Alpha$_1$-Adrenergic Receptor Induced Subsensitivy and Supersensitivity in Rabbit Iris-Ciliary Body

Effects on Myo-Inositol Trisphosphate Accumulation, Arachidonate Release, and Prostaglandin Synthesis

Sardar Y. K. Yousufzai and Ata A. Abdel-Latif

Topical treatment of the rabbit eye with three successive doses of 2% epinephrine resulted in attenuation of the in vitro drug response of the alpha$_1$-adrenoceptor-mediated phosphoinositide hydrolysis system and of alpha$_1$-adrenergic receptor-mediated contraction in the iris. In contrast, sympathetic denervation of the eye resulted in potentiation of these responses. Desensitized tissues showed a significant decrease in epinephrine-induced myo-inositol trisphosphate (IP$_3$) accumulation, 1,2-diacylglycerol (DG) formation, measured as phosphatidate, arachidonic acid (AA) liberation, measured by radiochromatography, prostaglandin (PG) E$_2$ release, measured by radiochromatography and radioimmunoassay, and muscle contraction. Adrenergic desensitization of the eye resulted in attenuation of: (1) The polyphosphoinositide response in the iris, measured both as loss of $^{32}$P-radioactivity from phosphatidylinositol 4,5-bisphosphate (PIP$_2$) and as IP$_3$ accumulation; (2) the epinephrine-stimulated liberation of AA, from membrane phosphoinositides and other phospholipids, and PGE$_2$ release in the iris; and (3) the epinephrine-induced muscle contraction in the iris dilator. This adrenergic desensitization of the eye is reversible. Surgical sympathetic denervation, previously found to increase alpha$_1$-adrenoceptor mediated accumulation of IP$_3$ and contraction, increased AA liberation. Dexamethasone blocked the epinephrine-induced liberation of AA and PG release, both in vivo and in vitro. These data support the hypothesis that changes in the activity of the alpha$_1$-adrenergic receptor-mediated phosphoinositide hydrolysis system and its derived second messengers may underlie the mechanism of adrenergic subsensitivity and supersensitivity in the iris-ciliary body. How much the desensitization of alpha$_1$-adrenergic receptor-mediated responses contribute to the therapeutic action of epinephrine in the eye remains to be determined.


Adrenergic supersensitivity in the iris smooth muscle has been reported to develop after chronic interruption of sympathetic neurotransmission by surgical denervation, and adrenergic subsensitivity, or desensitization, has been shown to develop in this tissue as a result of topical or systemic long-term treatment with epinephrine and other adrenergic drugs. Agonist-induced receptor desensitization in the iris has also been investigated for muscarinic cholinergic receptors. It develops within hours, and can persist for several days. Desensitization, which may limit the clinical use of adrenergic drugs, can occur rapidly (seconds to minutes) and it is not necessarily associated with a reduction in the number of neurotransmitter receptors (for review see Sibley and Lefkowitz). In the rabbit iris-ciliary body, the epinephrine-induced desensitization of beta-adrenergic receptor-coupled adenylate cyclase has been associated with a functional impairment of the guanyl nucleotide regulatory protein and possibly with a small decrease in the number or affinity of beta-adrenergic receptor ligand binding sites.

In addition to receptors that regulate cyclic AMP formation, there is another major type of cell surface receptors which mediates the receptors' effects via an

From the Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia.

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Reprint requests: Dr. Ata Abdel-Latif, Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-3331.
increase in cytosolic Ca\(^{2+}\). These are referred to as Ca\(^{2+}\)-mobilizing receptors, and there is experimental evidence which indicates that these receptors affect intracellular Ca\(^{2+}\) levels via hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into inositol 1,4,5-trisphosphate (IP\(_3\)) and 1,2-diacylglycerol (DG), and under the same experimental conditions there is liberation of arachidonic acid (AA) for eicosanoid biosynthesis (for reviews see Berridge and Irvine,\(^\text{10}\) Abdel-Latif, Green and Smith.\(^\text{11}\))

In the smooth muscles of the rabbit iris, activation of \(\alpha_1\)-adrenergic,\(^\text{5,12,13}\) muscarinic cholinergic\(^\text{12,13}\) and substance P\(^\text{14}\) receptors results in a rapid breakdown of PIP\(_2\) and the liberation of AA for prostaglandin (PG) biosynthesis.\(^\text{15,16}\)

In the iris dilator, sympathetic denervation increased the \(\alpha_1\)-adrenoceptor-mediated accumulation of IP\(_3\) and contraction, and this was suggested as a possible mechanism underlying the phenomenon of denervation supersensitivity in this tissue.\(^\text{5}\)

Catecholamines cause contraction of iris dilator by activating \(\alpha_1\)-adrenergic receptors.\(^\text{5}\) The present studies were designed to characterize \(\alpha_1\)-adrenergic receptor-mediated desensitization in the eye employing the iris-ciliary body as a model, and to investigate the possibility that changes in phosphoinositide hydrolysis could be involved in the mechanism responsible for the desensitization.

