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Differential regulation of the β-adrenoceptor density and cyclic AMP level with age and sex in turkey cardiac chambers

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ABSTRACT

Decreased responses of the heart to β-adrenoceptor stimulation with aging have been shown to occur merely in selected heart chambers in relation to increased catecholamine levels. However, there are no systematic studies that investigate all cardiac chambers with regard to receptor density and cAMP (adenosine 3′, 5′-cyclic monophosphate) responses. We used meat-type turkey poults (British United Turkey (B.U.T.) Big 6) with increasing age because their heart seems to decrease in weight in relation to body weight and they are often used as an animal model for heart failure. The receptor density and distribution were quantified by radioligand binding analysis using (-)-[^{125}I]-iodocyanopindolol and β-adrenoceptor subtype-specific antagonists (ICI 118.551 and CGP 20712 A) in membranes of four cardiac chambers (right and left atria and ventricles) of 6-week-, 12-week-, 16/21-week-, and 57-week-old B.U.T. BIG 6 turkeys. Receptor function was determined by measuring basal and stimulated cAMP production. In both sexes, the β-adrenoceptor density decreased significantly in all chambers with age without altered β-adrenoceptor subtype distribution. The receptor affinity (K_D) to the radioligand was similar in hearts of all age groups. β-adrenoceptor-(isoproterenol and guanosine 5′-triphosphate), G-protein-(NaF) and catalytic unit of adenylate cyclase (forskolin, Mn^{2+}) mediated cAMP responses were not chamber-dependent. Indeed, the cAMP level was significantly lower in 57-week-old hearts than in 6-week-, 12-week-, 16/21-week-old hearts. These data suggest that with increasing age and body weight, the β-adrenoceptor signal transduction pathway was highly blunted in all cardiac chambers, occurring by decreased receptor density and cAMP responses.

1. Introduction

In the elderly, there is an increased incidence of impaired neuroendocrine function in the cardiovascular system along with decreased cardiac inotropy and chronotropy, end-systolic and end-diastolic volume indexes and ejection fraction (Ferrara et al., 2014; Strait and Lakatta, 2012). β-adrenoceptor activation plays an important role in the facilitation of cardiovascular performance in several mammalian species including man and also avian species (Brodde et al., 2001; Klausner and Schwartz, 1985; Nilsson, 2011). With aging, it has been described that the heart muscle becomes hypertrophied and the β-adrenoceptor-mediated responses to catecholamines are significantly reduced in the heart from humans and rodent models (Fleg and Strait, 2012; White et al., 1994; Xiao et al., 1994). There are no studies available so far which show systematically the relationship between receptor density and adenylate cyclase activity in all four cardiac chambers.

Age-related alterations in myocardial responsiveness are strongly argued to occur as function of changes in β-adrenoceptor characteristics or events distal to the receptor related to the signal transduction pathways. Indeed, there have been conflicting reports about the effect of age on β-adrenoceptors: some studies have found decreased (Chevalier et al., 1991; Schocken and Roth, 1977; Sozmen et al., 2011), some unchanged (Abrass and Scarpace, 1981; Roth et al., 1998) or even increased (Schumacher et al., 1984) β-adrenoceptor density. Binding of an agonist to β-adrenoceptors leads to binding of guanosine 5′-triphosphate (GTP) to the stimulatory G-protein (G_s) which activates the catalytic component of the adenylate cyclase to enhance the synthesis of adenosine 3′, 5′-cyclic monophosphate (cAMP) (Gilman, 1995). In aging, the

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abolished myocardial β-adrenoceptor signal transduction pathways are presumably accompanied, as shown in aged rats, by a decrease in coupling efficiency of the receptor to G-proteins (Scarpace, 1986). In addition, in Sprague Dawley rats, the receptor- and forskolin-mediated activation of the adenylate cyclase and the number of this effector protein decreased in ventricular membranes with age (Robberecht et al., 1986; Shu and Scarpace, 1994). In failing human hearts, it was suggested that persistent sympathetic activation produces down-regulation of the main cardiac β-adrenoceptor subtype, i.e., β_1-adrenoceptor, and decreases agonist binding to cardiac β_1-adrenoceptors (Bristow et al., 1982), but these data were not age-related.

