Species Differences in the Effects of Endothelin-1 on Myo-Inositol Trisphosphate Accumulation, Cyclic AMP Formation and Contraction of Isolated Iris Sphincter of Rabbit and Other Species

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The authors investigated the effects of endothelin-1 (ET1) on inositol trisphosphate (IP3) production, 1, 2-diacylglycerol (DAG) formation, measured as phosphatidic acid (PA), cAMP formation, and contraction in iris sphincter of different mammalian species. They found that ET1 is a potent agonist for IP3 production, DAG formation, and contraction in rabbit, dog, cat, and pig iris sphincters, and for cAMP formation in all species that were investigated—rabbit, dog, cat, pig, bovine, monkey, and human sphincters. In the bovine model, ET1 induced cAMP formation in a dose-dependent manner, with an EC50 of 28 nM. This is the first report that showed an effect of the peptide on the adenylate cyclase system. In rabbit sphincter, ET1 induced a significant increase in IP3 production by 30 sec and reached a 6-fold level more than control within 1 and 5 min. ET1-stimulated IP3 production is dose dependent with an EC50 of 45 nM, this value is about 100- and 56-fold lower than those we reported for substance P and carbachol, respectively. ET1 also increased 32P labeling of PA more than 6-fold; and in rabbit sphincter, ET1 is a more potent agonist in contracting the sphincter than in contracting the dilator (the EC50 values for sphincter and dilator were 46 and 120 nM, respectively). L-type Ca2+ channels are not involved in IP3- and contraction responses because several blockers of these channels did not affect the ET1-induced responses, implying that in the iris sphincter, ET1 elicits the physiologic response through the G protein activation of phospholipase C and/or adenylate cyclase and not through the activation of voltage-dependent Ca2+ channels. The results of this study show the major species differences in biochemical and functional responsiveness to ET1 and support a modulatory role for the peptide in muscle response in the iris. However, the physiologic significance of this peptide in ocular tissues is undetermined. Invest Ophthalmol Vis Sci 32:2432-2438, 1991

Endothelin-1 (ET1) is a 21-residue vasoconstrictive peptide that was originally isolated and sequenced from the supernatant of cultured porcine aortic endothelial cells.1 Studies have shown the widespread existence of specific high-affinity binding sites for ET1 in a variety of tissues and cell types, suggesting diverse biologic effects of this peptide.2-4 ET1 produced concentration-dependent contractions in aorta, trachea, and bladder body that were obtained from rat and rabbit.5 Based on results of experiments in which the effects of calcium-free medium and a dihydropyridine calcium channel antagonist, nicardipine, were examined, it was hypothesized that ET1 produced contraction of vascular and airway smooth muscle by interacting with and activating voltage-dependent calcium channels. However, more recent studies showed that ET1 acts by a mechanism other than an interaction with dihydropyridine-sensitive calcium channels and stimulation of extracellular calcium influx.6,7 These studies suggest that ET1 produces contraction of smooth muscle by interacting with its specific receptor on the target cell and subsequent release of intracellular Ca2+ as a consequence of stimulation of hydrolysis of phosphatidyl inositol 4, 5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG).

In the rat eye, ET1 mRNA and binding sites are localized to the iris; however, prominent ET1 binding sites also occur in the corneal endothelium.8 In cultured human ciliary muscle cells, ET1 (10^-10-10^-6 M) induced a dose-dependent reversible membrane voltage depolarization and a dose-dependent rise in intra-
cellular calcium ion. In this study, we examined the effects of ET1 on IP3 accumulation, DAG production (measured as 32P labeling of phosphatidic acid [PA]), cAMP formation, and contraction of isolated iris sphincter of rabbit and other mammalian species, and IP3 accumulation and contraction of isolated rabbit iris dilator. In addition, we studied the effects of Ca2+ channel blockers on ET1-induced IP3 accumulation and contraction in the rabbit sphincter.

Materials and Methods

Chemicals

Myo-[3H] inositol (80–120 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL); [32P] orthophosphate, carrier-free, was obtained from New England Nuclear (Boston, MA). Endothelin-1 (human, porcine) was purchased from Peptides International (Louisville, KY). Nicardipine was purchased from Sigma Chemical Co. (St. Louis, MO). Nifedipine, verapamil, and diltiazem were gifts from Pfizer (New York, NY), Knoll Pharmaceuticals Co. (Whippany, NJ), and Marion Laboratories (Kansas City, MO), respectively. All other chemicals were of reagent grade.

