Activation of Beta-Adrenergic Receptors Causes Stimulation of Cyclic AMP, Inhibition of Inositol Trisphosphate, and Relaxation of Bovine Iris Sphincter Smooth Muscle

Biochemical and Functional Interactions between the Cyclic AMP and Calcium Signalling Systems

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We have investigated the interactions between the cAMP and inositol 1,4,5-trisphosphate (IP₃)-Ca²⁺ signalling systems in the bovine iris sphincter by measuring the effects of β-adrenergic and cholinergic muscarinic agonists and antagonists on cAMP formation, IP₃ accumulation and muscle contraction–relaxation. (1) Addition of 5 nM isoproterenol (ISO) or forskolin (5 μM) consistently produced stimulation of cAMP (540%), inhibition of IP₃ (34%) and complete relaxation of the muscle. The ISO effects were dose-dependent, with EC₅₀ values for cAMP formation, IP₃ inhibition and muscle relaxation of 2.8 × 10⁻⁷ M, 3.4 × 10⁻⁷ M and 0.45 × 10⁻⁷ M, respectively. (2) Timolol, a β-adrenergic antagonist, inhibited the ISO effects in a dose-dependent manner, with IC₅₀ values for cAMP formation, IP₃ accumulation and muscle relaxation of 1.8 × 10⁻⁵ M, 3.2 × 10⁻⁵ M and 2.2 × 10⁻⁵ M, respectively. (3) The effects of ISO (0.5 μM) were time-dependent, and they clearly indicate a temporal relationship between the agonist-induced stimulation of cAMP, inhibition of IP₃, and relaxation of the muscle. Within 15 sec following the addition of ISO, there was a marked increase in the level of cAMP, a decrease in IP₃, and this was accompanied by an equally rapid relaxation of the muscle. (4) Addition of carbachol (CCh) to iris sphincter pretreated with ISO decreased cAMP formation and reversed muscle relaxation to complete contraction. Thus, when the sphincter was first treated with ISO and then stimulated with different concentrations of CCh, there was a dose-dependent inhibition of cAMP formation, an increased production of IP₃, and a parallel development of muscle contraction. Taken together, the data presented suggest a reciprocal interaction between the cAMP and IP₃–Ca²⁺ signalling systems in the iris sphincter: activation of β-adrenergic receptors results in elevation of cAMP and inhibition of IP₃, and this leads to muscle relaxation, whereas stimulation of cholinergic muscarinic receptors lowers cAMP formation and increases IP₃ production, and this leads to Ca²⁺ mobilization and muscle contraction. We propose that in the iris sphincter cAMP may act as regulator of responses to neurotransmitters, hormones and pharmacological agents that exert their action through the IP₃–Ca²⁺ second messenger system. Invest Ophthalmol Vis Sci 30:2232–2239, 1989

There is now compelling experimental evidence that suggests that most tissues use at least two major signalling pathways for controlling cellular functions and proliferation. The A-kinase pathway is coupled to cAMP, while the Ca²⁺ messenger system [Ca²⁺-calmodulin and protein kinase C (PKC)] is coupled to the breakdown of polyphosphoinositides (for reviews, see refs. 1, 2). In the former, the extracellular signal through its receptor at the plasma membrane activates adenylate cyclase which generates intracellular cAMP. In the latter, the receptor-activated phospholipase C leads to the release, from phosphatidylinositol 4,5-bisphosphate (Pİ₂*), of two putative

* Abbreviations used are: Pİ₂, phosphatidylinositol 4,5-bisphosphate; DG, 1,2-diacylglycerol; IP₃, inositol 1,4,5-trisphosphate; ISO, isoproterenol; CCh, carbachol; MLC, myosin light chain, PKC, protein kinase C.

