Beta3-Adrenoceptor in the eel (Anguilla anguilla) heart: negative inotropy and NO-cGMP-dependent mechanism

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Summary

Neuroendocrine regulation of cardiac function involves a population of three types of β-adrenoceptors (ARs). In various mammalian species, β1- and β2-AR stimulation produces an increase in contractility; whereas β3-AR activation mediates negative inotropic effects. At the moment, nothing is known about the physiological role of β3-AR in fish.

Using an isolated working heart preparation, we show that a β3-AR selective agonist BRL37344 (0.1–100 nmol l⁻¹) elicits a dose-dependent negative inotropism in the freshwater eel Anguilla anguilla. This effect was insensitive to the β1/β2-AR inhibitor nadolol (10 μmol l⁻¹), but was blocked by the β3-AR-specific antagonist SR59230 (10 nmol l⁻¹). The analysis of the percentage of stroke work (SW) variations, in terms of EC50 values, induced by BRL37344 alone (10 nmol l⁻¹), and in presence of SR59230 (10 nmol l⁻¹), indicated a competitive antagonism of SR59230. In addition to the classic positive inotropy, the non-specific β agonist isoproterenol (100 nmol l⁻¹) induced, in 30% of the preparations, a negative inotropic effect that was abrogated by pre-treatment with SR59230, pointing to a β3-mediated pathway. The BRL37344-induced negative inotropic effect was abolished by exposure to a G₁₀ protein inhibitor pertussis toxin (PTx; 0.01 nmol l⁻¹), suggesting a G₁₀-dependent mechanism. Using L-N5(l-imino-ethyl)ornithine (L-NIO; 10 μmol l⁻¹), as a nitric oxide (NO) synthase (NOS) blocker and haemoglobin (Hb; 1 μmol l⁻¹), as a NO scavenger, we demonstrated that NO signalling is involved in the BRL37344-induced response. Pre-treatment with either an inhibitor of soluble guanylate cyclase (GC) 1H-(1,2,4) oxadiazolo-(4,3-a)quinoxalin-1-one (ODQ; 10 μmol l⁻¹), or an inhibitor of the cGMP-activated protein kinase (PKG) KT5823 (100 nmol l⁻¹), abolished the β3-dependent negative inotropism, indicating the cGMP-PKG component as a crucial target of NO signalling. Taken together, our findings provide functional evidence for the presence of β3-like adrenoceptors in the eel Anguilla anguilla heart identifying, for the first time in a working fish heart, the β3-AR-dependent negative inotropy discovered in mammals.

Key words: catecholamines, teleost, G₁₀ proteins, cGMP-dependent protein kinase, autocrine-paracrine regulation, myocardial performance, NOS.

Introduction

The sympathetic neurohumoral system is an essential regulator of cardiovascular homeostasis, metabolism and immune system in vertebrates. Its principal end products are nor-adrenaline and adrenaline, called catecholamines, which exert a variety of actions at their target cells, through interaction with the adrenoceptor system (Xiao et al., 1999; Brodde et al., 2006).

In 1967, Lands et al. classified the β-adrenergic receptors (β-ARs) into β1 and β2 based on the rank order, in different tissues, of the potency of the two catecholamines, adrenaline and nor-adrenaline (Lands et al., 1967). Both β-ARs have been identified in the mammalian heart where they are responsible for most of the adrenergic-mediated effects on cardiac performance, i.e. positive inotropic, chronotropic and lusitropic responses (Brodde, 1991). To a large extent these effects result from an elevation of intracellular cAMP after adenylyl cyclase stimulation through Gs proteins (Ishikawa and Homcy, 1997).