**Materials and Methods**

**Chemicals**

\([1-^{14}C]\)Arachidonic acid (56.5 mCi/mmol) and myo-[\(^3\)H]inositol (15.5 Ci/mmol) were purchased from Amersham Corp., Arlington Heights, IL; \([^{32}\text{Pi}]\)orthophosphate, carrier-free, was obtained from New England Nuclear, Boston, MA. L(-)Epinephrine bitartrate, norepinephrine, and dexamethasone were purchased from Sigma Chemical Co., St. Louis, MO; prazosin hydrochloride was a gift from Pfizer, NY; and PGE\(_2\)-\(^3\)H-RIA kit was obtained from Seragen Inc., Boston, MA. All other chemicals were of reagent grade.

**Animals**

Albino rabbits of both sexes weighing approximately 4 kg were used in the present study. All studies were carried out in accordance with the ARVO Resolution on the Use of Animals in Research.

**Sympathetic Denervation**

The procedure for surgical sympathetic denervation was the same as that previously described by Abdel-Latif, Green and Smith.\(^\text{4}\) The superior cervical ganglion on one side (the other served as control) was carefully isolated and removed. Two weeks following the above surgical procedure, NE (50 \(\mu\)l of 1% solution) administered topically to the eyes was used to verify denervation supersensitivity. An almost complete mydriasis in the denervated eye occurred within approximately 10 min. The pupil size in the normally innervated eye did not change. Animals were allowed at least 3 days to recover from the above test before they were killed. Eyes were enucleated, cooled in ice and the iris-ciliary body dissected free and immediately incubated under various conditions as discussed below.

**Adrenergic Desensitization**

This was carried out essentially as described by Mitter and Tormay.\(^\text{7}\) Briefly, rabbits were treated topically in one eye with 2% epinephrine in three successive doses (2 \(\times\) 50 \(\mu\)L) at 32, 24 and 6 hr before killing by exposure to CO\(_2\) gas. Control eyes received vehicle (saline) only. Eyes were enucleated, cooled in ice and the iris-ciliary body dissected free and immediately incubated under various conditions as discussed below.

**Incubation of Irides For Studies on the Effects of Catecholamines on PIP\(_2\) Breakdown and IP\(_3\) Accumulation**

Eyes (normal, denervated, or desensitized) were enucleated, the irides dissected free, and each iris was placed in modified Krebs-Ringer bicarbonate buffer of the following composition: 118 mM NaCl, 25 mM NaHCO\(_3\), 4.7 mM KCl, 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 1.25 mM CaCl\(_2\), 1.6 mM cytidine, and 10 mM D-glucose. The pH of the buffer was adjusted to 7.4 with 97% O\(_2\)-3% CO\(_2\).

**Incubation with \(^{32}\text{P}\) and phospholipid analysis:** Each iris muscle was incubated for 45 min in one ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 20 \(\mu\)Ci\(^{32}\text{Pi}\). The prelabeled irides were then halved (of the two halves, one served as control and the other as experimental) and each muscle incubated in 1 ml fresh buffer containing 20 \(\mu\)Ci\(^{32}\text{Pi}\) for 15 min in absence and presence of various concentrations of epinephrine. The reaction was stopped with 10% (w/v) trichloroacetic acid (TCA) and the phospholipids were extracted and analyzed for radioactivity by one- and two-dimensional thin-layer chromatography (TLC) as previously described.\(^\text{12}\)

**Incubation with myo-[\(^3\)H]inositol and analysis of IP\(_3\) accumulation:** Each iris muscle was incubated at 37°C in one ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5 \(\mu\)Ci myo-[\(^3\)H]inositol for 1 hr. The prelabeled muscles were then halved (of the two halves, one served as control and the other as experimental) and each muscle incubated in 1 ml fresh buffer containing 2.5 \(\mu\)Ci myo-[\(^3\)H]inositol for 15 min. The incubation was then continued in absence and presence of various concentrations of epinephrine for 15 min at...
37°C. The incubations were terminated with 10% (w/v) TCA and the radiolabeled myo-inositol phosphates were analyzed by anion-exchange chromatography.