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second messengers, 1,2-diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP3). DG activates PKC and IP3 induces Ca2+ release from the endoplasmic reticulum. Rather than serving as distinctly separate second messenger systems, the two pathways can participate in a synergistic manner to regulate cell function. Thus, in platelets cAMP has a marked inhibitory effect on phosphoinositide breakdown.1 Reciprocal effects were also reported for some phorbol esters, which are considered DG analogs, on the activity of receptors linked to cAMP.3,4

The mammalian iris sphincter is an ideal model for studies on the biochemical and functional interactions between the two second messenger systems. It is innervated by cholinergic nerve fibers, activation of which leads to muscle contraction and miosis.3 Histological investigations have revealed that the iris sphincter muscle is innervated by both adrenergic and cholinergic neurons in the monkey,6 rabbit7 and man.8 Physiological investigations have shown that the iris sphincter is functionally innervated by adrenergic nerve fibers in the dog,9 monkey,10 cattle,11,12 rat13 and rabbit.10,14 In the human iris sphincter muscle, exogenously applied epinephrine or norepinephrine relaxes the tissue.15,16 In addition, the human iris sphincter muscle possesses both α and β adrenoceptors.17 Considerable evidence suggests that the adrenergic nervous system plays a significant but complex role in the regulation of intraocular pressure (IOP) in the eye.18,19 Both sympathetic stimulation and locally applied β-adrenergic agonists such as epinephrine decrease IOP. Paradoxically, β-adrenergic antagonists such as timolol (which is effective in treating glaucoma) also decrease IOP.

In previous communications from this laboratory we have established that in the iris smooth muscle the stimulated-hydrolysis of PIP2 into DG and IP3 by cholinergic muscarinic and α1-adrenergic agonists is an early event which couples activated receptors to smooth muscle contraction20,21 (for review, see ref. 22). Activation of cholinergic muscarinic receptors in rabbit ciliary processes also leads to inhibition of cAMP formation.21 More recently, comparative studies on the effects of prostaglandins on DG and IP3 production, cAMP formation, myosin light chain (MLC) phosphorylation and contraction in the iris sphincter smooth muscle of rabbit, bovine and other species revealed biochemical and functional interactions between the IP3-Ca2+ and cAMP second messenger systems.24 The overall objective of the present work was to investigate whether an interaction exists between receptor-mediated cAMP production, IP3 accumulation and contraction—relaxation response of the bovine iris sphincter. In the present study we report on the reciprocal effects of isoproterenol (ISO) and other β-adrenergic agents and carbachol (CCh) on IP3 accumulation, cAMP formation and relaxation—contraction responses in the bovine iris sphincter.

Materials and Methods

The following compounds were purchased from Sigma Chemical Co. (St. Louis, MO): Carbamylcholine chloride (carbachol), indomethacin, L-isoproterenol-HCl, propranolol, timolol, and 3-isobutyl-1-methylxanthine (IBMX). Sources for other reagents were as follows: Antibody to cyclic AMP from ICN ImmunoBiologicals (Lisle, IL); reagents for radioimmunoassay (RIA) of cyclic AMP including succinyl cyclic AMP tyrosine (2200 Ci/mmol) from New England Nuclear (Boston, MA); myo-[3H]inositol (15 Ci/mmol) from American Radiolabeled Chemical Inc. (St. Louis, MO).

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

Bovine eyes were obtained from a local slaughterhouse, usually within 30 min after the death of the animals. After removal of the cornea, two sphincter muscle strips were prepared from each eye by making a radial cut about 3 mm from the pupillary margin at 3 o'clock and 9 o'clock positions and then cutting completely around the pupil on a line parallel to the pupillary margin. The paired sphincter strips from each eye were incubated at 37°C for 90 min in 1 ml of Krebs-Ringer bicarbonate buffer that contained 10 μCi of [3H]-inositol/ml.21 The pH of the buffer was adjusted to 7.4 with 97% O2-3% CO2. At the end of incubation, the sphincters were washed four times with 3 ml nonradioactive buffer and then suspended singly (of the paired strips, one was used as a control and the other as experimental) in 1 ml fresh nonradioactive buffer. At this time LiCl (10 mM final concentration) and IBMX (0.1 mM) were added to each incubation and 10 min later ISO or other pharmacological agents were added and incubations were continued for appropriate time intervals as indicated. Antagonists, when used, were added 5 min prior to the addition of the agonists. Incubations were terminated by the addition of 1 ml 10% (w/v) trichloroacetic acid (TCA), then homogenized in the same medium and [3H]-IP3 and cAMP in the TCA extract analyzed as given below.