In various mammalian tissues, including the heart, recent molecular and pharmacological studies have identified, besides the classic β1- and β2-ARs, a new receptor, called β3-AR (Gauthier et al., 1996; Gauthier et al., 1998; Varghese et al., 2000; Tavernier et al., 2003; Boivin et al., 2006), β3-AR, as well as β1- and β2-ARs, belongs to the G-protein coupled receptors characterized by seven transmembrane domains of 22–28 amino acids showing three intracellular and three extracellular loops. It shares 51% and 46% identity with β1- and β2-AR amino acid sequences, respectively, and is activated by selective pharmacological agonists (BRL37344-SR58611, CGP12177), which have little effect on β1- and β2-ARs (Skeberdis, 2004). Using synthetic β3-AR agonists, several studies have shown that β3-AR activation induces
metabolic and functional processes in many tissues (Bianchetti and Manara, 1990; McLaughlin and MacDonald, 1990; Norman and Leathard, 1990; Langin et al., 1991; Yoshida et al., 1991; Arch and Kaumann, 1993; Berlan et al., 1993). In the mammalian heart, functional response to β3-AR stimulation differs among species and depends on the anatomical region within the myocardium (Gauthier et al., 2000). For example, whereas in human and canine ventricles β3-AR stimulation induces negative inotropic effects (Gauthier et al., 2000); in human atrium, specific β3-AR agonists produce positive inotropic actions (Arch and Kaumann, 1993; Sennit et al., 1998), as well as an increase in heart rate under in vivo conditions (Wheeldon et al., 1994). However, in human atrial preparations expressing β3-AR subtype (Chamberlain et al., 1999), no cardiac effects could be detected (Kaumann et al., 1997). The β3-AR-induced intracellular signal pathways, which operate in cardiac tissues, have not been completely clarified. However, the negative inotropic effect has been attributed to an action mechanism that involves $G_{i/o}$ proteins and results from the production of nitric oxide (NO) by the endothelial isoform of NO synthase (eNOS) with the consequent increase in intracellular cGMP levels (Gauthier et al., 1998; Varghese et al., 2000).

Despite their importance in the vertebrate stress response, knowledge of β-ARs in fish is based on relatively few studies centred on a limited number of species, in which the classic cardiac adrenergic response has been mainly attributed to $β_2$-AR stimulation (Gamperl et al., 1994). In this context, the presence and role of $β_3$-ARs have, until now, received surprisingly little, or no, attention.

The expression of two previously unreported β-ARs in the teleost Oncorhynchus mykiss was recently shown (Nickerson et al., 2003). These two trout β-ARs were found to be homologous to the mammalian β3-AR and highly expressed in both gills and heart. However, no physio-pharmacological characterization of $β_3$-ARs in fish heart or its subsequent coupling to second messengers has been reported.

The aim of this study was to analyse in the European eel Anguilla anguilla heart the physiological role of $β_3$-AR and the downstream signal transduction mechanism. As in previous studies (Imbrogno et al., 2001; Imbrogno et al., 2003; Imbrogno et al., 2004), we used juvenile eel hearts with a compact outer ventricular layer and a poorly developed coronary circulation, which allowed us to analyse the effect of cardioactive substances without interferences from the coronary vasculature. We previously reported that in the eel heart the endogenous NO signalling cascade, through a cGMP-mediated mechanism, transduces the negative inotropic effects triggered by chemical stimuli such as acetylcholine (Imbrogno et al., 2001), angiotensin II (Imbrogno et al., 2003) and vasostatin I (Imbrogno et al., 2004). We now demonstrate that $β_3$-AR stimulation decreases cardiac mechanical performance through a PTX-sensitive $G_{i/o}$ protein mechanism that involves a NO-cGMP-cGMP-activated protein kinase (PKG) cascade.