Since coherent investigations that show the relationship between β-adrenoceptor density and adenylate cyclase activity of all four cardiac chambers (right and left ventricle and atria) with age and sex as well as fast increasing body weight are lacking, we evaluated 1) the relationship between body weight and heart weight, 2) the β-adrenoceptor density in crude membranes of left and right atria and ventricles and 3) isoproterenol (Iso)-, GTP-, forskolin-, sodium fluoride (NaF)- and manganese (Mn^{2+})-mediated activation of adenylate cyclase by measuring cAMP production in four heart chambers. We used meat-type B.U.T. (British United Turkey) Big 6 turkeys during the fast growing period between 6 and 21 weeks. A recent study from this laboratory has shown that all turkey cardiac chambers express exclusively the β_1-receptor subtype (Hoffmann et al., 2015).

2. Material and methods

2.1. Animals

All turkeys (origin B.U.T. BIG 6) were obtained from the same commercial local turkey farm and were kept and fed under conventional husbandry conditions for meat-type turkeys. All animal handling and experimental procedures were conducted following approval from the local committee for animal studies according to the German animal welfare act (Reg.-Nr. 15-104/13). For the purpose of the present study, male and female conventional meat-type turkeys in four age groups were used: 6-week- (beginning of fattening period), 12-week- (middle of fattening period) and 16-week- for female/21-week-old for male turkeys (end of fattening period) were used. Turkeys of both sexes aged 57 weeks represented the oldest group we could obtain from the slaughter house. Depending on the animal age, 5–30 birds per age- and sex-group were employed. Atrial tissues of 6- and 12-week-old birds needed to be pooled in order to obtain enough tissue material. At 6 and 12 weeks of age male and female poults were randomly picked out of the barn, anesthetized with ketamine and diazepam and thereafter euthanized with 7.45% potassium chloride. Shortly after killing the animals, hearts were quickly removed. Hearts of the age groups of 16 (female), 20 (male) and 57 weeks (male and female) were directly collected from the slaughter house. All hearts were excised into right and left atria as well as ventricles. After determining the wet weight of the whole heart and each chamber, samples were frozen and stored at −80 °C until use.

2.2. Preparation of heart crude membranes

Frozen atrial and ventricular (left, right) tissues (400 mg) were thawed in 10 ml of the lysis buffer containing 1 mM $KHCO_3$. All procedures were carried out at 4 °C. The tissues were minced with scissors and homogenized twice for 30 s at 20,000 g using an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen, Germany). The homogenates were centrifuged at 500 g for 15 min and the supernatant was filtered over four layers of gauze and centrifuged at 20,000 g for 30 min. After discarding the supernatant, pellets were resuspended in 10 ml of the incubation buffer containing 10 mM Tris-Base (pH 7.4), 154 mM NaCl and 0.55 mM ascorbic acid and centrifuged again at 20,000 g for 30 min. Finally, the pellets were resuspended in the incubation buffer to a final protein concentration of 4–6 mg/ml determined by the method of Lowry et al. (1951). Membrane preparations were stored at −80 °C.

2.3. Receptor binding study

Assays of total β-adrenoceptor binding were carried out in duplicates on washed crude membranes of all heart chambers of turkeys as recently described (Hoffmann et al., 2015). In brief, (-)-[^{125}I]-iodocyanopindolol (ICYP), a β-adrenoceptor antagonist, was used at 6 increasing concentrations to determine the receptor affinity and total number of β-adrenoceptors. The total assay volume amounted to 250 μl in which ICYP at concentrations ranging from 5 to 140 pM, 1 μM (±)-CGP-12177 and 150 μl tissue membranes (20 μg protein) were added as final concentrations. Samples were incubated at 37 °C for 90 min. The reaction was terminated by adding 10 ml of ice-cold washing buffer (10 mM Tris-Base, 154 mM NaCl, pH 7.4) and rapid vacuum filtering through glass fiber Whatman GF/C filters on a Brandl Cell Harvester (Brandl Biomedical Research and Development, Gaithersburg, MD, USA) followed by one additional wash using 10 ml of the same buffer. The filters were counted in a Wallace WIZARD 1470 Gamma Counter (Perkin-Elmer Life Sciences) at counting efficiency of 80%. Specific binding of ICYP was defined as the difference between total ICYP binding in absence and presence of 1 μM (±)-CGP-12177. The density of binding sites (B_{max}) and the dissociation constant (K_D) were determined by linear regression according to Scatchard (1949) and nonlinear analysis of the saturation binding data. For this purpose, the computer program GraphPad Prism (San Diego, USA) was used. A correlation coefficient >0.9 was considered acceptable.