Extraction and Analysis of [3H]-Inositol Phosphates

The method used to extract and analyze 3H-inositol phosphates was the same as described previously.21 Briefly, the tissues were homogenized in the 5% TCA-containing medium, the homogenate
contraction was investigated, the muscles were equilibrated. EC50 value is defined as that concentration of after the effect of the previous concentration had sta-
ted to equilibrate for 90 min under a resting tension of
physiological solution was changed every 20 min. At the end
of the equilibration, ISO or other drugs were added and
thereafter the tissue did not develop any further
developing force which continued until about 1 hr,
ner tone probably due to en-
dogenous prostaglandin synthesis. Because of this the
bovine sphincter does not respond maximally to cho-
linergic stimulation unless the tissue is pretreated with
indomethacin. To inhibit the endogenous for-
mation of prostaglandins, 1 μM indomethacin was
routinely added to the tissue baths.

Calculations and Statistical Analysis of the Data

To correct for variation in tissue size the data for
3H-inositol phosphates, cAMP and tension responses
were normalized to tissue protein content. Data are
expressed as the mean ± SE. Statistical differences
between the two means were determined by a paired
student’s t-test. When P was <0.05, the values were
considered to be significantly different.

Results

Effects of β-Adrenergic and Cholinergic Muscarinic
Agonists on cAMP Formation, IP3 Accumulation
and Muscle Tension in the Bovine Iris Sphincter

In the following studies we used IP3 accumulation
as an index of stimulated PIP2 hydrolysis by phos-
pholipase C, and cAMP formation as an index of
adenylate cyclase activity. As shown in Table 1, addi-
tion of ISO or forskolin to iris sphincter resulted in a
5- to 6-fold increase in cAMP formation. These ef-
fects were blocked by the β-adrenergic antagonists
timolol and propranolol. Addition of the antagonists

Table 1. Effects of β-adrenergic agonists and
antagonists on cAMP formation, IP3 accumulation
and muscle tension in the bovine iris sphincter*

<table>
<thead>
<tr>
<th>Addition</th>
<th>cAMP (pmol/mg protein)</th>
<th>IP3 (dpm/mg protein)</th>
<th>Tension† (mg/mg wet tissue wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>14 ± 1</td>
<td>3922 ± 254</td>
<td>24.8 ± 0.4</td>
</tr>
<tr>
<td>ISO (5 μM)</td>
<td>90 ± 8</td>
<td>2686 ± 168</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Forskolin (5 μM)</td>
<td>98 ± 4</td>
<td>2714 ± 108</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Timolol (50 μM)</td>
<td>13 ± 1</td>
<td>3020 ± 179</td>
<td>28.5 ± 0.05</td>
</tr>
<tr>
<td>ISO (5 μM) + Timolol (50 μM)</td>
<td>16 ± 2</td>
<td>3961 ± 234</td>
<td>19.9 ± 1.3</td>
</tr>
<tr>
<td>Propranolol (50 μM)</td>
<td>12 ± 1</td>
<td>2980 ± 147</td>
<td>27.2 ± 0.7</td>
</tr>
<tr>
<td>ISO (5 μM) + Propranolol (50 μM)</td>
<td>17 ± 3</td>
<td>3804 ± 151</td>
<td>15.6 ± 1.0</td>
</tr>
<tr>
<td>CCh (50 μM)</td>
<td>17 ± 2</td>
<td>9471 ± 803</td>
<td>26.5 ± 0.8§</td>
</tr>
</tbody>
</table>

* The data are means ± SE of three experiments, each conducted in tripli-
cate (N = 9). (For details of experiment, see Materials and Methods.)
† The concentrations of ISO and CCh used in measurement of the phar-
macological response were 1 μM.
§ The basal tension in the absence of carbachol was 4.4 mg/mg wet weight.