### Materials and methods

#### Isolated and perfused working heart preparations

We used freshwater specimens of European eel Anguilla anguilla L., weighing 85.5±2.7 g (mean ± s.e.m., N=66). Fish were provided by a local hatchery and kept at room temperature (18–20°C) without feeding for 5–7 days. In accordance with accepted standards of animal care, the experiments were organized to minimize stress and number of animals used. Experiments were performed from September to April. Each eel was anaesthetised in benzocaine (0.2 g l⁻¹) for about 15 min. The hearts, isolated and connected to a perfusion apparatus, as previously described (Imbrogno et al., 2001), received Ringer’s solution from an input reservoir and pumped against an afterload pressure given by the height of an output reservoir. The composition of the perfusate (in mmol l⁻¹) was: NaCl 115.17, KCl 2.03, KH₂PO₄ 0.37, MgSO₄ 2.92, (NH₄)₂SO₄ 50, CaCl₂ 1.27, glucose 5.55, NaHPO₄ 1.90; pH was adjusted to 7.7–7.9 by adding NaHCO₃ (about 1 g l⁻¹) (Imbrogno et al., 2001). The Ringer’s solution was equilibrated with a mixture of O₂/CO₂ at 99.5:0.5%. Experiments were carried out at room temperature (18–20°C). The controlled non-paced hearts operated at a frequency of about 50 beats min⁻¹ (see Imbrogno et al., 2001). Hearts were stimulated with an LE 12006 stimulator (frequency identical to that of control, non-paced hearts; pulse width fixed at 0.1 ms; voltage: 1.2±0.1 V; means ± s.e.m.).

#### Measurements and calculations

Pressure was measured through T-tubes placed immediately before the input cannula and after the output cannula, and connected to two MP-20D pressure transducers (Micron Instruments, Simi Valley, CA, USA) in conjunction with a Unirecord 7050 (Ugo Basile, Comerio, Italy). Pressure measurements (input and output) were expressed in kilopascals (kPa) and corrected for cannula resistance. Heart rate ($f_H$) was calculated from pressure recording curves. Cardiac output ($Q$) was collected over 1 min and weighed; values were corrected for fluid density and expressed as volume measurements. The afterload (mean aortic pressure) was calculated as two-thirds systolic pressure plus one-third maximum pressure. Stroke volume ($V_s$; ml kg⁻¹; $Q/ f_H$) was used as a measure of ventricular performance; changes in $V_s$ were considered to be inotropic effects. $Q$ and $V_s$ were normalised per kilogram of wet body mass. Ventricular stroke work [$W_s$; ml g⁻¹; (afterload-preload) × $V_s$/ventricle mass] served as an index of systolic functionality.

#### Experimental protocols

#### Basal conditions

Isolated perfused hearts were allowed to maintain a spontaneous rhythm for up to 15–20 min. In all experiments the control conditions were established at a mean output pressure of about 3 kPa, with a $Q$ set to 10 ml min⁻¹ kg⁻¹ body mass by appropriately adjusting the filling pressure. These values are within the physiological range [for references see Imbrogno et al. (Imbrogno et al., 2001)]. Cardiac parameters
were simultaneously measured during experiments. To analyse the inotropic effects distinct from the chronotropic actions of substances, the preparations were electrically paced. Hearts that did not stabilise within 20 min from the onset of perfusion were discarded.

**Drug application**

After the 15–20 min of control period, paced hearts were perfused for 20 min with Ringer’s solution enriched with BRL37344 at increasing concentrations (from 0.1 to 100 nmol l\(^{-1}\)) to construct cumulative concentration–response curves. Heart preparations were used to test the effects of 10 nmol l\(^{-1}\) of BRL37344 in the presence of \(\beta_1\), \(\beta_2\)-ARs antagonist nadolol, the specific \(\beta_3\)-AR inhibitor SR9230, the nitric oxide (NO) synthase (NOS) inhibitor [L-N\(^5\)-(1-iminoethyl)ornitine (L-NIO)], the NO scavenger haemoglobin (Hb), the soluble guanylate cyclase (GC)-specific inhibitor [1H-[1,2,4]oxadiazole-[4,3-a]quinoxalin-1-one (ODQ)] and the protein kinase G (PKG) blocker (KT5823). In another set of experiments the effects of isoproteinerol (ISO), a non-specific \(\beta_2\)-AR antagonist, nadolol, 1-[(1S)-1,2,3,4-tetrahydronaphth-1-yl]aminopropanol (SR59230) and the specific antagonist SR59230. In the above-mentioned protocols repeated doses of BRL 37344 revealed absence of receptor desensitization (data not shown). The effects of the agonist remained stable for 15 min then gradually decreased with time. Accordingly, cardiac parameters were measured after 10 min. BRL37344 (0.1–100 nmol l\(^{-1}\)) induced a concentration-dependent negative inotropic effect, revealed by a significant reduction of both \(V_s\), starting from the concentration of 1 nmol l\(^{-1}\), and \(W_s\), above 10 nmol l\(^{-1}\) (Fig. 1).