Incubation and Assay of PGE$_2$ Release by Radioimmunoassay

The muscles were halved (of the two halves, one was used as control and the other as experimental) then incubated in 1 ml of Krebs-Ringer bicarbonate buffer at 37°C for 15 min or as indicated. Epinephrine and other pharmacological agents were added as indicated. The reactions were ended by acidification of the medium with 10% formic acid to pH 3.5 and addition of ethyl acetate. The solvent was evaporated under N$_2$. The residue was dissolved in chloroform/methanol (2:1, v/v) and PG was determined by RIA as previously described.

The amount of PG in each sample was determined by interpolation from the standard curve. Data presented in the text and figures are means ± SEM.

Prelabelling of Iris Muscle With [14C]AA and Assay For the Release of Labeled Arachidonate and PGs

Iris muscles were preincubated singly in one ml of Krebs-Ringer bicarbonate buffer that contained 0.2 μCi of [14C]AA at 37°C for 1 hr. The prelabelled muscles were washed three times with nonradioactive medium, then halved (of the two halves, one was used as control), and incubated in the absence and presence of the epinephrine as indicated. In experiments where the effects of dexamethasone were investigated, the inhibitor was added 5 min prior to the addition of the agonist. At the end of incubation, the medium was analysed for radioactive AA and PGE$_2$. The medium was acidified with 10% formic acid to pH 3.5 and extracted three times with 3 ml of ethyl acetate. The solvent was evaporated under N$_2$. The residue was dissolved in chloroform/methanol (2:1, v/v), spotted on Whatman pre-coated silica gel LK6DF plates, and developed in a solvent system of ethyl acetate/acetic acid/trimethylpentane/water (11:2.5:10, by vol). After visualization of the PG standards by exposure to I$_2$ vapor, the radioactive PG spots were located by autoradiography and the radioactive contents of PGs and AA were measured by counting in a Beckman liquid-scintillation counter.

Changes in the release of PGE$_2$ into the medium are presented as the amount of PG in μg/g of tissue, and changes in the release of AA from prelabeled tissue into the medium are expressed as 14C radioactivity (dpm/g tissue). The data presented in the figures are mean of three or more experiments and the bars represent the SEM.

Measurement of Contraction Response in Iris Dilator Muscle

Isometric contractions were recorded using a Grass FT-03 transducer and Grass DC amplifier (Harvard Apparatus, South Natick, MA) as previously described. Two strips of the iris dilator muscle of about 2 mm width were isolated from each of the untreated and treated eyes by the method of Kern. The strips were then mounted in 25-ml baths containing Krebs-Ringer buffer which was continuously oxygenated (97% O$_2$-3% CO$_2$), and maintained at a constant temperature of 37°C. The strips were allowed to equilibrate for 90 min under a resting tension of 40 mg. During this period, the muscles were washed with fresh oxygenated Krebs-Ringer buffer (pH 7.4) every 30 min. After equilibration, the isometric contractions were recorded.

The effects of different concentrations of epinephrine on normal and desensitized iris dilator muscle were recorded as the increase in tension by the agonist. Maximum response was defined as the maximum tension recorded with epinephrine (plotted as 100%). All other responses were plotted as percentages of this maximum response against the log molar concentration of agonist.

Results

Effect of Epinephrine on Phosphoinositide Hydrolysis in Non-desensitized and Desensitized Irids

These experiments were designed to find if activation of adrenergic receptors by epinephrine provokes phosphoinositide hydrolysis in the iris-ciliary body, and if α$_1$-adrenoceptor-mediated hydrolysis of phosphoinositides desensitizes. As seen in Table 1 and Fig. 1, addition of epinephrine, like that of NE, provoked the hydrolysis of PIP$_2$ and the release of IP$_3$ and formation of DG, measured as phosphatidic acid (PA). In the undesensitized iris, epinephrine (50 μM) increased the breakdown of PIP$_2$ by 24% and the $^{32}$P labeling of PA by 149% (Table 1). As expected, the adrenergic agonist had no effect on the $^{32}$P labeling of phosphatidylycholine (PC). Similarly, the adrenergic agonist increased IP$_3$ accumulation by 36% of that of the control (Fig. 1). Addition of prazosin (1 μM) blocked the epinephrine-stimulated hydrolysis of PIP$_2$ and IP$_3$ accumulation (data not shown). In general, the basal $^{32}$P labeling of the iris phospholipids was slightly lower in the desensitized, as compared with the undesensitized eyes (Table 1). These data indicate that epinephrine, as with NE, behaves as a Ca$^{2+}$-mobilizing agonist, and that its actions could be mediated, in part, through the phosphoinositide response. Treatment of the eye with epinephrine resulted in a significant attenuation of the epinephrine-stimulated hydrolysis of PIP$_2$ (Table 1),
Table 1. Effect of epinephrine on $^{32}$P labeling of phosphoinositides and other phospholipids in irides from rabbit eyes after pretreatment with the agonist*