Measurement of Cyclic AMP

Measurement of cAMP was routinely performed in the
same incubations used for the determination of
3H-inositol phosphates. To protect cAMP from hy-
drolysis 0.1 mM IBMX, a cAMP phosphodiesterase
inhibitor, was routinely included in the incubation
mixture. This concentration of IBMX had no signif-
icant effect on 3H-inositol phosphates formation.
After appropriate dilution of the supernatant from
the previous step, cAMP in the sample was succiny-
lated and then assayed by RIA as described by
Frandsen and Krishna.26

Measurement of Agonist-Induced Tension
Response in the Iris Sphincter

For measurement of tension response, the two
sphincter strips from the same iris were mounted in
two separate 30 ml jacketed tissue baths that con-
tained Krebs–Ringer bicarbonate buffer at 37°C. A
mixture of O2 (97%) and CO2 (3%) was continuously
bubbled through the solution. The tissue was allowed
to equilibrate for 90 min under a resting tension of
300 mg. Shortly after mounting, the tissue started
developing force which continued until about 1 hr,
and thereafter the tissue did not develop any further
tension. During the equilibration period the physio-
logic solution was changed every 20 min. At the end
of equilibration, ISO or other drugs were added and
the mechanical responses were monitored contin-
uously using a Grass FT-03 force transducer con-
ected to a Grass d.c. preamplifier. Dose–response
curves for mechanical responses were constructed by
cumulative addition of the agonist in the tissue bath.
The concentration of the agonist was increased only
after the effect of the previous concentration had sta-
bilized. EC50 value is defined as that concentration of
the agonist that produces 50% of the maximum re-

centrifuged at 3000g for 15 min, and the supernatant
transferred to new tubes. The supernatant was ex-
tracted five times with 4 ml of anhydrous diethyl
ether. At this time suitable aliquots of the extract
were taken for cAMP determination (see below), the
remainder was first neutralized with NaOH and then
analyzed for 3H-inositol phosphates by means of
anion-exchange chromatography employing Biorad 1
× 8 resin (formate form, 200–400 mesh) as pre-
viously described.21

The TCA-insoluble pellet was solubilized in 1 ml
0.1 N NaOH and suitable aliquots used for determi-
nation of protein by the method of Lowery et al.25

Materials and Methods.

Table 1. Effects of β-adrenergic agonists and
antagonists on cAMP formation, IP3 accumulation
and muscle tension in the bovine iris sphincter*
alone had no effect on the basal formation of cAMP. In contrast to their effects on cAMP, both ISO and forskolin inhibited IP₃ accumulation by about 31% of that of the control, and these effects were reversed by the β-adrenergic antagonists. Interestingly, both timolol and propranolol significantly depressed (P < 0.01) IP₃ formation by about 23% of that of the control. Parallel studies on the effect of ISO and forskolin on iris sphincter, preequilibrated for 90 min under the same experimental conditions employed for the biochemical studies resulted in a complete relaxation of the muscle (Table 1, Fig. 1). As with the biochemical findings, the β-adrenergic antagonists inhibited the agonist-induced muscle relaxation, timolol being more potent than propranolol. In contrast to the effects of the β-adrenergic agonists, CCh, a muscarinic agonist, elicited a 140% increase in IP₃ accumulation and this was accompanied by a large increase in the contractile response (Fig. 1). The contractile response consisted of a rapid (30 sec) phasic component and this was followed by a slightly lower tonic response which lasted for several minutes. CCh had no effect on the basal level of tissue cAMP.

**Dose–Response Effect of ISO on cAMP Formation, IP₃ Accumulation and Muscle Relaxation in Bovine Iris Sphincter**

Dose–response studies on the effects of ISO on the biochemical and physiological responses showed that the β-adrenergic agonist dose-dependently increased the formation of cAMP with a maximal effect obtained at 1 μM of the agonist (Fig. 2). Under the same experimental conditions ISO inhibited IP₃ accumulation in a dose-dependent manner. Increasing concentrations of ISO resulted in increased relaxation of the muscle and a maximal relaxant effect of the agonist was observed at 0.2 μM. The finding that the dose–relaxation curve for ISO lies to the left of those for cAMP and IP₃ could suggest the presence of spare receptors for eliciting the pharmacological response. The EC₅₀ values were: 2.8 × 10⁻⁷ M for cAMP formation, 3.4 × 10⁻⁷ M for IP₃ inhibition and 4.5 × 10⁻⁸ M for muscle relaxation.