Animals

Albino rabbit, pig, and bovine eyes were obtained from local slaughterhouses, and dog and cat eyes were obtained through the courtesy of the Richmond County Animal Control (Augusta, Georgia). Generally, we obtain the eyes within 1 hr after the animals are killed. Eyes were enucleated immediately after death and were transported to the laboratory packed in ice. Human eyes were obtained from the Medical College of Georgia Eye Bank. Rhesus monkey eyes were obtained through the courtesy of Dr. T. C. Chu and Dr. Keith Green of the Department of Ophthalmology, Medical College of Georgia. Human eyes were obtained within 7–14 hrs after death, and monkey eyes were obtained 30 min after death. We routinely test the contractility of the muscle with carbachol before the effects of ET1 on the physiologic response are investigated. Thus, in human and monkey sphincters, the maximal responses obtained with 0.5 μM carbachol were 30 and 26 mg tension/mg tissue, respectively. From our experience with the iris, we find little change in the biochemical and physiologic responses when the tissue is kept for a 2-day period at 4°C.

Incubation of the Sphincter Muscle with Myo-[3H] Inositol

To prelabel the tissue with myo-[3H] inositol, the paired sphincter muscles (from the same animal) were incubated at 37°C for 90 min in 1 ml of Krebs-Ringer bicarbonate buffer that contained 10 μCi of myo-[3H] inositol. The sphincters were washed four times with 4 ml of nonradioactive Krebs-Ringer bicarbonate buffer and then suspended singly (of the paired sphincters, one was used as the control medium and the other as experimental) in 1 ml of fresh nonradioactive buffer. LiCl, to a final concentration of 10 mM, was added to each incubation. Ten minutes later, ET was added as shown, and incubation continued for 5 min or as indicated in the experiment. When used, Ca2+ channel antagonists were added 5 min before the addition of ET1. The incubations were stopped by the addition of 1 ml of 10% (w/v) trichloroacetic acid (TCA). The TCA extract was analyzed for 3H-labeled myo-inositol phosphates.

Incubation of Iris Sphincter Muscle with 32Pi and Analysis of Phospholipids

All experimental details for prelabeling the tissue with 32P were the same as described above for 3H-inositol, except that the amount of 32Pi used was 20 μCi/ml and no LiCl was added before the addition of ET1. The phospholipids were extracted and analyzed for radioactivity by two-dimensional thin-layer chromatography as described previously.12 Assay of cAMP

Measurement of cAMP was performed in the same incubations that were used for the determination of 3H-inositol phosphates, except that H-inositol and LiCl were omitted from the medium and 0.1 mM IBMX was added to the incubations 10 min before the addition of the agonist. After appropriate dilution
Measurement of Contraction Response in the Iris Sphincter Muscle

For measurements of the contraction response, the sphincters were mounted individually in separate organ baths (20 ml) containing Krebs-Ringer bicarbonate buffer that contained 1 μM indomethacin. A mixture of 97% O₂-3% CO₂ was bubbled continuously through the buffer, which was maintained at 37°C. The tissue was allowed to equilibrate for 90 min under a resting tension of 50 mg. During this period, the medium was replaced with fresh Krebs-Ringer bicarbonate buffer every 30 min. After equilibration of the tissue, the agonist was added and changes in tension were monitored continuously with a Grass FT-03 force transducer connected to a Grass d.c. amplifier.

Calculation of Data

Data for ³H-IP₃ are reported as DPM/mg of protein. Dose–response curves for muscle contraction were constructed by cumulative addition of the agonist in the organ bath. The concentration of the agonist was increased only after the response to the previous concentration had stabilized. EC₅₀ value is defined as that concentration of the agonist that produces 50% of the maximum response.

Results

Time Course of ET1-Induced IP₃ Formation in Rabbit Iris Sphincter

Time course studies on the effects of ET1 on IP₃ accumulation in the rabbit iris sphincter are shown in Figure 1. In this study we used anion-exchange chromatography to analyze for the inositol phosphates, and thus the radioactivity that was recovered in the IP₃ fraction represents the combined radioactivities of the two isomers, 1,4,5-IP₃ and 1,3,4-IP₃. ET1 (10⁻⁶ M) induced a significant increase in IP₃ production by 30 sec and reached a 6-fold level more than control within 1 and 5 min. Based on these findings, we used 5-min incubations with the agonist in the experiments described below.