**Statistics**

Percentage changes were evaluated as means ± s.e.m. of percentage changes obtained from individual experiments. Because each heart acted as its own control, a one-way ANOVA test was used for comparisons within groups \((P<0.05)\). Comparisons between groups were made using a two-way ANOVA, Duncan’s multiple-range test \((P<0.05)\).

The concentration–response curves of the reduction of \(V_s\) induced by BRL37344 alone and by BRL37344 plus SR9230 were fitted using GraphPad Prism 4.02. This provided the \(-\log\) of the concentration (in mol l\(^{-1}\)) that induced the 50\% effect (EC\(50\)) of BRL37344 alone and BRL37344 plus SR9230.

**Drugs and chemicals**

[4-[2-[[2-(3-chlorophenyl)-2-hydroxy-ethyl]amino]propyl]-phenoxy]acetic acid sodium (BRL37344), 3-(2-ethylphenoxy)-1-[[1S]-1,2,3,4-tetrahydro-naphth-1-yl]amino]-2S)-2-propanol oxalate salt (SR9230), isoproterenol (ISO), nadolol, haemoglobin (Hb), L-N\(^5\)-(1-iminoethyl)ornitine (L-NIO), 1H-[1,2,4]oxadiazole-[4,3-a]quinoxalin-1-one (ODQ) and pertussis toxin (PTx) were purchased from Sigma Chemical Company (St Louis, MO, USA). KT5823 (used in a darkened perfusion apparatus to prevent degradation) was purchased from Calbiochem (Milan, Italy). All the solutions were prepared in double-distilled water (ODQ and KT5823 were prepared in DMSO); dilutions were made in Ringer’s solution immediately before use.

**Results**

**Isolated heart preparation**

After equilibration, the *in vitro* isolated and perfused heart preparation works at physiological loads and generates values of output pressure, \(f_H\), \(Q\), \(V_s\) and \(W_s\) that mimic the physiological values of the *in vivo* animal [see Imbrogno et al. (Imbrogno et al., 2001) for references].

Baseline variables for the resting heart preparations were: output pressure (kPa) = 2.63±0.53; \(f_H\) (beats min\(^{-1}\)) = 56.47±13.5; \(Q\) (ml min\(^{-1}\) kg\(^{-1}\)) = 10.69±2.18; \(V_s\) (ml kg\(^{-1}\)) = 0.17±0.02; and \(W_s\) (mJ g\(^{-1}\)) = 0.57±0.07. Data are expressed as mean ± s.e.m., \(N=66\).

**Effects of \(\beta_3\)-adrenergic stimulation on basal cardiac performance**

Concentration–response curves of selective \(\beta_3\)-AR agonist were generated by exposing the cardiac preparations to increasing concentrations of BRL37344, since exposure to single repeated doses of BRL37344 revealed absence of receptor desensitization (data not shown). The effects of the agonist remained stable for 15 min then gradually decreased with time. Accordingly, cardiac parameters were measured after 10 min. BRL37344 (0.1–100 nmol l\(^{-1}\)) induced a concentration-dependent negative inotropic effect, revealed by a significant reduction of both \(V_s\), starting from the concentration of 1 nmol l\(^{-1}\), and \(W_s\), above 10 nmol l\(^{-1}\) (Fig. 1).

