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POSITIVE COUPLING OF β-LIKE ADRENERGIC RECEPTORS WITH ADENYLATE CYCLASE IN THE CNIDARIAN *RENILLA KOELLIKERI*

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Summary

Coupling of the previously characterized β_1- and β_2-like adrenoceptors in the sea pansy *Renilla koellikeri* with adenylate cyclase was examined in membrane preparations from this cnidarian. Adenylate cyclase activity was stimulated by several guanine nucleotides, such as GTP, Gpp(NH)p and GTPγS. Fluoride ions and cholera toxin greatly enhanced the enzyme activity, whereas forskolin had no effect on basal or isoproterenol-induced stimulation of the enzyme. The stimulation of adenylate cyclase activity by several β-adrenergic agonists in different parts of the animal reflected a positive coupling with the β_2- and β_1-like adrenoceptors in autozooid and peduncle tissues, respectively. In addition, isoproterenol-induced stimulation of adenylate cyclase activity was dependent on guanine nucleotides, suggesting coupling mediated by a G protein. The pharmacological profile of various antagonists on isoproterenol-sensitive adenylate cyclase in autozooid and peduncle tissues matched that of previous radioligand binding studies. Isoproterenol-induced stimulation of adenylate cyclase activity in rachidial tissues was partially inhibited by trifluoperazine or (±)CGP12177 and was completely blocked in the presence of both antagonists. This suggests that coupling of the enzyme occurs with β_1- and β_2-like adrenoceptors, both being present in the rachis. Serotonin and dopamine were also found to stimulate adenylate cyclase activity. Their stimulatory effect was additive to isoproterenol-induced activation, suggesting the presence of dopaminergic and serotonergic receptors in the tissues of the sea pansy. Along with the data presented previously on β-adrenergic binding, this study suggests that elements of receptor-dependent G protein signal transduction originated early in invertebrate evolution.

Introduction

Over the last 20 years or so, coelenterate neurobiology has attracted considerable attention from zoologists. This interest originated from the widely held view that early nervous systems emerged first in this group of animals. The application of intracellular recording techniques has greatly enhanced our understanding of the physiology of both

Key words: adenylate cyclase, β-like adrenoceptors, G protein, positive coupling, Cnidaria, sea pansy, *Renilla koellikeri.*

electrical and chemical synapses in these early metazoans (see review by Anderson and Spencer, 1989; Anderson and Schwab, 1982). Studying coelenterate neuropharmacology provided an opportunity to unravel the evolutionary origin of chemical neurotransmission. A great deal was learnt about the behavior of different species and the type of electrical signals associated with each behavior. However, information on the identity of neuroactive substances and pharmacological mechanisms of neurotransmission in coelenterates remain scarce.

Some evidence for the involvement of catecholaminergic-like mechanisms in the control of colonial behavioral responses comes from the bioluminescent sea pansy *Renilla koellikeri* (Cnidaria, Anthozoa). The colony is composed of two types of individuals called polyps. Luminescence originates in photocytes (light-emitting cells) located in the endoderm of autozooid (feeding) and siphonozooid (water-pumping) polyps (Morin, 1974).

The luminescent behavior of *R. koellikeri* is a colonial response mediated by a through-conducting nerve net (Anderson and Case, 1975). On the basis of a pharmacological study, Anctil *et al.* (1982) suggested the presence of adrenergic mechanisms associated with the neural control of bioluminescence in this species. Epinephrine, but not norepinephrine, was found to induce luminescence in polyps, and this response was depressed by the classical β-adrenergic blocker propranolol. Subsequently, a Ca^{2+}-dependent and desipramine-sensitive uptake mechanism for both epinephrine and norepinephrine was reported in *R. koellikeri* (Anctil *et al.* 1984). Later, De Waele *et al.* (1987) detected the presence of both epinephrine and norepinephrine in the tissues of the sea pansy, using radioenzymatic and HPLC-ED techniques.

Recently, Awad and Anctil (1993) have demonstrated the presence of two different classes of β-adrenergic binding sites, $site_1$ and $site_2$, in membrane preparations from *R. koellikeri*. Using two different β-adrenergic radioligands, dihydroalprenolol and (±)CGP12177, binding was found to be specific, rapid, reversible and saturable and to have a high affinity as well as a marked specificity for β-adrenergic agonists and antagonists. The major characteristics of these β-adrenergic receptor sites are as follows.

First, the distribution of the binding sites in various parts of the colony reflected their functions. $Site_1$ was detected in polyp tissues. The specificity pattern of the physiological effect of β-adrenergic drugs on luminescence mirrored that of the radioligand interaction with $site_1$, suggesting an association with bioluminescent activity. In contrast, radioligand binding corresponding to $site_2$ was observed in colonial tissues, namely the rachis (the muscular body of the colony) and the peduncle (the anchoring muscular organ; for the general organization of the colony see Awad and Anctil, 1993). $Site_2$ distribution substantiates previous pharmacological investigations (Anctil *et al.* 1982) and suggests that this site plays a role in muscle activity.

Second, the two sites displayed different radioligand specificity. [^{3}H]DHA was found to bind to both sites, whereas [^{3}H]CGP12177 recognized $site_1$ only.

Third, displacement studies with different drugs revealed different pharmacological properties for the sites. The pharmacological profile of β-adrenergic binding in *R. koellikeri* membrane fractions followed a pattern similar to β_2- and β_1-adrenoceptor subtypes for $site_1$ and $site_2$, respectively.