Dose–Response Effect of ET1 on IP₃ Production in Rabbit Iris Sphincter

ET1-stimulated IP₃ accumulation was dose dependent with an EC₅₀ of about 45 nM (Fig. 2). This value is about 100-fold lower than the EC₅₀ reported from this laboratory for substance P-induced IP₃ accumulation in this tissue,¹⁴ and about 56-fold lower than the EC₅₀ we reported for carbachol.¹⁰

Effects of ET1 on ³²P Labeling of Phospholipids in Rabbit Iris Sphincter

To assess the effect of ET1 on the production of DAG, the other second messenger formed as a result of the supernatant, cAMP in the sample was succinylated and then assayed by radioimmunoassay (RIA) as described by Frandsen and Krishna.¹³
of phospholipase C activation, we measured the increase in \(^{32}\)P radioactivity in PA in iris sphincter muscle that was prelabeled with \(^{32}\)P. As shown in Table 1, the addition of ET1 (1 \(\mu\)M) to the muscle caused more than a 6-fold increase in PA labeling, 45% decrease in PIP\(_2\) labeling, and indicated the activation of phospholipase C by the peptide. Under the same experimental conditions, little change occurred in the turnover of phosphatidyl inositol and phosphatidyl choline by the agonist.

**Dose–Response Effect of ET1 on Contraction in Rabbit Iris Sphincter and Dilator Muscles**

Cumulative addition of ET1 to isolated rabbit iris sphincter and dilator muscles elicited a concentration-dependent increase in contractile tension (Fig. 3). The EC\(_{50}\) values that were obtained for the sphincter and dilator muscles were 4.6 \(\times\) 10\(^{-8}\) and 1.2 \(\times\) 10\(^{-7}\) M, respectively. These values are about 6-fold lower than the EC\(_{50}\) that we reported for carbachol in the rabbit iris sphincter,\(^{10}\) and about 40-fold lower than that of norepinephrine in the rabbit dilator.\(^{15}\) As compared with carbachol, the contractile response to ET1 had a slow onset and was sustained for 6–20 minutes. The ET1 response was poorly reversible in that contractile tension of sphincter or dilator muscle fell very little during a 10-min wash with buffer. The maximum response of the dilator to ET1 (1 \(\mu\)M) was about 53% of that of the sphincter (14.9 mg tension/mg wet wt for the dilator as compared with 28.3 for the sphincter). We also observed differential effects of ET1 on IP\(_3\) accumulation in iris sphincter and dilator. Thus, ET1 (10 nM) increased IP\(_3\) accumulation in the sphincter by up to 113%, whereas the increase in the dilator was about 77%.

**Effects of ET1 on IP\(_3\) Accumulation, cAMP Formation, and on Contraction in Bovine Iris Sphincter and Other Species**

Studies on the effects of ET1 on IP\(_3\) accumulation and on contraction in iris sphincter of different mammalian species showed that only the rabbit, dog, cat, and pig sphincters, the same species that showed a pronounced IP\(_3\) response, contracted in response to the peptide (Table 2). The increase in IP\(_3\) accumulation and contraction due to ET1 were comparable in rabbit and dog sphincters; however, they were considerably lower in the cat, being 36% and 18.5% of those of the rabbit, respectively. The peptide did not induce IP\(_3\) accumulation or contraction in bovine, rhesus monkey, or human sphincters. Previously, we showed species differences in the effects of Ca\(^{2+}\)-mobilizing agonists on IP\(_3\) accumulation, cAMP formation, and muscle contraction, and concluded that IP\(_3\) producers are contracted by the agonists, whereas cAMP producers are not.\(^{16}\) Thus, it was interesting to investigate the effect of ET1 on cAMP formation in the bovine sphincter and other mammalian species. ET1 increased the intracellular cAMP formation in the bovine sphincter in a dose-dependent manner with an EC\(_{50}\) of 28 nM (Fig. 4). A 4.5-fold increase in cAMP formation was seen in the presence of 0.1 \(\mu\)M ET1. In contrast to our previous findings on the differential effects of eicosanoids and substance P on IP\(_3\) accumulation and cAMP formation and on contraction in iris sphincter of different mammalian species, ET1 increased cAMP formation in all of the species that were investigated (Table 2). This is the first report to show an increase in cAMP formation by ET1. In addition, the contractile response in the cat was much weaker than that of the pig, whereas IP\(_3\) accumulation was stronger in the cat than in the pig (Table 2). However, the basal release of IP\(_3\) in the pig was 193% greater than that of the cat, and the formation of cAMP in the pig was 80% of that of the cat.