![Fig. 1. Cumulative dose–response curve for BRL37344 on stroke volume (\(V_s\)) and stroke work (\(W_s\)) in isolated and perfused paced eel hearts. Percentage changes were evaluated as means ± s.e.m. (\(N=9\)). Significance of differences from control values (one-way ANOVA test) **\(P<0.01\).](image-url)
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SR₅₉₂₃₀ against the β₃-AR BRL₃₇₃₄₄-dependent stimulation, heart preparations were perfused with Ringer’s solution containing increasing concentrations of either BRL₃₇₃₄₄ (0.1–100 nmol l⁻¹) alone or with the addition of a single concentration of SR₅₉₂₃₀ (10 nmol l⁻¹). The computerized fitting of the curves provided the percentage of variations of Vs obtained in the presence of BRL₃₇₃₄₄ alone and BRL₃₇₃₄₄ plus SR₅₉₂₃₀. The resulting sigmoid concentration–response curves and EC₅₀ values (Fig. 3) indicate a competitive antagonism of SR₅₉₂₃₀ on the β₃-AR-mediated negative inotropism.

Effects of ISO after treatment with SR₅₉₂₃₀

Isoproterenol (ISO), a non-specific β-AR agonist, generally produces positive inotropic and chronotropic effects in the heart (Brodde, 1991). In our study, in addition to the classic positive inotropism (data not shown), ISO (100 nmol l⁻¹) stimulation, in 30% of preparations, induced a negative inotropic effect that was abolished by pre-treatment with SR₅₉₂₃₀ (Fig. 4).

G protein interaction

β₃-AR belongs to the guanine nucleotide-binding protein (G protein)-coupled receptor super family characterized by seven transmembrane segments (Skeberdis, 2004). To verify the involvement of G proteins in the β₃-AR-induced inotropic action, cardiac preparations were perfused with Ringer’s solution containing PTx (0.01 nmol l⁻¹) in presence of BRL₃₇₃₄₄. In the rat heart, PTx catalyzes the ADP-ribosylation of the alpha-subunit of Gᵢ₀ and uncouples the interaction between Gᵢ and several inhibitory receptors, including muscarinic receptors [Angelone et al. (Angelone et al., 2006) and references therein]. Whereas PTx alone did not modify basal cardiac performance (data not shown), its pre-treatment abolished the BRL₃₇₃₄₄-dependent negative inotropic effect (i.e. Vs and Ws; Fig. 5), suggesting the involvement of the Gᵢ₀ protein system.

Fig. 2. Effects of BRL₃₇₃₄₄ (10 nmol l⁻¹) before and after treatment with nadolol (10 μmol l⁻¹) on stroke volume (Vₛ) and stroke work (Wₛ) in isolated and perfused paced eel hearts. Percentage changes were evaluated as means ± s.e.m. (N=6). One-way ANOVA was used for comparisons within groups; significance of differences from control values *P<0.05. Comparison between groups (two-way ANOVA, Duncan’s test); §P<0.05.

Effects of BRL₃₇₃₄₄ after treatment with nadolol

To explore putative interactions between β₁/β₂- and β₃-adrenergic signalling, hearts were perfused with the β₁-AR antagonist BRL₃₇₃₄₄ (10 nmol l⁻¹) in presence of a specific β₁/β₂-ARs inhibitor, nadolol (10 μmol l⁻¹). Two-way ANOVA test showed no significant differences between the untreated (with antagonist) and antagonist-treated preparations suggesting that the negative inotropic effect induced by BRL₃₇₃₄₄ is not influenced by β₁/β₂-AR inhibition (Fig. 2).

Fig. 3. The sigmoid concentration–response curves of BRL₃₇₃₄₄-mediated inhibition on Vₛ of BRL₃₇₃₄₄ alone (0.1–100 nmol l⁻¹) and of BRL₃₇₃₄₄ plus a single concentration of SR₅₉₂₃₀ (10 nmol l⁻¹) on the isolated and perfused working eel heart preparation. Inhibition of contractility is expressed as a percentage of Vₛ [baseline=0%, peak inhibition by BRL₃₇₃₄₄ and BRL₃₇₃₄₄ plus SR₅₉₂₃₀=−100%]. The EC₅₀ values (in log mol l⁻¹) of BRL₃₇₃₄₄ alone was −7.68±0.12 (r²=0.98), of BRL₃₇₃₄₄ plus SR₅₉₂₃₀ (10 nmol l⁻¹) was −6.62±0.55 (r²=0.96). Comparison between groups (two-way ANOVA, Duncan’s test); §P<0.05 (N=8).