Fourth, the pharmacological properties of the β-adrenergic receptor sites in the sea pansy differ from those of mammals. Atenolol, a β_1-selective blocker, was found to act as an agonist on the β_2-like site$_1$ and to induce luminescence like the other β-adrenergic agonists. Similarly, the antidepressant and dopaminergic antagonist trifluoperazine acted as an antagonist on site$_2$.

In mammalian systems, interaction of a β-adrenergic agonist with its binding site on the cell surface stimulates a guanine-nucleotide regulatory protein (G protein) that binds GTP and acts to convey information from the receptor to the catalytic unit of the membrane-bound adenylate cyclase. When activated, this enzyme catalyses the formation of the second messenger cyclic AMP from ATP (Stadel, 1991). Therefore, this study was undertaken to examine whether, in this cnidarian, such coupling exists with adenylate cyclase and to examine the degree of pharmacological similarity between the cnidarian signal transduction system and its mammalian counterpart.

Materials and methods

Tissue preparation

Colonies of *Renilla koellikeri* Pfeffer were purchased from Marinus Inc. (California, USA). They were kept in aerated, filtered and circulating sea water (12–16°C; pH7.4–8.0; specific gravity 1.025). The colonies were exposed to 12h:12h light:dark daily cycles and were used within a few days of arrival. Autozooid polyps were excised after anesthetizing the colony in 0.37mol l^{-1} $MgCl_2$ mixed with artificial sea water (1:1). The peduncle and the rachis (including siphonozooid polyps) were then separated from each other and cut into small pieces. The tissues were homogenized at 0°C with a Brinkman polytron homogenizer in Tris–HCl (50mmol l^{-1}, pH7.4) containing 0.25mol l^{-1} sucrose, 2mmol l^{-1} EDTA and 1mmol l^{-1} dithiothreitol. The homogenates were centrifuged for 10min at 1000 ***g***. The supernatant was collected and centrifuged at 35000 ***g*** for 20min at 0°C. The pellet was resuspended in Tris–HCl buffer, centrifuged for 20min at 35000 ***g*** and washed three times by repeated centrifugation. The final pellet was resuspended in Tris–HCl containing 2mmol l^{-1} $MgCl_2$ and 1mmol l^{-1} EDTA.

Adenylate cyclase assay

The resuspended pellets were thawed immediately before use. The protein content of the crude membrane preparations was determined according to the method of Bradford (1976), using a Bio-Rad protein assay kit.

Adenylate cyclase activity was determined in an assay medium slightly modified from that of Salomon (1979). The reaction was conducted in Tris–HCl buffer (50mmol l^{-1}; pH 7.4) containing 4mmol l^{-1} $MgCl_2$, 1mmol l^{-1} cyclic AMP, 0.5mmol l^{-1} ATP, 1 mmol l^{-1} EDTA, 1mmol l^{-1} dithiothreitol, 1mmol l^{-1} theophylline, 10 μmol l^{-1} GTP and 1.2μCi of [^{3}H]ATP. The reaction was initiated by the addition of the membrane suspension (100–200 μg of protein) in a final volume of 100 μl and was carried out routinely for 30min at 25°C. The reaction was terminated by adding 200 μl of a stopping solution containing 2% SDS (sodium dodecyl sulfate) and 10mmol l^{-1} cyclic AMP. To determine cyclic AMP recovery, 25 μl of a standard ^{14}C-labelled cyclic AMP solution

was added to each tube. The tubes were boiled for 3min. Separation of ^{3}H-labelled cyclic AMP from its precursor [^{3}H]ATP and from the other metabolites of ATP was achieved by sequential chromatography with Dowex 50W-X4 ($^{+}$H form, 200–400 mesh; Bio-Rad) and then alumina (70–230 mesh; Sigma) columns.

[^{3}H]cyclic AMP and [^{14}C]cyclic AMP were eluted from alumina columns with 3ml of $0.1\,mol\,l^{-1}$ imidazole–HCl (pH7.5) into scintillation vials containing 10ml of Universol scintillation fluid (ICN Radiochemicals) and counted in a two-channel scintillation counter. The recovered [^{3}H]cyclic AMP was corrected by using [^{14}C]cyclic AMP as an internal standard. The recovery of [^{3}H]cyclic AMP from the Dowex alumina column system was consistently between 60 and 75%.

Data analysis

EC_{50} and IC_{50} values (concentration of agonist inducing half-maximal stimulation of adenylate cyclase or of antagonist inducing half-maximal inhibition of agonist-induced stimulation of adenylate cyclase, respectively) were determined by fitting a four-variable logistic equation to the data using the SigmaPlot computation procedure (Jandel Corporation, USA).

Statistical analyses of paired data were performed using Student's *t*-test. One-way analysis of variance (ANOVA) was used to compare multiple groups of means. Statistical significance was taken as $P<0.05$. Results are presented as means ± standard deviation. The number of animals used in each experimental condition (N) ranged between three and eight.

Chemicals

Most chemicals used were purchased from Sigma Chemical Co., St Louis, MO, including Antho-RFamide, atenolol, cholera toxin, dopamine, (−)epinephrine, forskolin, Gpp(NH)p, GTPγs, GDPβs, haloperidol, NaF, (−)norepinephrine, octopamine, pindolol, phentolamine, (−)propranolol, (+)propranolol, serotonin and yohimbine. [^{3}H]ATP ($1.1\,TBq\,mmol^{-1}$) and [^{14}C]cyclic AMP ($1.9\,GBq\,mmol^{-1}$) were obtained from New England Nuclear/Du Pont Canada Inc; (−)isoproterenol, (+)isoproterenol and (±)CGP12177 from Research Biochemicals Inc., MA, USA. Methysergide was a gift from Sandoz Canada Inc.

