-adrenoceptors (α_{1A}, α_{1B} and α_{1D}), corresponding to the cloned subtypes α_{1a}, α_{1b} and α_{1d} (Hieble et al., 1995; Docherty, 1998; Piascik and Perez, 2001). A fourth, atypical subtype (α_{1L}) exhibiting low affinity for prazosin in functional studies was also postulated (Flavahan and Vanhoutte, 1986; Docherty, 1998), which may represent a phenotype of the α_{1a} subtype displaying tissue-dependent characteristics (Ford et al., 1997; Daniels et al., 1999).

All three α_1-adrenoceptor subtypes have been detected from the iris, at mRNA and protein levels, with a slight predominance of the α_{1A}-adrenoceptor subtype, followed by the α_{1B}-adrenoceptor subtype (Vidovic and Hill, 1995; Grayson et al., 1998; Nakamura et al., 1999; Wikberg-Matsson et al., 2000). Minimal gene transcription of the α_{1D}-adrenoceptor is also observable (Vidovic and Hill, 1995).

Functionally, we have characterized α_{1A}-adrenoceptor as the predominant subtype in the mediation of mydriasis evoked by sympathetic nerve stimulation in rats, whereas α_{1D}-adrenoceptors are not involved (Yu and Koss, 2002). This is primarily based on effects of the α_{1A}-adrenoceptor antagonist, 5-methylurapidil, and the α_{1D}-adrenoceptor antagonist, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY-7378). However, the exact role of α_{1B}-adrenoceptors has not been adequately evaluated due to lack of specific ligands for this receptor subtype. It is possible that α_{1B}-adrenoceptors may play a minor role in the mediation of neurally evoked pupillary dilation. Alternatively, it has been hypothesized that α_{1A}-adrenoceptors reside in the synaptic region of neuromuscular junction within the iris dilator muscle where they are primar-

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ily activated by neuronal norepinephrine release (Eltze, 1997). Other α_1-adrenoceptors (α_{1B}, α_{1D} or α_{1L}) may be located in the peri-synaptic regions where they can be stimulated by exogenous α_1-adrenoceptor agonists or circulating catecholamines (Eltze, 1997).

The present series of in vivo experiments were designed to determine (1) the α-adrenoceptor subtypes mediating the mydriasis elicited by exogenous norepinephrine, and (2) the relative role of α_{1B}-adrenoceptors in the mediation of mydriasis produced by both norepinephrine administration and sympathetic nerve stimulation with the selective α_{1B}-adrenoceptor ligand, 4-amino-2-[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino]carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline (L-765314; Patane et al., 1998). Our results suggest that α_{1A}-adrenoceptors are almost exclusively involved, and do not support the hypothesis that mydriatic responses evoked by endogenous (neuronal) and exogenous norepinephrine are differentially mediated in this species.

2. Materials and methods

2.1. Animal preparation

Adult male Sprague–Dawley rats (340–588 g) were anesthetized with pentobarbital (60 mg/kg i.p. + 5 mg i.v. as needed). A femoral artery and vein were cannulated for monitoring blood pressure (Statham P23 pressure transducer; Statham, Murray Hill, NJ) and for i.v. drug administration, respectively. Heart rate was derived from the pressure wave using a Grass tachograph (7P4D; Grass Instruments, Quincy, MA). The trachea was intubated. Rectal temperature was maintained at approximately 37 °C with a Deltaphase isothermal pad (Braintree Scientific, Braintree, MA). Pupillary diameter was measured using a 0.1-mm ruler under a surgical microscope (Olympus, Tokyo, Japan). A green light filter was utilized to increase visual contrast as well as to reduce light-induced pupillary constriction. Studies were approved by the Institutional Animal Care and Use Committee of University of Oklahoma Health Sciences Center and were undertaken in accordance with the "NIH Guide to the Care and Use of the Laboratory Animals".

2.2. Norepinephrine challenge

Norepinephrine diluted in approximately 0.1 ml of physiological saline was administered intravenously as a bolus in increasing doses (1, 3.3, 10, 33, 100 μg/kg), which elicited transient pupillary dilations. The maximal mydriatic effect after each injection was recorded to establish dose–response relationships. Pupillary effects were allowed to recover to the basal level before the next higher dose of norepinephrine was administered. Initial saline control experiments indicated that reversible dose–response curves of pupillary dilation could be produced from the same animal, with 15-min intervals between each curve. Antagonists were administered (i.v.) slowly over 1–3 min in order to avoid rapid blood pressure changes. Dose–response curves were generated approximately 15 min after antagonist or saline administration.