In addition, competition binding assays were carried out to determine the β-adrenoceptor subtype distribution with aging in cardiac chambers of female and male turkey poults. With this regard, the selective β_1-adrenoceptor antagonist CGP 20712A and the selective β_2-adrenoceptor antagonist ICI 118.551 were used concentration-dependently to displace ICYP from specific binding sites.

2.4. Measurement of cyclic AMP production

Cyclic AMP was assayed in triplicates in 40 μg of cardiac membrane protein indirectly by the conversion of ATP (adenosine 5′-triphosphate) into cAMP in the presence of an ATP-regenerating system as described previously (Abraham et al., 2003). In brief, cAMP formation was determined under several experimental conditions: first, basal cAMP was determined only by incubating crude membranes with the reaction buffer (40 mM HEPES, 5 mM $MgCl_2$; 1 mM EDTA-Na_4, 0.5 mM ATP, 0.1 mM cAMP, 5 mM phosphocreatine, 50 U/ml creatine phosphokinase, 20 U adenosine deaminase, pH 7.4). Second, in addition to the reaction buffer cAMP formation was stimulated by either 10 μM (-)-isoproterenol + 10 μM GTP or 10 μM forskolin or 10 mM sodium fluoride (NaF) (adenylate cyclase stimulating agents). Third, membranes were incubated in the presence of 10 mM $MnCl_2$ without $MgCl_2$. Additionally, dose-response curves with forskolin were generated by adding concentrations ranging between 0.01 and 100 μM. After pre-incubation of samples for 10 min at 30 °C in a shaking water bath, the reaction was initiated by adding 0.5 mM [α-^{32}P]-ATP (about 200,000 cpm/tube) and samples were further incubated for another 10 min under same conditions. Reaction was stopped by adding 100 μl stopping solution (containing 70 mM

sodium dodecyl sulfate (SDS), 40 mM ATP, 1.4 mM cAMP, 50 mM Tris–HCl, 8500 cpm cyclic [^{3}H]-AMP, pH 7.4) and 800 μl distilled water. Cyclic [^{32}P]-AMP was separated from [α-^{32}P]-ATP by column chromatography combining Dowex® 50 WX 8 (anion-exchange) and neutral alumina columns and elution with 5 ml 0.1 m imidazoline. Radioactivity was measured in Rotiszint® eco plus scintillation fluid by liquid scintillation counting with 60% counting efficiency (Tri-Carb 2810TR LSA, Perkin Elmer). Less than 5% of the added [α-^{32}P]-ATP was converted to cyclic [^{32}P]-AMP in all experiments.

2.5. Statistical analysis

To assess the effect of age on β-adrenoceptor properties and basal and stimulated cAMP level in cardiac chambers of turkey poults, a one-factor analysis of variance (ANOVA) with post-hoc test for linear trend was performed to determine chamber specific differences in B_{max}, K_D and cAMP level between sex-matched age groups. Significance was accepted at a level of P-value smaller than 0.05. All binding data were analyzed using an iterative, non-linear curve fitting computer program GraphPad Prism (GraphPad Software, San Diego, CA, USA). The receptor density of ICYP binding (B_{max}) and the equilibrium dissociation constant (K_D) were calculated from individual saturation curves and also by Scatchard analysis (Scatchard, 1949). Dose-response curves, EC_{50} values and all statistical calculations were also assessed by GraphPad Prism. *F*-test was used comparing EC_{50} values among different age groups. Experimental data in text and figures are given as mean ± standard error of the means (S.E.M.) of the *n* number of experiments.

3. Results

The body weight and the heart weight of young turkey poults of both sexes were considerably less than those of older turkeys, as assumed (Table 1). Sex had measurable effect on body and heart weight, i.e., male birds gained more weight than female birds (Table 1). On the other hand, the heart to body weight ratio decreased with age in both sexes to a similar extent (k-value of exponential decay female: 0.17 vs. male: 0.18). Similarly, the ratios of left ventricular weight to body weight and left ventricular weight to right ventricular weight were lesser in older than in younger turkeys.