Results

Since site$_1$ (β_2-like) is localized in polyp tissues and site$_2$ (β_1-like) in rachidial and peduncle tissues (Awad and Anctil, 1993), the possible coupling of β-adrenoceptors with adenylate cyclase in *R. koellikeri* was investigated in autozooid tissues for site$_1$ and in peduncle tissues for site$_2$, unless otherwise stated.

Basal activity of adenylate cyclase in R. koellikeri

The basal activity of adenylate cyclase in membrane fractions of the sea pansy was measured in the presence of $10\,\mu mol\,l^{-1}$ GTP and $1\,mmol\,l^{-1}$ theophylline. In the absence of GTP and Mg^{2+}, basal activity was negligible. The activity in autozooid and

peduncle crude membrane preparations was found to increase linearly with protein content (35–425 μg) in the assay. In the experiments described below, protein content of the membrane suspensions was in the range 100–200 μg.

Accumulation of cyclic AMP increased linearly with time up to about 30min of incubation. An incubation time of 30min was routinely used. Adenylate cyclase activity was found to be temperature-dependent. It was negligible at 0˚C and optimal at 25˚C. The activities at 16˚C and 45˚C were about 80% and 20% of the optimal value, respectively. All experiments were routinely conducted at 25˚C. Cyclic AMP accumulation was found to be dependent on the pH of the assay medium. The activity did not change between pH 7.0 and 8.0 but decreased markedly below pH7.0 and above pH 8.0.

Under the standard assay conditions detailed above, the basal activities in crude membrane preparations extracted from three different parts of the colony were as follows: autozooids, 0.147±.0.008pmolmin^{-1} mg^{-1} protein; rachis, 0.069±0.016pmol min^{-1} mg^{-1} protein; peduncle, 0.310±0.057pmolmin^{-1} mg^{-1} protein (*N*=8).

Effects of guanine nucleotides, cholera toxin and fluoride ions on adenylate cyclase activity

To investigate the possible involvement of a G protein in mediating the activation of adenylate cyclase by the receptors, the effect of guanine nucleotides, cholera toxin and NaF on the activation of the enzyme were tested.

GTP and its stable analogue Gpp(NH)p were found to stimulate autozooid adenylate cyclase activity in a dose-dependent fashion (Fig. 1). Half-maximal stimulation of basal activity occurred at 14.38±0.87 μmol l^{-1} for GTP and at 1.31±0.08 μmol l^{-1} for Gpp(NH)p. At saturation concentration, stimulation was about threefold above basal for GTP and fivefold above basal for Gpp(NH)p.

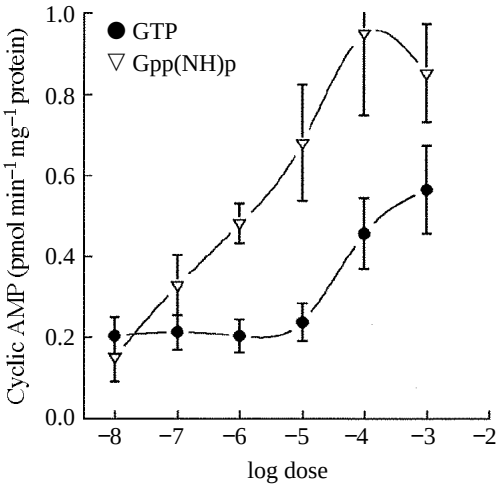

Fig. 1. Effect of increasing concentrations of GTP and Gpp(NH)p (in mol l^{-1}) on adenylate cyclase activity in crude membrane preparations from autozooid tissues. Mean ± S.D., *N*=4.

Table 1. *The effects of guanine nucleotides, NaF, forskolin and cholera toxin on adenylate cyclase activity in autozooid and peduncle crude membrane preparations*

	Autozooids		Peduncle	
	Specific activity ($pmol\,min^{-1}\,mg^{-1}$ protein)	Stimulation†	Specific activity ($pmol\,min^{-1}\,mg^{-1}$ protein)	Stimulation†
Basal*	0.15±0.08	1.0	0.31±0.06	1.0
Gpp(NH)p ($10\,\mu mol\,l^{-1}$)	0.68±0.14	4.6	1.55±0.16	5.0
GTPγs ($100\,\mu mol\,l^{-1}$)	0.87±0.12	5.9	1.49±0.14	4.8
GDPβs ($10\,\mu mol\,l^{-1}$)	0.14±0.02	0.9	0.27±0.03	0.8
NaF ($100mmol\,l^{-1}$)	1.10±0.13	7.5	1.64±0.13	5.3
Forskolin ($100\,\mu mol\,l^{-1}$)	0.16±0.04	1.0	0.35±0.03	1.1
Cholera toxin ($1.5mg\,ml^{-1}$)	1.74±0.14	11.6	1.72±0.01	5.5

*In the presence of $10\,\mu mol\,l^{-1}$ GTP.
†Stimulation is the level of activity compared with the basal level.
Values are mean ± S.D., *N*=8.

Sodium fluoride ($100mmol\,l^{-1}$) and the GTP analogue GTPγs ($100\,\mu mol\,l^{-1}$) caused a five- to sevenfold increase in adenylate cyclase activity in crude membrane fractions extracted from autozooid and peduncle tissues. In contrast, GDPβs was found to depress adenylate cyclase activity slightly at $10\,\mu mol\,l^{-1}$. The effects of these GTP analogues on the enzyme are summarised in Table 1.

To assess the possible involvement of a stimulatory G protein in the coupling between the receptor and the adenylate cyclase, the effect of cholera toxin was tested on the enzyme activity in both autozooid and peduncle membrane fractions (Table 1). Autozooid adenylate cyclase activity was increased 10- to 11-fold over basal levels by cholera toxin ($1.5mg\,ml^{-1}$). In contrast, a five- to sixfold increase was observed in the peduncle.