2.3. Sympathetic nerve stimulation

One cervical preganglionic sympathetic nerve was carefully separated from the vagus nerve and crushed proximally. A bipolar electrode was placed under the separated cervical sympathetic nerve and covered with mineral oil. The stimuli, generated by a Grass S88 stimulator, consisted of 10-s trains of 5-V pulses (width: 2 ms, frequency: 1–64 Hz), which elicited reproducible pupillary dilations. Each mydriatic response was allowed to recover to the basal level before the next higher frequency of stimulation was applied. Frequency–response curves were generated approximately 15 min after i.v. drug (L-765314) or saline administration.

2.4. Drugs and data analysis

(−)-Norepinephrine (bitartrate salt, hydrate), phentolamine hydrochloride, phenoxybenzamine hydrochloride, prazosin hydrochloride, 2-([2,6-dimethoxyphenoxyethyl]aminomethyl)-1,4-benzodioxane (WB-4101) and 5-methylurapidil were purchased from Sigma (St. Louis, MO). Rauwolscine hydrochloride was obtained from Sigma/RBI (Natick, MA). L-765314 was from Merck (West Point, PA). BMY-7378 dihydrochloride was from Tocris Cookson (Ballwin, MO). Solutions of drugs were prepared in sterile physiological saline or distilled water, with the exception of prazosin (2.5% glucose w/v: 2.5% glycerol v/v) and L-765314 (first dissolved in 95% alcohol, then diluted with distilled water). Doses administered represent the respective salts.

Values are reported as means ± S.E.M. Statistical significance was determined using one-way analysis of variance followed by Dunnett's *t*-test. *P*-values of less than 0.05 are considered significant.

3. Results

3.1. Effects of nonselective α-adrenoceptor antagonists

Multiple norepinephrine dose–response curves were generated, at 15-min intervals, in anesthetized rats. In these control experiments, norepinephrine (1–100 μg/kg, i.v.) produced mydriatic responses that were reproducible for up to four successive trials (data not shown). Initial experiments were undertaken to determine if the rat has "typical" adrenoceptors in the pupil dilator muscle mediating norepinephrine-elicited mydriasis. Nonselective, prototypical α-adrenoceptor antagonists, phentolamine (0.3–3 mg/kg) and phenoxybenzamine (0.03–0.3 mg/kg), as well as the selec-

tive α_1-adrenoceptor antagonist, prazosin (0.3 mg/kg), and the α_2-adrenoceptor antagonist, rauwolscine (0.5 mg/kg), were administered systemically. Norepinephrine dose–response curves were generated before and 15 min after cumulative antagonist administration. As shown in Fig. 1, the mydriasis produced by norepinephrine was antagonized