**Effect of Timolol on ISO-Induced cAMP Formation, IP₃ Accumulation and Muscle Relaxation in Bovine Iris Sphincter**

Timolol is a potent β-adrenergic antagonist and is widely used as an antiglaucoma agent. However, the mechanism of its hypotensive effect is still unclear. Thus, it was of interest to investigate its effect on ISO-induced cAMP formation, IP₃ accumulation and muscle relaxation in the iris sphincter. As shown in Figure 3, the biochemical and pharmacological responses induced by ISO (0.5 μM) were dose-depen-dently inhibited by timolol. The IC₅₀ values for cAMP formation, IP₃ accumulation and muscle relaxation were 1.8 × 10⁻⁵ M, 3.2 × 10⁻⁵ M, and 2.2 × 10⁻⁵ M, respectively.

**Time Course of the Effects of ISO on cAMP Formation, IP₃ Accumulation and Muscle Relaxation in Bovine Iris Sphincter**

In these experiments we have investigated the temporal relationships between ISO-induced cAMP formation, inhibition of IP₃ accumulation and muscle relaxation. Upon addition of ISO (0.5 μM) the level of intracellular cAMP was increased by 40% within 15 sec, and the maximal effect was observed at about 5 min (Fig. 4). In contrast to its stimulatory effect on cAMP formation, ISO inhibited IP₃ accumulation in
a time-dependent manner. Inhibition of IP$_3$ accumulation by ISO was evident after 30 sec of incubation and reached maximal (30% inhibition) at about 5 min (Fig. 4). In general the time course profile for ISO-induced muscle relaxation parallels that of cAMP formation. About 50% of muscle relaxation by the agonist is achieved within 15 sec and it is complete within 2 min. These data clearly demonstrate a temporal relationship between the ISO-induced stimulation of cAMP, inhibition of IP$_3$ and relaxation of the bovine iris sphincter.

**Reversal by CCh of ISO-Induced cAMP Formation, IP$_3$ Inhibition and Muscle Relaxation in the Bovine Iris Sphincter**

Further support for interaction between the cAMP and IP$_3$–Ca$^{2+}$ signalling systems came from studies on the effects of CCh on ISO-induced stimulation of cAMP, inhibition of IP$_3$ and relaxation of the iris sphincter (Fig. 5). In this experiment the sphincter strips were pretreated with ISO (0.5 μM) prior to the addition of various concentrations of CCh. Under these experimental conditions the muscle: (1) is relaxed; (2) has an elevated level of cAMP (540% increase); and (3) has a reduced level of IP$_3$ (30% decrease). Addition of CCh reversed the three responses which resulted in: (1) a decrease in cAMP formation, with maximal effect (40% inhibition) obtained at 20 μM CCh; (2) a dose-dependent increase in IP$_3$ accumulation; and (3) a dose-dependent reversal of muscle relaxation to maximal contraction obtained at 50 μM CCh.