<table>
<thead>
<tr>
<th>Epinephrine</th>
<th>Non-desensitized</th>
<th></th>
<th></th>
<th></th>
<th>Desensitized</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PA</td>
<td>PI</td>
<td>PIP$_2$</td>
<td>PC</td>
<td>PA</td>
<td>PI</td>
<td>PIP$_2$</td>
<td>PC</td>
</tr>
<tr>
<td>None</td>
<td>Control</td>
<td>123 ± 4</td>
<td>239 ± 3</td>
<td>687 ± 93</td>
<td>247 ± 13</td>
<td>95 ± 3</td>
<td>202 ± 18</td>
<td>395 ± 4</td>
</tr>
<tr>
<td>1 μM</td>
<td>Exp.</td>
<td>127 ± 2</td>
<td>254 ± 4</td>
<td>635 ± 29</td>
<td>260 ± 6</td>
<td>97 ± 1</td>
<td>200 ± 20</td>
<td>403 ± 6</td>
</tr>
<tr>
<td></td>
<td>% of</td>
<td>Control</td>
<td>(103)</td>
<td></td>
<td>(92)</td>
<td></td>
<td>(105)</td>
<td></td>
</tr>
<tr>
<td>10 μM</td>
<td>Exp.</td>
<td></td>
<td>117 ± 13</td>
<td>192 ± 7</td>
<td>741 ± 104</td>
<td>264 ± 9</td>
<td>109 ± 4</td>
<td>180 ± 17</td>
</tr>
<tr>
<td></td>
<td>% of</td>
<td>Control</td>
<td>(140)</td>
<td></td>
<td>(161)</td>
<td></td>
<td>(108)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>94 ± 9</td>
<td>193 ± 6</td>
<td>732 ± 59</td>
<td>295 ± 24</td>
<td>97 ± 8</td>
<td>184 ± 15</td>
</tr>
<tr>
<td>50 μM</td>
<td>Exp.</td>
<td></td>
<td>234 ± 30</td>
<td>375 ± 24</td>
<td>558 ± 55</td>
<td>339 ± 12</td>
<td>161 ± 3</td>
<td>279 ± 20</td>
</tr>
</tbody>
</table>

* Rabbits were desensitized topically in one eye with 2% epinephrine in three successive doses (2 x 50 μl) at 32, 24, and 6 hr before killing. Control eyes received saline only. Non-desensitized and desensitized irides were incubated singly in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2.5 μCi myo-[3H]inositol for 15 min (total pre-incubation time was 75 min). Various concentrations of epinephrine were added, and incubation continued for an additional 15 min. The incubations were terminated with 10% (wt/vol) TCA followed by extraction and analysis of $^3$H-radioactivity in IP3 as described in Materials and Methods. In general, the basal accumulation of IP3 in non-desensitized and desensitized irides was (dpm/g tissue): 512,072 ± 15496 and 698,266 ± 25368, respectively. The data are means ± SEM of three separate experiments conducted in triplicate.

Effect of Epinephrine on AA Liberation and PGE$_2$ Release in Non-desensitized and Desensitized Irides

In addition to the stimulation of phosphoinositide hydrolysis, activation of Ca$^{2+}$-mobilizing receptors also leads to the release of AA for PG synthesis. The precise relationship between AA liberation and the phosphoinositide response is unclear. In the iris-ciliary body NE$^{15,16}$ acetylcholine, substance P$^{14}$, and the platelet-activating factor$^{18}$ increase the release of AA, and consequently PGE synthesis. In these studies it was found that PGE$_2$ was the most affected by these agonists, and this was also true of the present work on epinephrine (data not shown). As with NE$^{15,16}$, epinephrine-stimulated PGE$_2$ release is also mediated through α$_2$-adrenoceptors. Thus, epinephrine increased PGE$_2$ release by 46%, and was blocked by prazosin, but not by yohimbine (Table 2).