Effects of BRL₃₇₃₄₄ after treatment with SR₅₉₂₃₀, β₃ specific antagonist

To obtain information on the antagonistic behaviour of

Fig. 4. Effects of isoproterenol (ISO; 100 nmol l⁻¹) before and after treatment with SR₅₉₂₃₀ (10 nmol l⁻¹) on stroke volume (Vₛ) and stroke work (Wₛ) in isolated and perfused paced eel hearts. Percentage changes were evaluated as means ± s.e.m. (N=7). One-way ANOVA was used for comparisons within groups; significance of differences from control values *P<0.05. Comparison between groups (two-way ANOVA, Duncan’s test) §P<0.05.
Involvement of NO-cGMP-PKG signal transduction pathway

NO, via activation of GC, is an important modulator of cardiac performance in the in vitro working eel heart (Imbrogno et al., 2001; Imbrogno et al., 2003; Imbrogno et al., 2004).

The involvement of the NO-cGMP-PKG signalling in the BRL37344-dependent inotropism, was examined by perfusing cardiac preparations with either Hb (1 μmol l⁻¹), or L-NIO (10 μmol l⁻¹), or ODQ (10 μmol l⁻¹). All these treatments abolished the effect of the β3-AR agonist demonstrating its dependence on a NO-cGMP mechanism (Fig. 6). Since cGMP modulates cardiac contractility via activation of a cGMP-PKG pathway (Hove-Madsen et al., 1996), and this is known to be the case also in the eel heart (Imbrogno et al., 2003), we pre-treated the heart with a specific PKG inhibitor (KT5823, 100 nmol l⁻¹). This abrogates the BRL37344-dependent reduction of $V_s$ and $W_s$ (Fig. 6), indicating the involvement of PKG in the β3-AR-induced inotropic response.

Discussion

This study provides physio-pharmacological evidence for the existence of functional β3-like adrenoceptors in the heart of A. anguilla. On the isolated working eel heart, we show that activation of β3-AR induces a clear negative inotropic action. This negative inotropic effect is coupled with PTx-sensitive inhibitory Gi proteins and involves an NO-cGMP-PKG signal transduction pathway.

Effects of β3-AR stimulation

In the eel, β3-AR stimulation negatively affects cardiac performance. This conclusion is based on several lines of evidences. Treatment with BRL37344, a preferential β3-AR agonist (Arch and Wilson, 1996; Balligand, 2000), induces a dose-dependent negative inotropic effect. As revealed by the analysis of the percentage of $W_s$ variations in terms of EC₅₀ values, this negative inotropism was blocked by the β3-AR specific antagonist SR59230 (Trochu et al., 1999), which in the eel heart acts in a competitive manner. Inversely, it was not modified by nadolol, a β1- and β2-ARs inhibitor, free of β3-AR antagonist properties (Emorine et al., 1989; Galitzky et al., 1993), ruling out the involvement of β1- and β2-ARs. Moreover, according to the results obtained in dog (Pelat et al., 2003) and in human ventricular biopsy (Gauthier et al., 1996), in the eel heart, in addition to the classic ISO-dependent positive inotropic effect (Tota et al., 2004), non-specific β-AR stimulation induced, in about 30% of preparations, a negative inotropism which was abrogated by SR59230 pre-treatment. No data are currently available regarding the factors that can influence the sensitivity of the eel heart to isoproterenol stimulation (i.e. specific agonist concentration, seasonal factors, etc). However, our observations further support the pharmacological evidence of the β3-AR functional expression in the eel heart.