Finally, forskolin had no significant effect on cyclic AMP accumulation in either tissue at $100\,\mu mol\,l^{-1}$ (Table 1).

Stimulation of adenylate cyclase by β-adrenergic agonists and the β_1-selective blocker atenolol

The ability of several β-adrenergic agonists to stimulate adenylate cyclase activity in the sea pansy was tested. In autozooid tissues, (−)isoproterenol, epinephrine and atenolol were found to stimulate adenylate cyclase activity with a rank order of efficacy of (−)isoproterenol = atenolol > (−)epineprine (Fig. 2A; Table 2). At a saturation concentration of $100\,\mu mol\,l^{-1}$, stimulation was about sixfold. The effects of (−)isoproterenol and atenolol on adenylate cyclase activity were not additive. (−)Norepinephrine and the less active isomer (+)isoproterenol induced a small but significant increase (1.5-fold) in adenylate cyclase activity.

All three agonists, (−)isoproterenol, epinephrine and norepinephrine, induced a

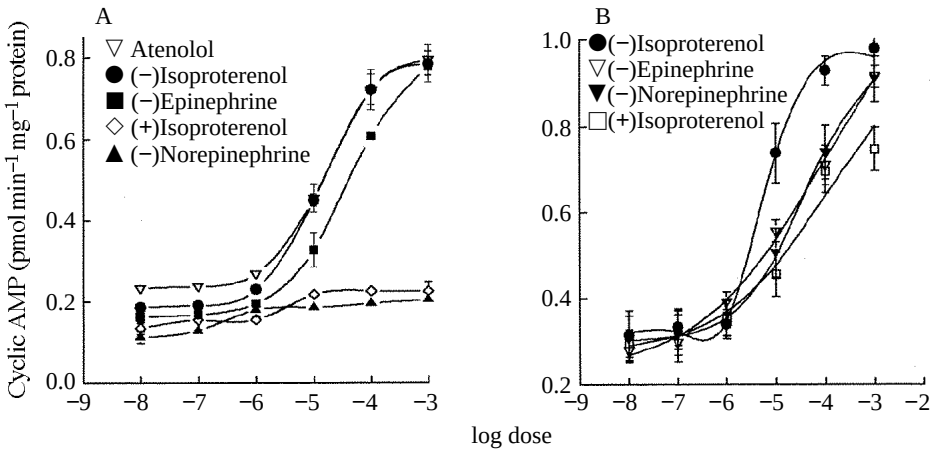

Fig. 2. Effects of β-adrenergic agonists and the β_1-adrenergic antagonist atenolol on adenylate cyclase activity in crude membrane preparations extracted from autozooid (A) and peduncle (B) tissues. Mean ± S.D., *N*=8.

threefold increase of adenylate cyclase activity in the peduncle with an order of efficacy of (−)isoproterenol > (−)epinephrine = (−)norepinephrine (Fig. 2B; Table 2). (+)Isoproterenol was about four times less active than the (−) isomer. Atenolol, however, had no significant effect on the enzyme activity in the peduncle.

Effects of guanine nucleotides, theophylline and forskolin on isoproterenol-induced stimulation of adenylate cyclase

The following experiments were undertaken to investigate the dependency of agonist-induced increases in adenylate cyclase activity on guanine nucleotides and to test for the possible presence of an endogenous phosphodiesterase in the assay medium.

In the absence of GTP, the activity stimulated by isoproterenol and atenolol in autozooids and by isoproterenol in the peduncle was about twofold greater than basal levels. Addition of 10 μmol l^{-1} GTP enhanced the stimulatory effect of these agonists (Fig. 3). Agonist-induced stimulation of the enzyme in both tissues was completely blocked by the addition of 10 μmol l^{-1} GDPβs to the incubation medium. Finally, the

Table 2. *Efficacy values (EC_{50}, μmol l^{-1}) of different* β*-adrenergic agonists on stimulation of adenylate cyclase activity in autozooid and peduncle tissues*

Agonist	Autozooids	Peduncle
(−)Isoproterenol	8.40±0.79	5.74±0.56
(+)Isoproterenol	>1000	19.65±2.03
(−)Epinephrine	22.02±2.28	23.92±2.34
(−)Norepinephrine	>1000	25.63±2.47
Atenolol	11.08±1.04	>1000

Values are mean ± S.D., *N*=8.