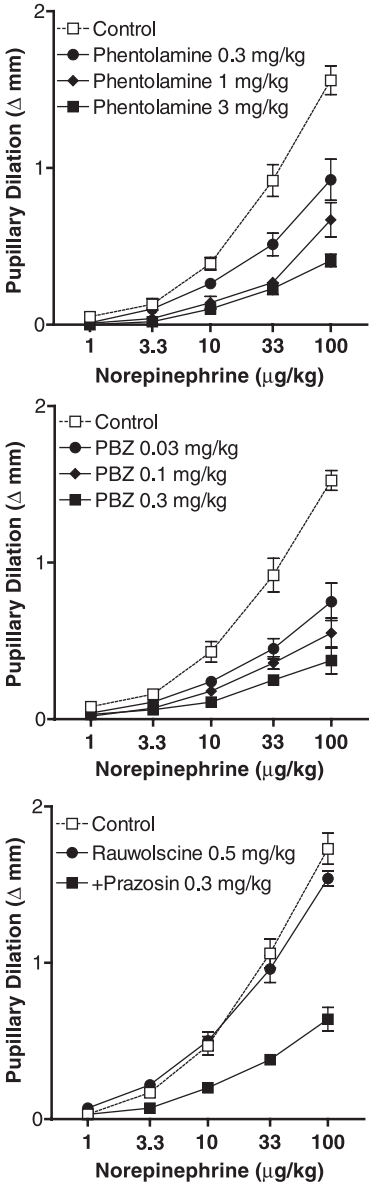

Fig. 1. Effects of α-adrenoceptor antagonists on norepinephrine-elicited mydriasis in anesthetized rats. Dose–response curves were generated before and 15 min after cumulative antagonist administration. All drugs were given intravenously. Values in each panel represent means ± S.E.M. for five animals. Statistics were determined at 10, 33 and 100 μg/kg as compared with respective control values. In upper two panels, norepinephrine-elicited pupillary responses were dose-dependently inhibited by nonselective α-adrenoceptor antagonists, phentolamine (0.3–3 mg/kg; $P<0.01$) and phenoxybenzamine (PBZ, 0.03–0.3 mg/kg; $P<0.01$). In the lower panel, animals were initially given rauwolscine (0.5 mg/kg), followed by addition of prazosin (0.3 mg/kg). Note that evoked pupillary responses were inhibited by the α_1-adrenoceptor antagonist, prazosin ($P<0.01$), but not by the α_2-adrenoceptor antagonist, rauwolscine ($P>0.05$).

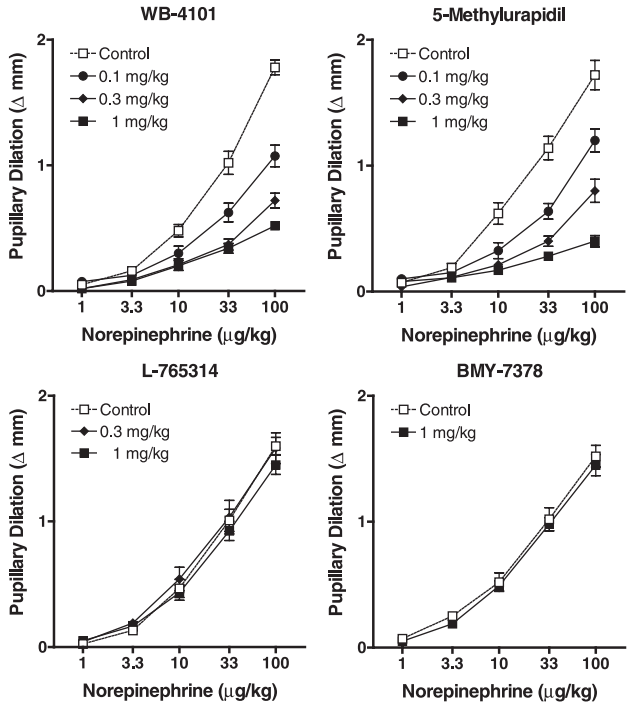

Fig. 2. Effects of subtype-selective α_1-adrenoceptor antagonists on norepinephrine-elicited mydriasis in anesthetized rats. Dose–response curves were generated before and 15 min after cumulative antagonist administration. All drugs were given intravenously. Values in each panel represent means ± S.E.M. for 5–6 animals. Statistics were determined at 10, 33 and 100 μg/kg as compared with respective control values. Note that elicited pupillary responses were dose-dependently inhibited by α_{1A}-adrenoceptor selective antagonists, WB-4101 (0.1–1 mg/kg; $P<0.01$) and 5-methylurapidil (0.1–1 mg/kg; $P<0.01$), but neither by the α_{1B}-adrenoceptor selective antagonist, L-765314 (0.3–1 mg/kg; $P>0.05$) nor by the α_{1D}-adrenoceptor selective antagonist, BMY-7378 (1 mg/kg; $P>0.05$).

by both phentolamine and phenoxybenzamine in a dose-dependent fashion. Rauwolscine had no significant effect on pupillary dilations, which, however, were blocked by prazosin.

3.2. Effects of subtype-selective α_1-adrenoceptor antagonists

To delineate which subtypes of α_1-adrenoceptors are involved, animals were challenged with cumulative doses of α_{1A}-adrenoceptor selective antagonists, WB-4101 (0.1–1 mg/kg) and 5-methylurapidil (0.1–1 mg/kg), given intravenously. The selective α_{1B}-adrenoceptor antagonist, L-765314 (0.3–1 mg/kg, i.v.), and the selective α_{1D}-adrenoceptor antagonist, BMY-7378 (1 mg/kg, i.v.), were utilized to determine the involvement of α_{1B}- and α_{1D}-adrenoceptors, respectively. Norepinephrine dose–response curves were generated before and 15 min after antagonist administration. Norepinephrine-elicited pupillary dilations were reduced in magnitude by WB-4101 and 5-methylurapidil in a dose-dependent fashion, but were not significantly affected by either L-765314 or BMY-7378 (Fig. 2).