In mammals, the cardiac response to β3-AR stimulation depends not only by species-related differences, but also by the organizational level under study (i.e. in vivo cardiovascular system vs isolated and denervated working heart) and the functional interactions among its components (Gauthier et al., 1996 and references therein). For example, beyond the negative inotropism observed in human (Gauthier et al., 1996), dog (Gauthier et al., 1999) and guinea pig (Kitamura et al., 2000) cardiac preparations, positive inotropic and chronotropic effects have been reported in in vivo experiments [see Gauthier et al. (Gauthier et al., 1996) for...
references]. However, these in vivo effects were not related to a direct stimulation of cardiac β3-AR but to reflex mechanisms (Tavernier et al., 1992; Wheeldon et al., 1993; Wheeldon et al., 1994; Shen et al., 1996). Thus, the eel heart preparation used in this study being free of extrinsic nervous and humoral influences, it is a very appropriate model to analyse the cardiac effects of β3-AR. Our data clearly indicate that, as reported in human (Gauthier et al., 1996), dog (Gauthier et al., 1999) and guinea pig (Kitamura et al., 2000), also in the eel β3-AR stimulation depresses cardiac contraction. This effect has been observed beginning from a BRL37344 concentration of 0.1 nmol l⁻¹, obtaining a reduction of 27±3.6% for Vₛ and of 27.9±3% for Wₛ at the higher concentration tested (100 nmol l⁻¹). Notably, these concentrations are similar to those observed in mammals, in which the maximum effect was obtained by BRL37344 (1 μmol l⁻¹) in human myocardium, CL-316243 (1 μmol l⁻¹) in guinea-pig, CGP12177 (0.1 μmol l⁻¹) in dog and BRL37344 (0.1 μmol l⁻¹) in rat (Gauthier et al., 1999). Moreover, in light of the classification proposed by Gauthier et al. (Gauthier et al., 1999) for mammalian hearts as hyper-responders (human and dog), hypo-responders (rat and guinea pig) and non-responders (ferret) to β3-AR agonists stimulation, the elevated sensitivity of the eel heart to BRL37344, shown by Vₛ reduction of about 30%, allows it to be classified within the hyper-responders group. In mammals, the interspecies variability and the heterogeneous pharmacological profile of β3-AR agonists were correlated to cardiac β3-AR expression. For example, it has been reported that the negative inotropic effect induced by β3-AR agonists in human and dog is associated with the presence of β3-AR transcripts (Gauthier et al., 1999). By contrast, no β3-AR mRNA was detected in the hypo-respondent rat ventricular myocardium (Gauthier et al., 1999) and rat right ventricle (Evans et al., 1996). Interestingly, Nickerson et al. (Nickerson et al., 2003) have recently cloned and characterized in the rainbow trout, Oncorhynchus mykiss, two previously unreported β-ARs (i.e. β3a-AR and β3b-AR), homologous to the mammalian β3-AR. Analysis of tissue expression patterns indicated elevated levels of β3a-AR mRNA in both gills and heart and lower levels in red muscle (Nickerson et al., 2003). However, the receptor expression level may not be the unique factor governing the cardiac response to β3-AR stimulation. In fact, important interspecies differences in the amino acid sequences of β3-ARs have been reported in mammals (Strosberg and Pietri-Rouxel, 1996; Gros et al., 1998). These differences concern transmembrane regions that are considered crucial for ligand binding and G protein interaction (Strosberg and Pietri-Rouxel, 1996). The presence of β3-AR transcripts in the heart of O. mykiss, together with the high functional sensitivity to BRL37344, that we have documented in A. anguilla, emphasize the importance of a putative cardiac β3-AR control in teleosts.