Studies on the effects of epinephrine on AA liberation and PGE$_2$ release in the desensitized iris-ciliary body revealed the following: (1) In the non-desensitized iris, epinephrine (50 μM) increased PGE$_2$ release by 49%, measured by RIA, and in the desensitized iris this decrease was observed at all concentrations of epinephrine (Fig. 2). This decrease was observed at all concentrations of epinephrine investigated (1–100 μM); (2) In the non-desensitized iris, epinephrine (50 μM) increased AA liberation and PGE$_2$ release (measured by radiochromatography) by 48% and 43%, respectively, and in the desensitized iris, these values decreased to 19% and 23%, respectively (Fig. 3). The decreases in epinephrine-induced AA liberation and PGE$_2$ release in desensitized
### Table 2. Effects of prazosin and yohimbine on epinephrine-stimulated release of PGE$_2$ by rabbit iris

<table>
<thead>
<tr>
<th>Additions</th>
<th>PGE$_2$ (μg/g tissue)</th>
<th>Effect of drug (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.07 ± 0.07</td>
<td>100</td>
</tr>
<tr>
<td>Epinephrine (50 μM)</td>
<td>4.48 ± 0.53</td>
<td>146</td>
</tr>
<tr>
<td>Epinephrine + Yohimbine (20 μM)</td>
<td>4.40 ± 0.56</td>
<td>143</td>
</tr>
<tr>
<td>Epinephrine + Prazosin (1 μM)</td>
<td>3.27 ± 0.12</td>
<td>107</td>
</tr>
</tbody>
</table>

*One iris from each pair was incubated in 1 ml of Krebs-Ringer buffer (pH 7.4) in the absence and presence of the pharmacological agents as indicated at 37°C for 15 min. At the end of incubation, PGE$_2$ in the medium was quantified by RIA. Results are means ± SEM of three different experiments, and each experiment was run in triplicate.

Iridectes were observed at all concentrations of epinephrine investigated.

#### Epinephrine-induced PGE$_2$ Release at Various Time Intervals Following Adrenergic Desensitization of the Iris

These experiments were designed to determine whether desensitization of PGE$_2$ release in the iris is reversible. Desensitization of the iris with epinephrine resulted in a 23% decrease in the agonist-stimulated release of PGE$_2$, and this difference between the non-desensitized and desensitized irides fell after 72 hr and 120 hr to 16% and 5%, respectively (Fig. 4). These data suggest that adrenergic desensitization of the polyphosphoinositide response in the iris is reversible.

#### Time-Course of the Effects of Topical Application of Epinephrine and/or Dexamethasone on PGE$_2$ Release by the Iris, and PGE$_2$ Accumulation in the Aqueous Humor

To examine the molecular mechanism underlying the epinephrine-induced PGE$_2$ release in the anterior segment of the eye, we investigated the time-course of the adrenergic agonist and/or dexamethasone, a phospholipase A$_2$ inhibitor, on PGE$_2$ release by the iris, and on PGE$_2$ accumulation in aqueous humor. Three hr after the drugs were administered, epinephrine increased PGE$_2$ release in the iris by 42%, while dexamethasone depressed it by 30%, and the epinephrine...
The effect on PGE₂ release was blocked by the phospholipase A₂ inhibitor (Fig. 5A). Under the same experimental conditions the adrenergic agonist increased PGE₂ accumulation in the aqueous humor by 72%, dexamethasone depressed it by 25%, and the stimulatory effect of epinephrine was blocked by the phospholipase A₂ inhibitor (Fig. 5B). At longer time intervals (6–9 hr) the effects of these drugs on PGE₂ release were significantly reduced.

**Effect of Dexamethasone on Arachidonate Release From Iris Glycerolipids**

The observation that dexamethasone blocks PG synthesis in the iris by inhibiting AA liberation from membrane phosphoinositides and other phospholipids, presumably by inhibiting phospholipase A₂, was confirmed by the studies on the effects of the glucocorticoid on AA release in irides prelabeled with ¹⁴C-AA. As seen in Table 3, dexamethasone blocked the liberation of AA from membrane PI and PC, and consequently inhibited the parallel release of PGE₂ into the medium. By inhibiting the activity of phospholipase A₂, which increased the level of radioactivity in PI and PC, 19% and 23%, respectively, the glucocorticoid limited the availability of AA (28% inhibition) for PGE₂ synthesis (26% inhibition).

**Effect of Adrenergic Desensitization on Contraction of the Rabbit Iris Dilator Muscle to Epinephrine**

To establish a correlation between the biochemical and physiological responses in non-desensitized and desensitized irides, the dilator strips from both were also equilibrated for 90 min in the Krebs-Ringer buffer before the tension responses to epinephrine were recorded. The maximum tensions developed by the non-desensitized and desensitized dilator muscles in response to maximal effective dose of epinephrine were different from each other (Fig. 6). The increase in tension by epinephrine was as follows (mg tension/mg wet wt): non-desensitized, 9.22 ± 0.43, and desensitized, 6.30 ± 0.22. The dose-response curve to epinephrine in desensitized eyes was significantly shifted to the right as compared with that for non-desensitized eyes [EC₅₀(M): non-desensitized = 3.1 × 10⁻⁶; desensitized = 1.7 × 10⁻⁵].