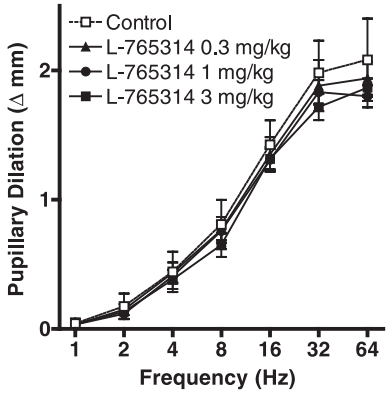

Fig. 3. Effects of the α_{1B}-adrenoceptor selective antagonist, L-765314, on mydriatic responses evoked by cervical preganglionic sympathetic nerve stimulation in six anesthetized rats. Frequency–response curves were generated before and 15 min after cumulative intravenous administration of L-765314. Values represent means ± S.E.M. Note that the evoked pupillary response was not inhibited by L-765314 at 0.3–3 mg/kg.

3.3. Effect of L-765314 on neurally evoked mydriasis

To further assess whether L-765314 has any effect on the mydriasis elicited by neuronal norepinephrine release, frequency–response curves of pupillary dilation were generated before and 15 min after cumulative intravenously administration of the selective α_{1B}-adrenoceptor antagonist, L-765314 (0.3–3 mg/kg). As shown in Fig. 3, L-765314 had no significant effect on the mydriasis evoked by direct cervical sympathetic nerve stimulation at 1–64 Hz.

4. Discussion

This study showed that pupillary dilations in response to exogenous norepinephrine were sensitive to antagonism by prazosin, phentolamine and phenoxybenzamine, but were not inhibited by rauwolscine. This antagonistic profile suggests that, similar to neurally elicited mydriasis in this species (Yu and Koss, 2002), "typical" α_1-adrenoceptors are involved. Although in a strict sense it is difficult to relate our in vivo data directly to the in vitro definition of the putative α_{1L}-adrenoceptor (Flavahan and Vanhoutte, 1986; Ford et al., 1997; Docherty, 1998; Daniels et al., 1999), a review of species variations led us to conclude that a more classical α_1-adrenoceptor is involved in the rat. Adrenoceptors in the iris dilator muscle of rabbits and cats have been reported not to be inhibited by the prototypical α_1-adrenoceptor antagonist, prazosin, up to an intravenous dosage of 1 mg/kg (Murray and Leopold, 1985; Hey et al., 1988; Koss et al., 1988, 1990). The cat irideal adrenoceptors are also refractory to the nonselective α-adrenoceptor antagonist, phentolamine (Hey et al., 1988; Koss et al., 1988, 1990). In humans, adrenoceptors in the iris dilator muscle have been found to be insensitive to prazosin (Ishikawa et al., 1996) and phentolamine (Weiner, 1985), although in other cases they are blocked by both antagonists (Yoshitomi et al., 1985; Mortlock et al., 1996). In addition, Nobata et al. (2002) reported that in guinea pigs, α_{1L}-adrenoceptors mediate the allergic bronchoconstriction, which is not attenuated by prazosin at 1–10 mg/kg, doses higher than that used in our study.

In the present study, norepinephrine-elicited mydriatic responses in rats were blocked by α_{1A}-adrenoceptor selective antagonists, WB-4101 and 5-methylurapidil, but were not antagonized either by the α_{1B}-adrenoceptor selective antagonist, L-765314, or by the α_{1D}-adrenoceptor selective antagonist, BMY-7378. The selectivity of these ligands for α_1-adrenoceptor subtypes has been previously established (Morrow and Creese, 1986; Hanft and Gross, 1989; Goetz et al., 1995; Patane et al., 1998). L-765314 also did not antagonize the mydriasis elicited by electrical stimulation of the preganglionic cervical sympathetic nerve. Previously, we have reported that the mydriasis elicited by sympathetic nerve stimulation was blocked by 5-methylurapidil but not by BMY-7378 in this species (Yu and Koss, 2002). All together, these results suggest an exclusive involvement of α_{1A}-adrenoceptors in sympathetic mydriasis in the rat.

The novel α_{1B}-adrenoceptor antagonist, L-765314, displays relatively high affinity for α_{1B}-adrenoceptors (5.4 nM on cloned rat α_{1B}-adrenoceptors and 9.3 nM on rat liver tissue), and has approximately 93-fold selectivity for cloned α_{1B}-adrenoceptors over cloned α_{1A}-adrenoceptors (Patane et al., 1998). This drug has been reported to selectively block the α_{1B}-adrenoceptor mediating electrically evoked vasoconstriction of isolated canine splenic arteries at 0.1–1 μM concentrations (Yang and Chiba, 2002). Willems et al. (2001) found that intravenous administration of 0.3 mg/kg of L-765314, but not of either 5-methylurapidil or BMY-7378, blocks the constrictor effect of phenylephrine on pig carotid arteriovenous anastomoses, suggesting that 0.3 mg/kg of L-765314 may be selective on α_{1B}-adrenoceptors. Therefore, we believe that the dosage used in our study should be able to antagonize the elicited mydriasis, if α_{1B}-adrenoceptors contribute to the response. However, we cannot exclude the possibility that a lack of action may be due to some pharmacokinetic factors. The present result is consistent with our previous finding that 1 mg/kg of 5-methylurapidil appears to block all of the neurally evoked pupillary dilation mediated by α_1-adrenoceptors (Yu and Koss, 2002).