**Discussion**

The data presented suggest a reciprocal relationship between the two second messenger systems in the bovine iris sphincter: activation of muscarinic re-
cAMP and smooth muscle contraction, whereas stimulation of β-adrenergic receptors results in elevation of cAMP production, inhibition of IP3 accumulation and muscle relaxation. This conclusion is supported by the following findings: (1) Addition of ISO or forskolin to the sphincter muscle increased cAMP formation, inhibited IP3 accumulation, and caused muscle relaxation. These effects of the β-agonist were dose-dependent (Figs. 2, 4) and were blocked by the β-antagonists, propranolol and timolol (Table 1). The observation that the dose–response curve for relaxation (EC50 = 0.045 μM) was shifted to the left of those for IP3 inhibition (EC50 = 0.34 μM) and cAMP production (EC50 = 0.28 μM) could be attributed to the presence of spare receptors in this tissue. Lack of receptor reserve for agonist-mediated increase in inositol phosphate accumulation has been reported in several types of smooth muscle including rabbit aorta,27,28 guinea pig visceral longitudinal smooth muscle29 and rabbit iris dilatator.30 A possible receptor reserve for α1-adrenergoreceptor-mediated [3H]inositol phosphate accumulation has been reported in rat vas deferens.31 In the present study it is also possible that other events may be responsible for relaxation at lower concentrations of ISO and that cAMP and IP3 effects are more important at higher concentrations of the β-agonist (0.5–5 μM). (2) The effects of ISO on the biochemical and physiological responses were time dependent, and they clearly demonstrated a temporal relationship between the ISO-induced stimulation of cAMP, inhibition of IP3, and relaxation of the bovine iris sphincter (Fig. 4). (3) Addition of CCh to iris sphincter pretreated with ISO decreased cAMP formation, with maximal effect (40% inhibition) obtained at 20 μM of the muscarinic agonist, induced a dose-dependent increase in IP3 accumulation, and caused a dose-dependent reversal of muscle relaxation (Fig. 5). These data show that in the iris sphincter, as with other ocular tissues,23 muscarinic receptors are negatively coupled to adenylate cyclase, and that this inhibition is significant only when adenylate cyclase is activated by β-adrenergic agonists. Further, as with other tissues,32 CCh alone produced little if any significant effect on basal cAMP metabolism, although it did inhibit adrenergic stimulation (Fig. 5). In light of these and previous findings on the iris sphincter,2,22,24 we propose the following scheme on possible interactions between the IP3–Ca2+–DG and cyclic AMP signalling systems in the iris sphincter (Fig. 6). Over the past 2 years, the importance of the interaction between the two signalling...
systems in the regulation of Ca\textsuperscript{2+}-dependent functions, such as smooth muscle contraction and secretion, has been well documented. In smooth muscle (Fig. 6), activation of Ca\textsuperscript{2+}-mobilizing receptors leads to the generation of IP\textsubscript{3} and DG. The increase in intracellular Ca\textsuperscript{2+} leads to activation of MLC kinase, phosphorylation of MLC and consequently phasic contraction. This lasts for about 30 sec in the iris sphincter (Fig. 1). DG activates PKC to: (1) Stimulate MLC phosphatase and consequently tonic contraction; (2) feedback-inhibit phospholipase C activity; and (3) stimulate adenylate cyclase to raise cAMP production and consequently inhibit phospholipase C and MLC kinase activities. The tonic response lasts for several minutes in the iris sphincter (Fig. 1). Furthermore, the iris muscle is highly enriched in the enzyme PKC.\textsuperscript{33} Recently, Mittag et al\textsuperscript{34} reported that intravitreal injection of 50 pmoles of phorbol ester into rabbit eyes produced approximately a 40% decrease of intraocular pressure relative to the control eye, lasting for more than 72-hr. Very recently, we demonstrated that ISO activates MLC phosphatase in the iris sphincter to release Pi (S. D. Tachado, R. A. Akhtar and A. A. Abdel-Latif, unpublished data). This could underlie the mechanism of relaxation in this smooth muscle (Fig. 6).

Timolol depressed basal IP\textsubscript{3} formation by 23% (Table 1), and both biochemical and pharmacological responses induced by ISO were dose-dependently inhibited by the drug (Fig. 3). More recently, we found that timolol (10 \mu M) inhibited IP\textsubscript{3} formation in bovine ciliary processes by 47% after 1 min of incubation (S. D. Tachado, R. A. Akhtar and A. A. Abdel-Latif, unpublished data). The mechanism of the hypertensive effect of this antiglaucoma drug is still unknown.\textsuperscript{35-38} The present study indicates, however, that in addition to acting as a \beta-adrenergic antagonist timolol exerts a significant inhibitory effect on the IP\textsubscript{3}-Ca\textsuperscript{2+} signalling system. The formation of aqueous humor appears to decrease after topical treatment with timolol, while the outflow facility seems to remain unchanged (for reviews, see refs. 37, 38). Since secretion and muscle contraction are Ca\textsuperscript{2+}-dependent functions, it is possible that timolol by inhibiting IP\textsubscript{3} formation and consequently Ca\textsuperscript{2+} mobilization could reduce the formation of aqueous humor in the ciliary processes.

In conclusion, the data presented demonstrate biochemical and functional interactions between the Ca\textsuperscript{2+} and cAMP systems in the bovine iris sphincter. In this tissue cAMP may act as regulator of responses to neurotransmitters, hormones and pharmacological agents which exert their action through the IP\textsubscript{3}-Ca\textsuperscript{2+}-DG second messenger system. This conclusion is in accord with recent findings on the species difference in the effects of prostaglandins on the two second messenger systems and on muscle contraction in the iris sphincter.\textsuperscript{24}

**Key words:** bovine iris sphincter, adrenergic agonists and antagonists, cyclic AMP, inositol trisphosphate-Ca\textsuperscript{2+}, muscle tension

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