**Effects of Various Ca\(^{2+}\)-Channel Antagonists on ET1-Induced IP\(_3\) Accumulation in Rabbit Iris Sphincter**

The effects of various Ca\(^{2+}\)-channel antagonists on ET1-induced IP\(_3\) accumulation are shown in Table 3.
Table 2. Effects of ET1 on IP3 accumulation and cAMP formation and on contraction in iris sphincter of different animal species*

<table>
<thead>
<tr>
<th>Response</th>
<th>Rabbit (dpm/mg tissue proteins)</th>
<th>Dog (dpm/mg tissue proteins)</th>
<th>Cat (dpm/mg tissue proteins)</th>
<th>Pig (dpm/mg tissue proteins)</th>
<th>Bovine (dpm/mg tissue proteins)</th>
<th>Rhesus monkey (dpm/mg tissue proteins)</th>
<th>Human (dpm/mg tissue proteins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP3 accumulation</td>
<td>3715 ± 125</td>
<td>2553 ± 17</td>
<td>601 ± 62</td>
<td>1762 ± 120</td>
<td>1762 ± 120</td>
<td>1762 ± 120</td>
<td>1762 ± 120</td>
</tr>
<tr>
<td>cAMP formation</td>
<td>146 ± 9.5</td>
<td>56 ± 3.2</td>
<td>45.4 ± 5.5</td>
<td>36.4 ± 10</td>
<td>54.1 ± 9.7</td>
<td>33.5 ± 3</td>
<td>33.5 ± 3</td>
</tr>
<tr>
<td>Contraction</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The concentrations of ET1 used for the biochemical and physiologic responses were 1 and 0.1 nM, respectively. In the IP3 and cAMP studies, the tissue was exposed to the agonist for 5 min. The basal values for 3H-IP3 accumulation for rabbit, dog, cat, and pig sphincters were 3715 ± 125, 2553 ± 17, 601 ± 62, and 1762 ± 120, respectively. The basal values for cAMP formation for rabbit, dog, cat, pig, monkey, human, and bovine were 146 ± 9.5, 56 ± 3.2, 45.4 ± 5.5, 36.4 ± 10, 54.1 ± 9.7, 33.5 ± 3, and 33.5 ± 3, respectively. The data are means ± SEM that were obtained from four to eight determinations.

Although there was a modest increase in basal IP3 accumulation (19–41%) by the blockers alone, at higher concentrations, nifedipine, verapamil, and diltiazem inhibited ET1-induced IP3 accumulation by only 11–33% of their respective controls (in the absence of the blocker). Nicardipine had no effect on basal or ET1-stimulated IP3 accumulation. Neither nifedipine nor nicardipine had any effect on ET1 concentration–contractile response curves in the rabbit iris sphincter. Thus, in a typical experiment, contraction of the sphincter by ET1 (100 nM) alone was 70% of that of the maximal response, and in the presence of nicardipine (1 μM), it was 72%.

Fig. 4. Dose–response effect of ET1 on intracellular cAMP formation in bovine iris sphincter. Sphincters were incubated in Krebs–Ringer bicarbonate buffer for 90 min, washed with the same buffer, then incubated for 10 min in buffer that contained 0.1 mM IBMX. Different concentrations of the agonists were added as indicated, and incubations were continued for 5 min. cAMP was determined in the TCA-soluble extract by means of RIA. The basal value for cAMP formation in the bovine iris sphincter was 23.6 ± 2.1 pico-moles/mg of protein. The results are means ± SEM that were obtained from three determinations.

Table 3. Effects of various Ca2+-channel antagonists on ET1-induced IP3 accumulation in rabbit iris sphincter*