**Effect of Sympathetic Denervation on AA Liberation and PGE₂ Release in Iris-ciliary Body to NE**

We reported that surgical sympathetic denervation increased α₁-adrenoceptor-mediated accumulation of IP₃ and muscle contraction in the rabbit iris dilator smooth muscle. If AA liberation and PG synthesis are linked to the agonist-enhanced phosphoinositide hydrolysis, then the release of arachidonate and eicosanoids by α₁-adrenergic agonists, such as NE, should also be enhanced in the sympathetically denervated iris. We investigated the effects of NE concentration on the release of AA from irides prelabeled with radioactive arachidonate, and the effects on PGE₂ synthesis measured by radiochromatography and RIA. NE (50 μM) increased AA liberation in normal and denervated irides by 52% and 115%, respectively, and increased PGE₂ release by 47% and 80%, respectively (Fig. 7). Similarly, when PG release was measured by RIA the NE-induced PGE₂ release in the normal and denervated iris was 52% and 92%, respectively (Fig. 8). This link among denervation supersensitivity, AA liberation (Fig. 7), and PGE₂ release (Figures 7 and 8) was observed at all concentrations of NE investigated (0.5 to 50 μM).

**Discussion**

Desensitization of the rabbit eye with epinephrine resulted in attenuation of the agonist-stimulated breakdown of polyphosphoinositides in the iris-ciliary...
ADRENERGIC DESENSITIZATION OF THE PIP2 RESPONSE IN THE IRIS / Yousufzai and Abdel-Larif

Fig. 5A. Release of PGE2 by irides from rabbit eyes after pretreatment in vivo with epinephrine and/or dexamethasone for various time intervals. Three groups of rabbits, three in each group, received topically in one eye either 100 μl of 2% epinephrine, or 50 μl of 0.125% dexamethasone suspended in 2.5% Tween 80 in saline, or with epinephrine and dexamethasone. Control eyes received vehicle only (saline or 2.5% Tween 80 in saline). Dexamethasone was applied 30 min prior to epinephrine administration. Rabbits were killed 3, 6 and 9 hr after the agonist was administered. Eyes were enucleated, the iris-ciliary body dissected, and aqueous humor collected (Fig. 5B). Irides were washed three times with buffer, then incubated singly in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) for 15 min at 37°. At the end of incubation, PGE2 in the medium was quantified by RIA. After 15 min of incubation, the basal release of PGE2 by irides from the eyes was 2.1 ±0.15 μg/g tissue. The effects of the drugs on PGE2 release are expressed as percentages of their respective controls. The data are means ± SEM of three separate experiments conducted in triplicate.

The release of PGE2, accompanied by decreases in IP3 accumulation, AA liberation, PG release, and muscle contraction. Sensitization of the iris, through surgical sympathetic denervation, resulted in potentiation of these biochemical and pharmacological responses (summarized in Fig. 9). These findings suggest that Ca2+-mobilizing receptors, such as α1-adrenergic and muscarinic cholinergic which utilize phosphoinositide hydrolysis as their effector system, are probably involved in the mechanism underlying the phenomenon of subsensitivity and supersensitivity in the iris-ciliary body. Several years ago, Bito and Dawson,21 working on the site and mechanism of the control of cholinergic sensitivity in the iris sphincter, suggested that subsensitivity and supersensitivity could represent opposing expression of the same phenomenon. We propose that changes in the activities of the α1-adrenergic receptor-mediated phosphoinositide hydrolysis system and its derived second messengers may underlie the mechanism of subsensitivity and supersensitivity in the iris-ciliary body (Fig. 9). Inactivation of the adenylate cyclase complex has previously been reported to be involved in the mechanism of desensitization of β-adrenergic receptors in the iris-ciliary body,7 as well as in other tissues.9 However, the relationship between the receptors which control cyclic AMP formation, and those which control phosphoinositide hydrolysis, is still under active investigation in several laboratories. The following findings support the concept that changes in the phosphoinositide response may constitute the biochemical mechanism underlying the phenomenon of subsensitivity and supersensitivity in the iris-ciliary body: (1) Adrenergic desensitization of the eye resulted in a significant attenuation of the polyphosphoinositide response, measured both as loss of 32P radioactivity from PIP2 (Table 1), and as accumulation of IP3 (Fig. 1). A decrease in epinephrine-induced IP3 release could result in a decrease in Ca2+ mobilization from the sarcoplasmic reticulum, and consequently in a lowering of the intracellular Ca2+ concentration required for Ca2+-dependent responses such as muscle contraction.