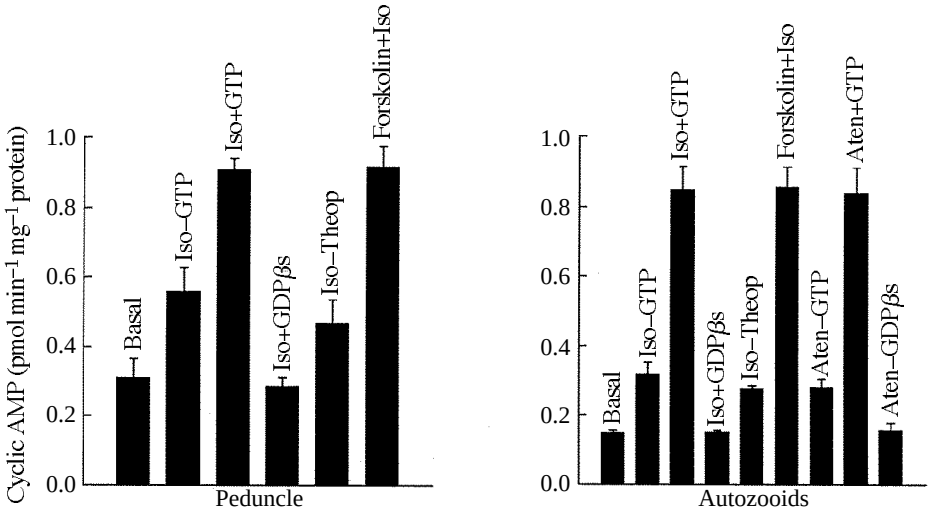

Fig. 3. Effect of GTP, GDPβs, theophylline and forskolin on isoproterenol- and atenolol-induced stimulation of adenylate cyclase activity in autozooid and peduncle tissues. Basal adenylate cyclase activity was measured in the presence of 10 μmol l^{-1} GTP. The minus sign indicates the omission of a substance from the assay medium. The plus sign indicates the addition of the substance to the assay medium. 10 μmol l^{-1} GTP and GDPβs and 100 μmol l^{-1} forskolin were added to the assay medium whenever indicated. Iso, isoproterenol (100 μmol l^{-1}); Aten, atenolol (100 μmol l^{-1}); Theop, theophylline (1mmol l^{-1}). Mean ± S.D., *N*=4.

absence of theophylline substantially reduced the stimulatory effect of the agonists on adenylate cyclase activity in autozooid and peduncle membrane preparations.

In contrast, forskolin had no significant effect on the isoproterenol-induced increase of the enzyme activity in either tissue of the sea pansy (Fig. 3).

Table 3. *Potency values (pKac) of different blockers on isoproterenol-induced stimulation of adenylate cyclase activity in autozooid and peduncle tissues*

Compound	Autozooids	Peduncle
(−)Propranolol	8.45±0.51	8.10±0.49
(+)Propranolol	6.64±0.64	7.59±0.48
(−)Alprenolol	7.70±0.59	7.41±0.44
Pindolol	8.20±0.50	8.57±0.47
(±)CGP12177	7.21±0.41	<3.00
Trifluoperazine	<3.00	7.44±0.74
Atenolol	–	<3.00

Values are mean ± S.D., *N*=8.

pKac is the inverse logarithm of Kac values calculated, assuming competitive inhibition, from the equation $IC_{50}=Kac[1+(S/K_m)]$, where K_m is the concentration of isoproterenol required for half-maximal stimulation of adenylate cyclase activity (8.404 μmol l^{-1} in autozooids and 5.736 μmol l^{-1} in the peduncle) and *S* is the concentration of isoproterenol used (100 μmol l^{-1})

Effects of antagonists on isoproterenol-induced stimulation of adenylate cyclase

To compare the isoproterenol-sensitive adenylate cyclase in autozooid and peduncle tissues, the effects of various antagonists on the isoproterenol-induced increase in adenylate cyclase activity were investigated (Table 3).

A number of β-adrenergic antagonists were tested for their activities as inhibitors of the isoproterenol-sensitive adenylate cyclase in autozooid and peduncle tissues (Fig. 4). The rank order of potency of the different β-antagonists tested on isoproterenol-induced stimulation of adenylate cyclase activity in autozooid tissues was: pindolol = (−)propranolol > (−)alprenolol > (±)CGP12177 > (+)propranolol. (+)Propranolol was 1–2 orders of magnitude less potent than the (−) isomer. Trifluoperazine, however, was completely inactive at 100 μmol l^{-1}.

In the peduncle, the (+) isomer of propranolol was slightly less effective than the (−) isomer. Of special interest is the inhibition of isoproterenol-stimulated adenylate cyclase activity by the antidepressant and dopaminergic antagonist trifluoperazine in peduncle tissues. The rank order of potency of the different antagonists tested on isoproterenol-induced stimulation of adenylate cyclase activity in peduncle tissues was: pindolol > (−)propranolol > (+)propranolol = (−)alprenolol = trifluoperazine. Atenolol and (±)CGP12177 were ineffective at 100 μmol l^{-1}.

Finally, the α-adrenergic antagonists phentolamine and yohimbine, and both methysergide and haloperidol, had little or no effect on isoproterenol-induced stimulation of adenylate cyclase in either autozooid or peduncle tissues.

Resolution of two different β*-adrenoceptor-adenylate cyclase couplings in rachidial membrane preparations*

Taking advantage of the selective action of (±)CGP12177 as an antagonist on $site_1$

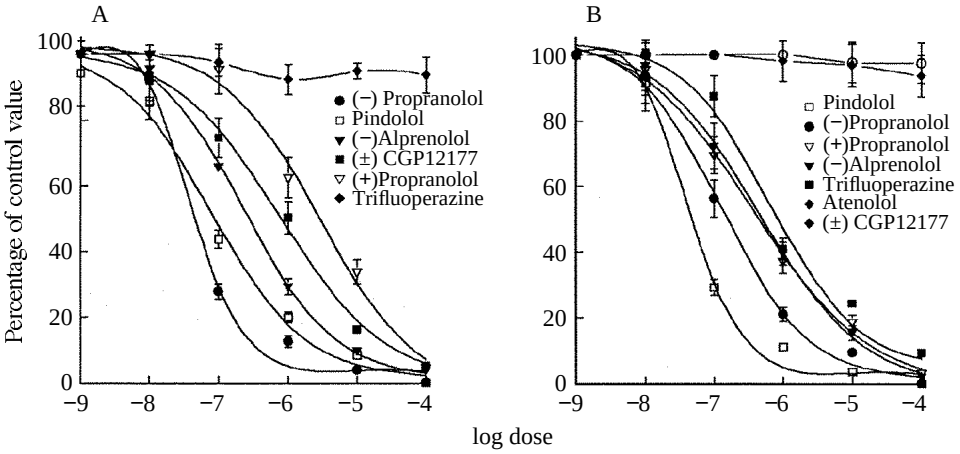

Fig. 4. Dose–response inhibition curves for isoproterenol-stimulated adenylate cyclase activity in autozooid (A) and peduncle (B) crude membrane preparations exposed to different β-adrenergic antagonists and trifluoperazine. The stimulation of adenylate cyclase by 100 μmol l^{-1} isoproterenol in the absence of blockers was taken as 100%. Mean ± S.D., *N*=8.