To investigate whether changes in β-adrenoceptor density and affinity occur in heart chambers (left and right atria and ventricles) of meat-type turkey poults with aging, radioligand binding studies were performed in heart chamber membranes of 6–57 weeks old turkeys of both sexes during the fattening period. There was a significant difference and decrease in β-adrenoceptor number with age in all four cardiac chambers of both male and female turkey poults (Fig. 1A-D). The data of total receptor density (B_{max}) and affinity (K_D) are summarized in Table 2 (A and B). From saturation ICYP binding studies, a progressive decrease (10–30%) in B_{max} for 12-week-old turkey hearts (~50 fmol/mg protein for male and female birds) compared with 6-week-old turkey hearts (~60 fmol/mg protein for male and female birds) could be identified (Table 2A). In 57-week-old turkey hearts, B_{max} was up to 68% less than that of 6-week-old turkey hearts. Indeed, with regard to the receptor density within one age group, we could not observe any difference between cardiac chamber membranes. Even if ranges of the receptor density were wider in female turkey poults than in male turkey poults, there was no correlation between receptor density and sex (Fig. 1A-D). The receptor affinity to the ligand (K_D) (~30 pM 6 weeks vs. ~35 pM 57 weeks old) was unaffected by cardiac chamber, sex and age of the animals (Table 2B). Competition binding studies using the β_1-adrenoceptor selective antagonist CGP 20712A and β_2-selective antagonist ICI 118.551 detected similar population of the β_1-subtype in all cardiac chambers of all age groups and sexes without affecting the subtype distribution (data not shown), indicating that the age-related decline in total β-adrenoceptor density in cardiac chamber membranes is due to the loss of the mere β_1-adrenoceptor subtype.

To assess the effects of age on β-adrenoceptor- and G-protein-mediated level of cAMP, crude membranes of four cardiac chambers were incubated with substances that stimulate β-adrenoceptors (isoproterenol), G-proteins (GTP, NaF) and the catalytic subunit of the adenylate cyclase (forskolin, Mn^{2+}). In both male and female turkey poults and in all age groups, the basal cAMP level remained similar in all cardiac chamber membranes (~16 pmol/mg protein/min 6-week-old turkey hearts vs. ~14 pmol/mg protein/min 57-week-old turkey hearts) (Figs. 2A-D and 3A-D). In all cardiac chambers and for all substances tested, stimulation of cAMP was significantly decreased in hearts from older turkey compared with hearts from younger turkeys but with differing responses depending on the cAMP activating agents; for example, isoproterenol/GTP-, Mn^{2+}-, NaF- and forskolin-stimulated cAMP level was reduced with age to percentage values ranging 14–57%, 34–55%, 51–78% and 73–94%, respectively. These differences reached statistical significance for almost all chambers (Figs. 2A-D and 3A-D). However, no significant differences were observed in cAMP accumulation between chambers (e.g. right and left ventricles as well as right and left atria) of one age group and in relation to sex. The isoproterenol-stimulated cAMP production was higher in cardiac membranes of all age groups than the basal cAMP level; indeed, it did not reach statistical significance. The direct β-adrenoceptor activation by the nonselective β-receptor agonist isoproterenol combined with GTP and the direct adenylate cyclase activation by Mn^{2+} resulted in a similar age-dependent pattern of adenylate cyclase activation but the net effect was less than NaF and forskolin (Figs. 2A-D and 3A-D). Accordingly, cAMP formation was activated at highest with forskolin followed by NaF and isoproterenol + GTP. Mn^{2+}-stimulated cAMP level was similar

Table 1
Allometric parameters for male and female B.U.T. Big 6 turkey poults.

	6 weeks		12 weeks		20/16 weeks		57 weeks	
	Male	Female	Male	Female	Male	Female	Male	Female
Body weight (kg)	3.12 ± 0.06	2.64 ± 0.09	8.17 ± 0.24	7.08 ± 0.14	20.43[a]	9.59[a]	31.20[a]	12.73[a]
Heart weight (g)	16.30 ± 0.37	13.21 ± 0.29	35.76 ± 1.00	30.39 ± 0.66	66.12 ± 1.42	33.64 ± 1.04	117.82 ± 11.56	45.71 ± 5.52
Heart weight/Body weight (%)	0.52	0.50	0.44	0.43	0.32	0.35	0.38	0.36
Left ventricular weight/Body weight (%)	0.38	0.37	0.31	0.31	0.23	0.26	0.25	0.25
Left ventricular weight/Right ventricular weight	4.61	4.33	4.61	4.91	3.75	3.80	3.38	3.99

Data are given as means ± S.E.M. of n = 10 per age group and sex.