Transduction mechanism

G-proteins interaction

The mechanisms by which β3-AR activation induces cardio-depressing effects are largely unknown. We show here that in the eel heart, the negative inotropism induced by BRL37344, is abolished by the pre-treatment with PTx, the toxin which uncouples signal transduction between several families of receptors and Gₓₒ proteins [Ai et al. (Ai et al., 1998) and references therein]. Accordingly, there is indication that the β3-AR-dependent negative inotropy involves PTx-sensitive G proteins. Our data agree with those obtained in human ventricular biopsies, in which PTx abolished the effect of β3-AR stimulation on cardiac contraction (Gauthier et al., 1996; Gauthier et al., 1998). In the heart, PTx-sensitive G proteins, located at the interface between receptor-response coupling, are involved in various inhibitory transduction cascades triggered by both chemical and physical stimuli (Hare et al., 1998). In the rat heart, β3-ARs, acting through a G protein, mediate the inhibitory effect of BRL on L-type Ca²⁺ current (Zhang et al., 2005). Moreover, in human heart the PTx-sensitive-β3-AR-induced negative inotropic effect was associated with decreased action potential amplitude and reduced action potential duration (Gauthier et al., 1996). Whether in the eel heart the BRL-induced cardio-suppressive effect involves these or other mechanisms remains to be elucidated.

NO-cGMP-PKG signal transduction pathway

In human ventricle, the β3-AR-induced negative inotropic action results from the production of NO by the endothelial isoform of NO synthase (eNOS) and the consequent increase in intracellular cGMP (Gauthier et al., 1998; Varghese et al., 2000). The role of NO in mediating inotropic effects induced by cardioactive agents has been extensively demonstrated in the eel by our lab. For example, direct stimulation of M₂ muscarinic acetylcholine receptors (Imbrogno et al., 2001) and AT₁ angiotensin II receptors (Imbrogno et al., 2003), or exposure to vasostatin I (Imbrogno et al., 2004) decreased contractility through NOS stimulation and a consequent increased cGMP production. Consistent with the expression of NOS in the eel heart (Tota et al., 2005), we have found that the β3-AR-induced inotropism is mediated by the NO-cGMP-PKG pathway. This was demonstrated by its abrogation following pre-treatment with either the NO scavenger (Hb), or NOS (L-NIO), or soluble GC (ODQ), or PKG inhibitors (KT5823). In the eel heart, an important intramyocardial target of NO signalling is PKG (Imbrogno et al., 2003; Imbrogno et al., 2004). In isolated mammalian ventricular cardiomyocytes (Méry et al., 1991), PKG affects Ca²⁺ influx and, through phosphorylation of troponin I, reduces the affinity of troponin C for calcium, thereby negatively regulating cardiac contractility (Hove-Madsen et al., 1996).

In conclusion, the present study, for the first time, extends to a working fish heart the negative inotropic action induced by β3-AR stimulation previously reported in mammals. This effect occurs via a Gₓₒ-NO-cGMP-PKG signal transduction pathway. The functional evidence for the presence of β3-AR in the heart of an ectotherm vertebrate suggests its ancient evolutionary origin and strongly supports important functions independent from thermogenic response. In view of the postulated role of β3-AR as a homeostatic regulator of the
cardiovascular system, possibly counteracting the excitatory adrenergic influences (Sterin-Borda et al., 2006), it is of interest that in the teleost heart, activation of β3-ARs induces cardio-suppressive actions. Indeed, the heart of many teleosts, including the eel, are exposed to stimulatory effects of circulating and intracardiac catecholamines, particularly under stress conditions [Imbrogno et al. (Imbrogno et al., 2003) for references], which may become harmful in the absence of local counter-regulatory mechanisms. The possibility that in fish β3-AR could exert cardio-inhibitory protection versus systemic and/or intracardiac cascades of excitatory stimuli targeting the heart is a challenge for future studies.

List of abbreviations

AR adrenoceptor
Q cardiac output
GC guanylate cyclase
Hb haemoglobin
fH heart rate
ISO isoproteonel
L-NIO L-N(1-imino-ethyl)ornithine
NO nitric oxide
NOS nitric oxide synthase
ODQ 1H-(1,2,4) oxadiazolo-(4,3-a)quinoxalin-1-one
PKG cGMP-activated protein kinase
PTx pertussis toxin
Vs stroke volume
W stroke work

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References