(β_2-like) and of trifluoperazine as an antagonist on site$_2$ (β_1-like) (Awad and Anctil, 1993), adenylate cyclase coupling with both sites in rachidial tissues was studied separately (Fig. 5).

(−)Isoproterenol stimulated adenylate cyclase activity by about fivefold at 100 μmol l^{-1}. A threefold increase, however, was observed with atenolol at 100 μmol l^{-1}. In the presence of 10 μmol l^{-1} (±)CGP12177, (−)isoproterenol induced only a twofold increase in adenylate cyclase activity. In contrast, atenolol-induced stimulation was almost completely blocked by 10 μmol l^{-1} (±)CGP12177.

Addition of 10 μmol l^{-1} trifluoperazine reduced the increase in adenylate cyclase activity induced by isoproterenol to threefold, but had no effect on atenolol-induced stimulation of the enzyme. The presence of both trifluoperazine and (±)CGP12177 in the assay medium abolished the adenylate cyclase activity stimulated by 100 μmol l^{-1} (−)isoproterenol in the rachis. Finally, the stimulatory effect of isoproterenol and atenolol on the enzyme was found not to be additive.

Effects of other putative neuroactive substances on adenylate cyclase activity in the tissues of the sea pansy

Several additional putative neuroactive substances were tested for their ability to stimulate adenylate cyclase in different parts of the colony (Table 4).

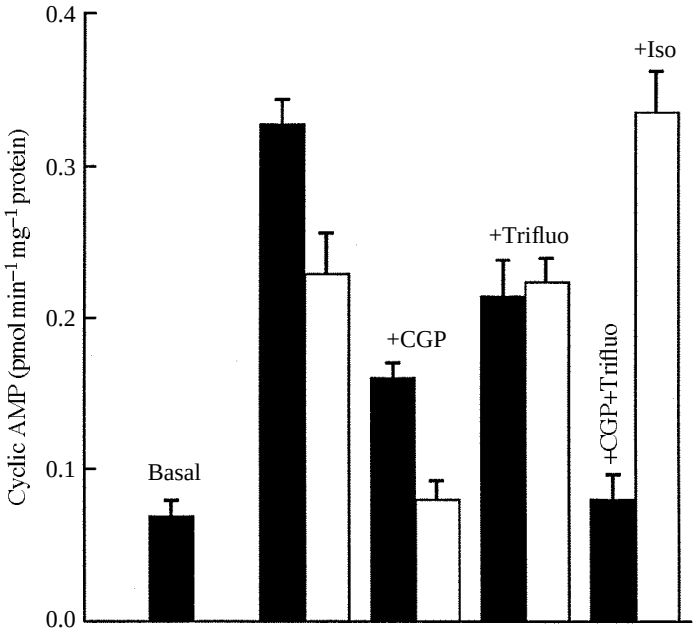

Fig. 5. Effects of (±)CGP12177 (10 μmol l^{-1}) and trifluoperazine (10 μmol l^{-1}) on isoproterenol-induced (100 μmol l^{-1}, filled bars) and atenolol-induced (100 μmol l^{-1}, open bars) stimulation of adenylate cyclase activity in crude membrane preparations extracted from rachidial tissues. The plus sign indicates the addition of the blocker to the assay medium. Enzyme activity was measured in the presence of 10 μmol l^{-1} GTP. CGP, (±)CGP12177; Trifluo, trifluoperazine; Iso, (−)isoproterenol. Mean ± S.D., *N*=6.

Table 4. *Effect of putative neuroactive substances (100 μmol l^{-1}) on adenylate cyclase activity in the tissues of the sea pansy* Renilla koellikeri

	Specific activity (pmolmin^{-1} mg^{-1} protein)		
Treatment	Autozooids	Rachis	Peduncle
Basal	0.15±0.01	0.07±0.02	0.31±0.06
Isoproterenol	0.85±0.07	0.33±0.04	0.93±0.03
Dopamine	0.39±0.07	0.55±0.01	1.01±0.05
Dopamine+isoproterenol	1.26±0.14	0.91±0.01	1.85±0.07
Serotonin	1.17±0.07	0.69±0.01	2.04±0.49
Serotonin+isoproterenol	2.08±0.17	0.95±0.04	2.68±0.12
Octopamine	0.18±0.03	0.08±0.01	0.35±0.03
Octopamine+isoproterenol	0.86±0.11	0.39±0.08	0.95±0.05
Antho-RFamide	0.32±0.09	0.08±0.01	0.57±0.03
Antho-RFamide+isoproterenol	0.85±0.06	0.35±0.05	0.90±0.09

Values are mean ± S.D., *N*=5.

Dopamine stimulated adenylate cyclase activity two- to threefold at 100 μmol l^{-1} in autozooid and peduncle tissues, and six- to eightfold in the rachis. Serotonin stimulation of the enzyme activity was also variable. An eightfold increase was observed in autozooid tissues, a six- to sevenfold increase in the peduncle and about a 10-fold increase in rachidial membrane preparations. Dopamine and serotonin stimulation of adenylate cyclase activity were additive with the isoproterenol effect in all three parts of the colony.