[a] Body weights of 16, 20 and 57 week old poults are presented as obtained from abattoir; therefore, SE could not be calculated.

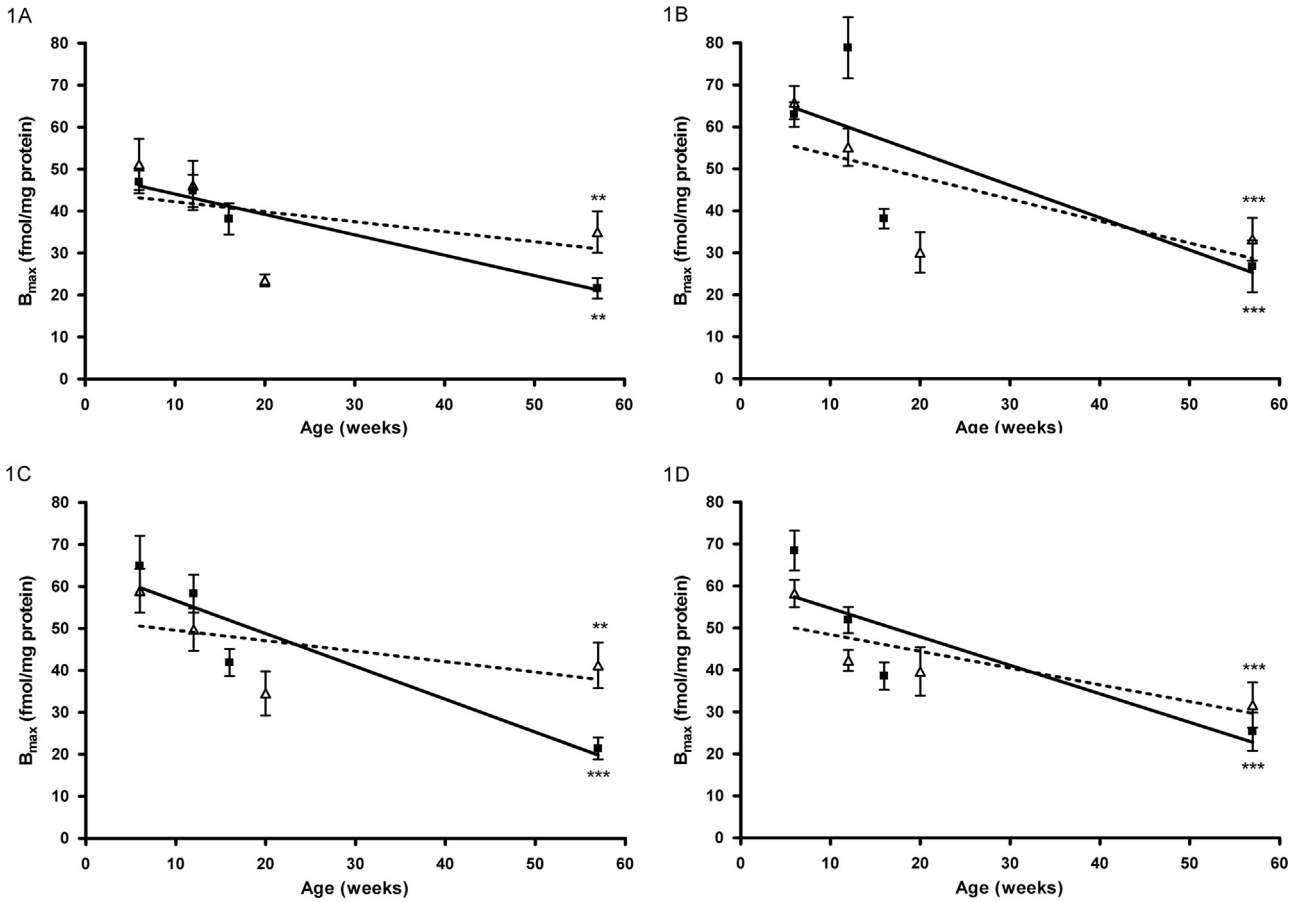

Fig. 1. Correlation between age and β-adrenoceptor density in right atrium (A), left atrium (B), right ventricle (C) and left ventricle (D) of male (△) and female (■) turkey poults. Data are given as means ± S.E.M. of n=5 experiments (minimum of 5 different animals per age group and sex). Statistical significant decrease of β-adrenoceptor density with age was obtained within each cardiac chamber for male and female poults $^{**}P < 0.01$; $^{***}P < 0.001$ (One-way ANOVA with *post hoc* for linear trend).

Table 2
β-Adrenoceptor density (2A) and affinity (2B) measured in cardiac chamber membranes of B.U.T. Big 6 turkeys with age.

2A	**Male**				**Female**			
Age	RA	LA	RV	LV	RA	LA	RV	LV
6 weeks	51.1 ± 6.1	65.7 ± 4.0	59.0 ± 5.2	58.2 ± 3.3	47.0 ± 2.7	62.9 ± 2.9	64.9 ± 7.1	68.4 ± 4.7
12 weeks	46.1 ± 5.9	55.1 ± 4.5	49.8 ± 5.1	42.2 ± 2.5	44.8 ± 3.8	78.8 ± 7.3	58.3 ± 4.5	51.9 ± 3.1
6/20 weeks	23.5 ± 1.4	30.1 ± 4.8	34.5 ± 5.2	39.6 ± 5.8	38.1 ± 3.7	38.1 ± 2.4	41.9 ± 3.2	38.5 ± 3.3
57 weeks	35.0 ± 4.9	33.2 ± 5.1	41.2 ± 5.4	31.6 ± 5.4	21.6 ± 2.4	26.7 ± 6.2	21.4 ± 2.6	25.3 ± 4.6
2B								
6 weeks	29.2 ± 1.0	35.4 ± 4.0	24.1 ± 1.1	31.7 ± 1.6	36.6 ± 2.6	35.0 ± 7.0	25.8 ± 3.0	22.9 ± 1.3
12 weeks	18.4 ± 2.0	23.6 ± 2.5	40.8 ± 11.1	26.2 ± 2.4	25.7 ± 1.6	43.4 ± 5.7	24.6 ± 1.7	37.4 ± 6.8
6/20 weeks	26.0 ± 8.0	38.6 ± 7.3	28.3 ± 4.2	66.3 ± 16.7	26.0 ± 4.6	26.8 ± 2.5	29.3 ± 4.1	32.8 ± 6.8
57 weeks	35.8 ± 10.1	35.5 ± 6.3	27.6 ± 3.3	24.0 ± 3.6	42.5 ± 10.5	49.1 ± 8.0	26.1 ± 4.1	38.2 ± 4.2