<table>
<thead>
<tr>
<th>Additions</th>
<th>3H-IP3 (dpm × 10^3/mg protein)</th>
<th>Effects of blockers (% of corresponding control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>27.4</td>
<td>100</td>
</tr>
<tr>
<td>ET1 (0.1 μM)</td>
<td>114.5</td>
<td>418</td>
</tr>
<tr>
<td>Nifedipine (0.1 μM)</td>
<td>32.7</td>
<td>119</td>
</tr>
<tr>
<td>ET1 and nifedipine (0.1 μM)</td>
<td>134.0 ± 9</td>
<td>410</td>
</tr>
<tr>
<td>ET1 and nifedipine (1 μM)</td>
<td>126.0 ± 7</td>
<td>385</td>
</tr>
<tr>
<td>Verapamil (5 μM)</td>
<td>32.3</td>
<td>118</td>
</tr>
<tr>
<td>ET1 and verapamil (5 μM)</td>
<td>128.9 ± 11</td>
<td>399</td>
</tr>
<tr>
<td>ET1 + verapamil (25 μM)</td>
<td>128.6 ± 11</td>
<td>398</td>
</tr>
<tr>
<td>Diltiazem (1 μM)</td>
<td>38.7</td>
<td>141</td>
</tr>
<tr>
<td>ET1 and diltiazem (1 μM)</td>
<td>156.9 ± 13</td>
<td>405</td>
</tr>
<tr>
<td>ET1 and diltiazem (5 μM)</td>
<td>157.7 ± 14</td>
<td>407</td>
</tr>
<tr>
<td>Nicardipine (1 μM)</td>
<td>26.5</td>
<td>97</td>
</tr>
<tr>
<td>ET1 and nicardipine (1 μM)</td>
<td>106.5 ± 6</td>
<td>402</td>
</tr>
<tr>
<td>ET1 and nicardipine (5 μM)</td>
<td>103.7 ± 5</td>
<td>301</td>
</tr>
</tbody>
</table>

This tissue was labeled with 3H-inositol, washed with buffer, and then preincubated with the Ca2+ channel antagonist for 5 min and with the agonist for 5 min. Each value represents mean ± SEM and was obtained from three determinations that were each run in triplicate.

Discussion

These data show that ET1 is a potent agonist for IP3 accumulation and muscle contraction in iris sphincters that are obtained from rabbit, dog, cat, and pig. The peptide had no effect on these biochemical and physiologic responses in sphincters of bovine, rhesus monkey, and human. Instead, in these species, it increased intracellular cAMP formation. In addition, ET1 increased cAMP formation in the sphincter muscle of all of the species that were studied (Table 2). This finding is in contrast to our previous findings with the eicosanoids and substance P, where we found that in those species in which these agonists produced IP3 and contraction, there were no effects on cAMP formation. The ET1 receptors in these species could
be linked to different G proteins that can activate the phospholipase C and adenylate cyclase systems. In those species in which ET1 receptors are coupled to phospholipase C and adenylate cyclase, the latter enzyme could be stimulated by the increase in intracellular Ca\(^{2+}\) concentration that results from the activation of PIP\(_2\) hydrolysis into IP\(_3\), the Ca\(^{2+}\) mobilizer, and DAG, the protein kinase C activator. In rat atria, ET1 did not modify intracellular levels of cAMP under basal conditions or after stimulation with isoproterenol, but it stimulated the formation of inositol phosphates.17 In rabbit sphincter, ET1 (1 \(\mu\)M) induced a significant increase in IP\(_3\) production by 30 sec and reached a 6-fold level more than control within 1 and 5 min. ET1-stimulated IP\(_3\) accumulation is dose dependent with an EC\(_{50}\) of about 45 nM. This result is about 100-fold lower than the EC\(_{50}\) we reported previously for substance P-induced IP\(_3\) accumulation in this tissue and about 56-fold lower than the EC\(_{50}\) value we reported for carbachol. The EC\(_{50}\) value we found for ET1 in the rabbit sphincter (45 nM) is comparable to those reported for IP\(_3\) production in porcine coronary artery and in guinea pig trachea, 40.3 and 45.9 nM, respectively. The finding that ET1 increased the \(^{32}\)P labeling of PA more than six-fold shows that the peptide acts by stimulating phospholipase C to hydrolyze PIP\(_2\) into IP\(_3\) and DAG. Thus, we conclude that ET1 is a Ca\(^{2+}\)-mobilizing agonist in the rabbit, dog, cat, and pig iris–ciliary body. The 35% increase in \(^{32}\)P labeling of phosphatidylinositol is about 5% of that seen in PA (637%, Table 1). Previously, we reported that substance P (1 \(\mu\)M) increased the \(^{32}\)P labeling of PA and phosphatidylinositol in the rabbit sphincter by only 30 and 27%, respectively. This finding could suggest that ET1 may be acting on other enzymes of the phosphoinositide cycle.