Octopamine had no significant effect on adenylate cyclase activity and was not observed to have any inhibitory effect on isoproterenol-induced stimulation of the

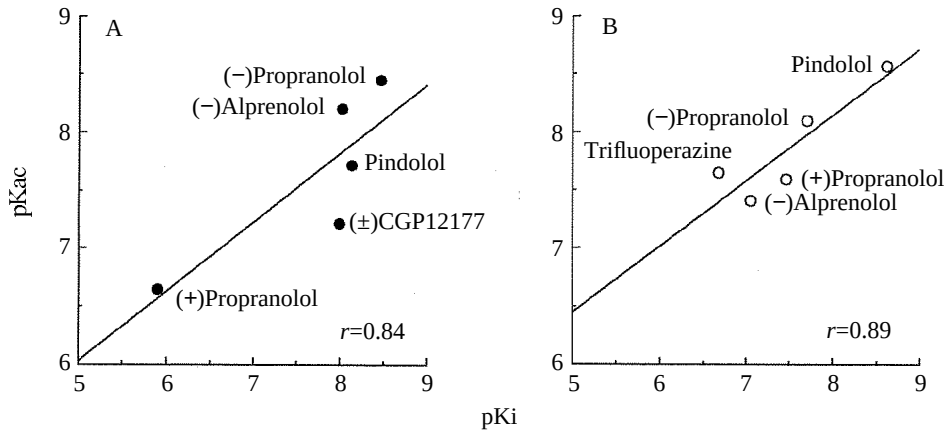

Fig. 6. Correlation between the potency values of different antagonists tested on adenylate cyclase activity (pKac) and on β-adrenergic binding sites (pKi) in autozooid (A) and peduncle (B) tissues. pKac values are those listed in Table 3. pKi values are the inverse logarithm of affinity values of the corresponding blockers reported in Awad and Anctil (1993).

enzyme. The native neuropeptide Antho-RFamide induced a 1.5-fold increase in adenylate cyclase activity in autozooid and peduncle tissues, but failed to cause any such response in the rachis. Antho-RFamide stimulation was not additive with the isoproterenol effect in autozooid and peduncle tissues and had no significant effect on isoproterenol stimulation of adenylate cyclase in the rachis.

Discussion

The presence of β-like adrenoceptors associated with neuroeffector activities in the tissues of *R. koellikeri* has been demonstrated previously (Awad and Anctil, 1993). In this study we report the positive coupling of these receptors with adenylate cyclase, apparently *via* a Gs protein.

Adenylate cyclase activity in the sea pansy is stimulated by both fluoride ions and the non-hydrolysable analogues of GTP, GTPγS and Gpp(NH)p, whereas the GDP analogue GDPβs inhibits the enzyme activity slightly. This suggests that the adenylate cyclase system in *R. koellikeri* is activated by GTP *via* a G protein. Adenylate cyclase systems sensitive to guanine nucleotides and fluoride ions have been reported in other invertebrate species, such as flatworms (Ferreti *et al.* 1989; Ribeiro and Webb, 1987; McNall and Mansour, 1985), annelids (Robertson and Osborne, 1979), molluscs (Drummond *et al.* 1985; Weiss and Drummond, 1985) and insects (Morton, 1984; Uzzan and Dudai, 1982; Harmar and Horn, 1977).

The stimulatory effect of cholera toxin on adenylate cyclase activity might be an indication of the involvement of a stimulatory (Gs) protein in the activation of the enzyme. Cholera toxin produced by the bacterium *Vibrio cholerae* is reported to act on adenylate cyclase systems in nearly all types of eukaryotic cells (Gill, 1977), including yeast (Nakafuku *et al.* 1987) and slime molds (Johnson *et al.* 1989). The presence of a Gs protein in the abdominal ganglion of *Aplysia californica*, where ADP-ribosylation of a G protein was observed, was proposed by Schwartz *et al.* (1983). It was also postulated to be present in the protozoan *Tetrahymena*, where cholera toxin stimulated phagocytosis (De Jesus and Renaud, 1989). The bacterial toxin is known to activate adenylate cyclase by catalysing the transfer of the ADP–ribose moiety of NAD^+ to the α-subunit of Gs proteins. The ADP-ribosylation of Gs_α markedly inhibits the GTPase activity and hence prolongs the lifetime of the Gs_α–GTP complex, which in turn stimulates adenylate cyclase (Stadel, 1991). Since the site of ADP-ribosylation is an arginine residue in the middle of the α-subunit (Kaziro *et al.* 1992), the activation of adenylate cyclase by cholera toxin in *R. koellikeri* suggests a structural homology between the Gs protein transduction system of this coelenterate and that of mammals.

Our results indicate that the diterpene forskolin, which is known to act directly on adenylate cyclase (Seamon *et al.* 1981), at $100\,\mu mol\,l^{-1}$, had no effect on basal or isoproterenol-induced stimulation of the enzyme. Forskolin-insensitive adenylate cyclase systems have been reported in several invertebrates, including flatworms (Ferreti *et al.* 1989), molluscs (Weiss and Drummond, 1985) and insects (Gole *et al.* 1986). The lack of a forskolin effect on the adenylate cyclase system in *R. koellikeri* might be due to a

difference in pharmacological selectivity between the adenylate cyclase enzyme of this early metazoan and that of higher vertebrates.