Fig. 5B. In vivo accumulation of PGE2 in aqueous humor after pretreatment of rabbit eyes with epinephrine and/or dexamethasone for various time intervals. Aqueous humor was obtained from the same rabbits used in the experiments described in Figure 5A. Accumulation of PGE2 in aqueous humor obtained from the untreated eyes was 5.2 ± 0.13 ng/ml.
Table 3. Effect of dexamethasone on the release of arachidonic acid from glycerolipids in rabbit iris prelabeled with [1-14C]arachidonic acid in vitro and its conversion into prostaglandins*

<table>
<thead>
<tr>
<th>Additions</th>
<th>Tissue</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI</td>
<td>PC</td>
</tr>
<tr>
<td>None</td>
<td>4072 ± 284</td>
<td>33,338 ± 1325</td>
</tr>
<tr>
<td>Dexamethasone (10 μM)</td>
<td>4860 ± 257</td>
<td>41,024 ± 1659</td>
</tr>
<tr>
<td>% of control</td>
<td>119</td>
<td>123</td>
</tr>
</tbody>
</table>

* Irides (in pairs) were pre-incubated for 1 hr in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.2 μCi of 14C-AA/ml. The prelabeled irides were washed twice with nonradioactive buffer, then incubated singly (one of the pair served as control, and the other as experimental) in the absence and presence of dexamethasone (10 μM) for 10 min at 37°C. At the end of incubation, the tissue was analyzed for phospholipids and the medium for AA and PGE2 as described in Materials and Methods. Results are means ± SEM of two different experiments, and each experiment was run in triplicate.

(2) Adrenergic desensitization of the eye resulted in attenuation of the epinephrine-stimulated liberation of AA from membrane phosphoinositides and other phospholipids (Fig. 3), and in the agonist-stimulated release of PGE2, measured by both RIA (Fig. 2) and by radiochromatography (Fig. 3). While the effects of epinephrine on phosphoinositide hydrolysis, AA liberation, and PG release are mediated through α1-adrenergic receptors (Table 2), the actual relationship between AA liberation and the phosphoinositide cycle is unknown (for reviews see Irvine,22 Abdel-Latif11). In rabbit iris NE,15,16 acetylcholine,16 substance P14 and the platelet-activating factor18 increase AA liberation from phosphoinositides, predominantly through Ca2+ activation of phospholipase A2. If adrenergic desensitization does impair the phosphoinositide response in the iris-ciliary body, a decrease in diacylglycerol, a potential source for AA release,23 coupled with a decrease in IP3 accumulation, required to mobilize Ca2+.

![Graph](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933363/)

Fig. 6. Effect of adrenergic desensitization on contraction of the rabbit iris dilator muscle to epinephrine. Non-desensitized and desensitized rabbit dilator muscle strips were equilibrated for 90 min at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4) which was continuously oxygenated with 97% O2-3% CO2. Various concentrations of epinephrine were added and isometric contractions recorded with a Grass polygraph as described in Materials and Methods. A supramaximal dose of epinephrine elicited a contractile response of 9.22 ± 0.43 and 6.30 ± 0.22 mg tension/mg tissue in the non-desensitized and desensitized dilator respectively, and were taken as 100% response. The results are means ± SEM obtained from six to eight different muscle strips.

![Graph](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933363/)

Fig. 7. Effect of sympathetic denervation on 14C-AA and 14C-PGE2 release in rabbit iris-ciliary body to NE. Normal and denervated irides were incubated singly for 1 hr in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.2 μCi of 14C-AA. The prelabeled irides were washed three times with nonradioactive buffer, then halved (of the two halves, one served as control and the other as experimental), and each muscle incubated in 1 ml of buffer in the absence and presence of different concentrations of NE for 15 min at 37°C. At the end of incubation, radioactive AA and PGE2 in the medium were analyzed by radiochromatography as described in Materials and Methods. In general, after 15 min of incubation, the basal release of 14C-AA for the normal and denervated irides was (dpm/g tissue): 168,986 ± 4571, and 183,296 ± 6730, respectively, and the basal releases for 14C-PGE2 in the normal and denervated irides were 24,931 ± 521 and 32,316 ± 446, respectively. The effects of NE on release of AA and PGE2 are expressed as percentages of their respective controls. The data are means ± SEM of three separate experiments conducted in triplicate.
for activation of phospholipase A₂, could limit the availability of AA for PG synthesis. The finding that dexamethasone, a phospholipase A₂ inhibitor, inhibited AA liberation from PI and PC (Table 3), inhibited the epinephrine-induced PGE₂ release in the iris (Fig. 5A), and also blocked the epinephrine-induced accumulation of PGE₂ in aqueous humor (Fig. 5B), suggests the involvement of phospholipase A₂ in AA release in the iris-ciliary body both in vivo and in vitro.