Several studies have identified α_{1B}-adrenoceptors from the iris. For instance, Vidovic and Hill (1995) found an abundant mRNA expression of α_{1B}-adrenoceptors in the rat iris, only slightly less than that of α_{1A}-adrenoceptors. The protein of α_{1B}-adrenoceptors has also been localized by specific antibodies in the iris dilator muscle of this species (Grayson et al., 1998). In rabbits, Nakamura et al. (1999) found a strong expression of α_{1A}-adrenoceptors, a weak expression of α_{1B}-adrenoceptors and an undetectable expression of α_{1D}-adrenoceptors at the mRNA level in the iris, consistent with the data from the rat (Vidovic and Hill,

1995). In a ligand-binding study in rabbits, both α_{1A}- and α_{1B}-adrenoceptors are detected in the iris, with approximately 60% and 40% in proportion, respectively (Wikberg-Matsson et al., 2000). Therefore, it is reasonable to speculate that both α_{1A}- and α_{1B}-adrenoceptors may play an important role in the iris. However, our current results do not support a role of α_{1B}-adrenoceptors in the mediation of sympathetic mydriasis.

The adrenergic mechanism of the iris dilator muscle appears to agree with the adrenergic regulation of the vascular smooth muscle. Although multiple α_1-adrenoceptors have been found in peripheral arteries, in most situations, a single α_1-adrenoceptor subtype (either α_{1A} or α_{1D}) is responsible for mediating the contraction of each blood vessel type (Piascik and Perez, 2001). The protein of α_{1B}-adrenoceptors is abundantly expressed in the smooth muscle of rat renal and femoral arteries. However, inhibition of the α_{1B}-adrenoceptor expression using antisense oligonucleotides has little effect on the vascular contractile response, suggesting that α_{1B}-adrenoceptors do not participate in vascular contractile regulation (Piascik et al., 1997; Hrometz et al., 1999).

It has been speculated that different α_1-adrenoceptor subtypes may mediate endogenous and exogenous norepinephrine elicited mydriasis (Eltze, 1997). This is supported by the clinical finding that the mydriasis induced by neuronally released norepinephrine is attenuated by the calcium entry blocker, nimodipine, whereas that evoked by phenylephrine is not (Alessandri et al., 1992). Such a differential mechanism of activation has also been found in some sympathetic organs including rat vas deferens and canine splenic artery (Honner and Docherty, 1999; Yang and Chiba, 2000, 2001). However, using receptor-specific pharmacological ligands, Nakamura et al. (1999) observed no difference in isolated rabbit iris dilator muscle in response to endogenous and exogenous norepinephrine. Our present in vivo study in rats provides further support of their conclusion.

More recently, Suzuki et al. (2002) revisited the adrenoceptor subtypes in the rabbit eye and mainly found the α_{1A}-adrenoceptor subtype in the iris. They also revealed more α_{1B}-(60–70% of total α_1-adrenoceptors) than α_{1A}-adrenoceptors in the ciliary body. These findings are consistent with our current functional study that the α_{1A}-adrenoceptor is probably the exclusive subtype that mediates the mydriasis. In addition, the concentration of α_{1B}-adrenoceptors in the ciliary body suggests that a possible role of α_{1B}-adrenoceptors in the modulation of aqueous humor dynamics needs to be clarified (Suzuki et al., 2002).

Taken together, the present investigation examined the adrenoceptor subtypes in the mediation of pupillary dilation in rats. The results showed that, together with our previous cervical sympathetic nerve stimulation study (Yu and Koss, 2002), the pharmacological profiles of adrenoceptors mediating mydriasis elicited by exogenous norepinephrine and sympathetic nerve stimulation are similar. The receptors involved may be characterized as being almost exclusively of the α_{1A}-adrenoceptor subtype. Although α_{1B}-adrenoceptors have been shown to exist in the iris in various studies, they do not appear to contribute to the modulation of pupillary size.

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