Generally, we found that ET1 is a more potent agonist in contracting the rabbit iris sphincter than in contracting the dilator muscle (the EC\(_{50}\) values for sphincter and dilator were 46 and 120 nM, respectively). This difference was also found in the IP\(_3\) response. These EC\(_{50}\) values are higher than those reported for the contractile responses in guinea pig trachea (EC\(_{50}\) = 30.9 nM) and porcine coronary artery (EC\(_{50}\) = 7-21 nM).18 In the rabbit iris sphincter, the EC\(_{50}\) value for ET1-induced IP\(_3\) production (45 nM) was comparable to that for the ET1-induced contraction (46 nM), thus supporting the hypothesis that there is a linkage between the two parameters in this tissue.19 Our data show that in the responsive species (rabbit, dog, cat, and pig), one of the initial membrane events in the action of ET1 is to activate phospholipase C-stimulated PIP\(_2\) hydrolysis, which liberates DAG and IP\(_3\). The latter substance then mobilizes Ca\(^{2+}\) from the sarcoplasmic reticulum and consequently leads to muscle contraction. Reports showed that ET1 increased intracellular Ca\(^{2+}\) in isolated rabbit aortic rings,20 cultured A10 smooth muscle cells,21 and rat atrial cells.22 In the unresponsive species (bovine, monkey, and human) the ET1 receptor appears to be coupled only to the adenylate cyclase system. The CAMP that is generated then inhibits muscle contraction by blocking the production of IP\(_3\). The finding that ET1 also induced CAMP formation in the contracting species could explain the observation that compared with carbachol, the contractile response to ET1 had a slow onset and was sustained for 6–20 minutes. We showed that substance P, which activates the adenylate cyclase system in dog, cat, and human sphincters, is involved in the modulation of carbachol-induced muscle contraction in these species (S. De, S. Y. K. Yousufzai, and A. A. Abdel-Latif, unpublished work). We suggest that ET1-stimulated CAMP formation functions to regulate the contractile responses in the iris ciliary body.

In their earlier studies, Yanagisawa et al1 proposed that ET1 may be a direct activator of voltage-dependent Ca\(^{2+}\) channels. However, results obtained with the use of calcium entry blockers such as nicardipine, nifedipine, diltiazem, and verapamil in rat aorta4–7 and in isolated rabbit aortic rings23 are not compatible with this hypothesis. Nicardipine (0.01 or 0.1 \(\mu\)M) or incubation in Ca\(^{2+}\)-free medium +0.1 \(\mu\)M EGTA for 30 min had little or no effect on ET1 concentration–contractile response curves in guinea pig intact trachea, but it markedly inhibited the responses produced by ET1 in the endothelium-denuded aorta of the rat.2 Our finding that these Ca\(^{2+}\) channel blockers have negligible effect on ET1-induced IP\(_3\) accumulation and on muscle contraction in the rabbit iris sphincter provides evidence that ET1 acts via a mechanism other than activation of dihydropyridine-sensitive channels in this tissue. Thus, in the iris sphincter, ET1 elicits the physiologic response through the G protein activation of phospholipase C and not through the activation of voltage-dependent Ca\(^{2+}\) channels. Arai et al24 and Sakurai et al25 reported on cloning and expression of cDNA encoding endothelin receptor subtypes. The two distinct receptors that were described probably serve different functions. Each belongs to the superfamily of rhodopsin-like receptors, with seven transmembrane domains, and each is coupled to a G protein.

In summary, we provided evidence that ET1 is a potent agonist for IP\(_3\) production, DAG formation, and contraction in rabbit, dog, cat, and pig iris sphincters, and for CAMP formation in all species studied. L-type Ca\(^{2+}\) channels are not involved in these responses because blockers of these channels did...
not affect the ET1-induced biochemical and physiologic responses. Further study is needed to determine whether the IP<sub>3</sub>-Ca<sup>2+</sup> and cAMP signalling systems are coupled to different subtypes of the ET receptor, or to a single ET1 receptor through different G proteins. This investigation is timely in light of reports that ET receptor subtypes are coupled to G proteins.<sup>24,25</sup> This investigation shows the major species differences in biochemical and functional responsiveness to ET1, and supports a modulatory role for the peptide in muscle response in the iris. However, the physiologic significance of this peptide in ocular tissues is undetermined.

**Key words:** iris sphincter, endothelin-1, inositol trisphosphate, cyclic AMP, contraction, species differences

**References**