(3) Adrenergic desensitization of the eye, as measured by a decrease in the phosphoinositide response and AA liberation and PG release, appears to be reversible. Within 72 to 120 hr after desensitization, the epinephrine-induced PGE₂ release in irides from non-desensitized and desensitized eyes was almost similar (Fig. 4). The mechanism of this resensitization and the return of normal functioning receptors has not been investigated in this tissue. (4) Adrenergic desensitization of the eye decreased the epinephrine-induced contraction in the iris dilator. The EC₅₀ values, obtained from the dose-response curves, were markedly higher in the desensitized as compared to the normal muscle (Fig. 6). If adrenergic desensitization does impair the receptor-stimulated phosphoinositide-hydrolysis, then a decrease in IP₃-induced Ca²⁺ release will result in lowering the intracellular Ca²⁺ concentration required for contraction. (6) Surgical sympathetic denervation, which decreases α₁-adrenoceptor mediated accumulation of IP₃ and muscle contraction in the iris dilator, also increased AA liberation from membrane phospholipids (Fig. 7), and PGE₂ release, measured both by radiochromatography (Fig. 7) and by RIA (Fig. 8). An increase in α₁-receptor IP₃ accumulation by NE results in an increase in intracellular Ca²⁺ concentration and subsequently in muscle contraction (Fig. 9).

Phosphoinositide hydrolysis does not desensitize in 1321 N₁ astrocytoma cell. In contrast, Lurie et al reported that incubation of rabbit aorta ring segments with epinephrine (10⁻⁶ M) for 7 hr resulted in a 10-fold loss in sensitivity of the tissue to α₁-adrenergic receptor-mediated contraction with no change in maximal force of contraction, and no changes in receptor number or affinity; however, desensitization was associated with a blunting of α₁-receptor stimulation of phosphatidylinositol (PI) turnover. More recently, Lefkowitz and his colleagues reported that pretreatment of DDT, M2 hamster vas deferens smooth muscle cells with 100 μM NE resulted in a drastic attenuation of the NE response on phosphatidylinositol metabolism.

There is evidence which indicates that some antiglaucoma drugs such as epinephrine may affect intraocular pressure (IOP) by influencing the production of prostaglandins. PGE₂ and PGF₂α are the major prostanooids produced by the iris-ciliary body, and their release has been shown to be stimulated by NE, epinephrine (the present study), and phenylephrine in this tissue. Dexamethasone, which is known to inhibit PG production in mammalian tissues, including ocular tissues, has been reported
to elevate IOP in rabbits.\textsuperscript{34,35} In the present study, dexamethasone blocked the epinephrine-induced liberation of AA and PG release, both in vivo and in vitro. In the eye, PGs, other eicosanoids and their derivatives have been suggested as potential antiglaucoma agents (see Bitto for review).\textsuperscript{36} There are several reports which indicate that moderate and low doses of PGE\textsubscript{2} and PGF\textsubscript{2\alpha} reduce IOP in several species including rabbits, cats, and monkeys.\textsuperscript{36-39} While the mechanisms involved in desensitization of responses mediated by \(\beta\)-adrenergic receptors that activate adenylyl cyclase may involve the loss of \(\beta\)-receptors from the plasma membrane or the uncoupling of the \(\beta\)-adrenergic receptor from its effector protein,\textsuperscript{7,9,40} the mechanism involved in desensitization of \(\alpha\text{-}\)adrenergic receptor-mediated responses remains uninvestigated. The present study suggests that in the iris-ciliary body, adrenergic desensitization is associated with attenuation of \(\alpha\text{-}\)adrenergic receptor stimulation of polyphosphoinositide turnover, while sympathetic denervation suppersensitivity is associated with the potentiation of this biochemical response. Attendant with adrenergic desensitization of phosphoinositide hydrolysis, there was a decrease in IP\textsubscript{3} release, AA liberation, PG synthesis, and smooth muscle contraction. How much the desensitization of \(\alpha\text{-}\)adrenergic receptor-mediated responses contribute to the therapeutic action of epinephrine in the eye remains to be established.

\textbf{Key words:} desensitization, sensitization, \(\alpha\text{-}\)adrenergic receptors, epinephrine, iris, phosphoinositides, inositol trisphosphate, arachidonate, prostaglandins

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