In this study, the responsiveness of adenylate cyclase to various β-adrenergic agonists suggests coupling of the β-adrenoceptors previously reported in the sea pansy (Awad and Anctil, 1993) with this enzyme. In autozooid tissues, the stimulation of adenylate cyclase activity by isoproterenol and epinephrine, but not norepinephrine, reflects a coupling with the β_2-like adrenoceptor in *R. koellikeri*. The agonist action of the β_1-selective blocker atenolol is not surprising since it was found to act on the β_2-like adrenoceptor and to induce luminescence in this species (Awad and Anctil, 1993). The pharmacological specificity of β-adrenergic agonists on adenylate cyclase activation is identical to that of the β_1-like adrenoceptor reported to be present in peduncle and rachidial tissues of the sea pansy. The dependency of isoproterenol-induced and atenolol-induced stimulation of adenylate cyclase activity on guanine nucleotides suggests that the coupling of the β-like adrenoceptors in *R. koellikeri* with this enzyme is *via* a G protein. In addition, the effect of the phosphodiesterase inhibitor theophylline on the isoproterenol-induced increase in cyclic AMP production might indicate the presence of a native phosphodiesterase in the assay medium that is sensitive to that methylxanthine.

Although a substantial amount of work has been done on the effect of serotonin, dopamine and octopamine on adenylate cyclase systems in invertebrate tissues, few studies have been carried out on the stimulation of this enzyme activity by adrenergic agents. To our knowledge, this study represents the first evidence of the presence of a catecholamine-sensitive adenylate cyclase system in Cnidaria. One of the very few examples of the effect of adrenergic agents on adenylate cyclase systems in invertebrates comes from the study of Biondi *et al.* (1982), who demonstrated a slight increase in cyclic AMP production caused by norepinephrine in muscle tissues of the leech *Hirudo medicinalis*. Propranolol-sensitive β-adrenergic binding sites involved in the stimulation of phagocytosis were reported in the unicellular eukaryote *Paramecium aurelia* (Wyroba, 1989), but their possible coupling with adenylate cyclase was not investigated.

In order to correlate previous radioligand binding studies (Awad and Anctil, 1993) with functional responses, the effects of several β-antagonists were tested for their potency as inhibitors of isoproterenol-induced stimulation of adenylate cyclase activity in tissues of the sea pansy. Our studies had previously associated different pharmacological profiles of β-adrenergic antagonists with β-like adrenergic binding sites in autozooid and peduncle tissues (Awad and Anctil, 1993). The effect of these blockers (reported here) on catecholamine-sensitive adenylate cyclase in *R. koellikeri* confirms the presence of two different β-like adrenoceptors in the colony. From a comparison of the K_D values of the various blockers tested on adenylate cyclase activity *versus* β-adrenergic binding sites (Fig. 6), it can be concluded from the significant correlation that the β_1-like adrenoceptor present in peduncle and rachidial tissues and the β_2-like adrenoceptor present in polyp tissues are both directly coupled to an adenylate cyclase system.

The partial inhibition of isoproterenol-induced stimulation of adenylate cyclase activity by trifluoperazine or (±)CGP12177, and the complete blockage of this stimulatory effect when both antagonists are present in the assay medium, suggests the presence of both site$_1$

(β_2-like) and site$_2$ (β_1-like) in the rachis. Their dual presence in this tissue was previously proposed by Awad and Anctil (1993). This is further demonstrated by the differential effect of atenolol on adenylate cyclase activity, the antagonistic effect of (±)CGP12177, but not trifluoperazine, on atenolol-stimulated enzyme activity and the non-additive effect of isoproterenol and atenolol on adenylate cyclase activity in rachidial tissues.

Although Venturini *et al.* (1984) failed to detect any change in adenylate cyclase activity of the cnidarian *Chlorohydra viridissima* following exposure to serotonin and dopamine, adenylate cyclase activity in the anthozoan *R. koellikeri* is greatly stimulated by these biogenic amines. The additive effects of dopamine and serotonin to the isoproterenol-induced stimulation of the enzyme activity suggest that these amines act *via* receptors other than β-adrenergic receptors in the tissues of the sea pansy, possibly through dopaminergic and serotonergic receptors. The presence of serotonin and dopamine has been reported in *R. koellikeri* (Umbriaco *et al.* 1990; De Waele *et al.* 1987). In addition, a cyclic-AMP-mediated serotonergic mechanism involved in the modulation of rhythmic contractions and peristalsis in the sea pansy has been described by Anctil (1989). Stimulation of adenylate cyclase by dopamine and serotonin has been reported in several invertebrate species, including insects (Lafon-Cazal and Bockaert, 1984; Uzzan and Dudai, 1982; Kilpatrick *et al.* 1980; Bodnaryk, 1979), molluscs (Drummond *et al.* 1980, 1985), annelids (Robertson and Osborne, 1979) and flatworms (McNall and Mansour, 1985; Biondi *et al.* 1982).

Although octopamine receptors and their coupling to adenylate cyclase are quite ubiquitous in arthropods, octopamine had no apparent effect on the cnidarian adenylate cyclase system. Octopamine has not been detected by HPLC-ED in *R. koellikeri* (Pani and Anctil, 1993). The small or ineffective stimulation of adenylate cyclase activity by the native neuropeptide Antho-RFamide might suggest that its action on rhythmic contractions in *R. koellikeri* (Anctil and Grimmelikhuijzen, 1989) is not likely to be associated with cyclic AMP.

This paper presents the first biochemical and pharmacological characterization of an adenylate cyclase system coupled to β-adrenoceptors in a coelenterate. Along with the data presented previously on β-adrenergic binding, this study suggests that the basic elements of the receptor-dependent G protein signal transduction systems described in mammals are also present in early metazoans. It implies that β-adrenergically mediated transduction mechanisms originated early in invertebrate evolution and have been conserved in higher animals.

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