B_{max} is given as means ± S.E.M. of n=5 experiments (minimum of 5 different animals per age group, cardiac chamber and sex); RA=right atrium, LA=left atrium, RV=right ventricle, LV=left ventricle; K_D is given as means ± S.E.M. of n=5 experiments (minimum of 5 different animals per age group and sex).

with that of isoproterenol in the presence of GTP.

The extent of cAMP production with age was less when receptor and G-proteins were stimulated; and thus, we further sought to provide data of cAMP production by activating the catalytic units of the adenylate cyclase by forskolin. Generally, forskolin highly stimulated the cAMP level in all cardiac chamber membranes (Figs. 2A-D and 3A-D). All cardiac chambers of older turkeys exhibited significantly less cAMP accumulation with increasing age in response to forskolin (Figs. 2A-D and 3A-D). A similar pattern of activation of cAMP level was obtained when membranes were incubated with increasing concentrations of forskolin. Dose-response curves of cAMP activation by forskolin were assessed in left ventricular membranes of male (Fig. 4A) and female (Fig. 4B) turkey poults. The half-maximal stimulation (EC_{50}) was similar in all cardiac chambers of all ages, indicating the same adenylate cyclase sensitivity for forskolin activation. However, dose-response curves in older turkey hearts were shallower than in younger turkey hearts. This age-related decrease in maximum forskolin-stimulated cAMP production was greater in male turkey hearts (95% reduction 6 weeks vs. 57 weeks) compared to female

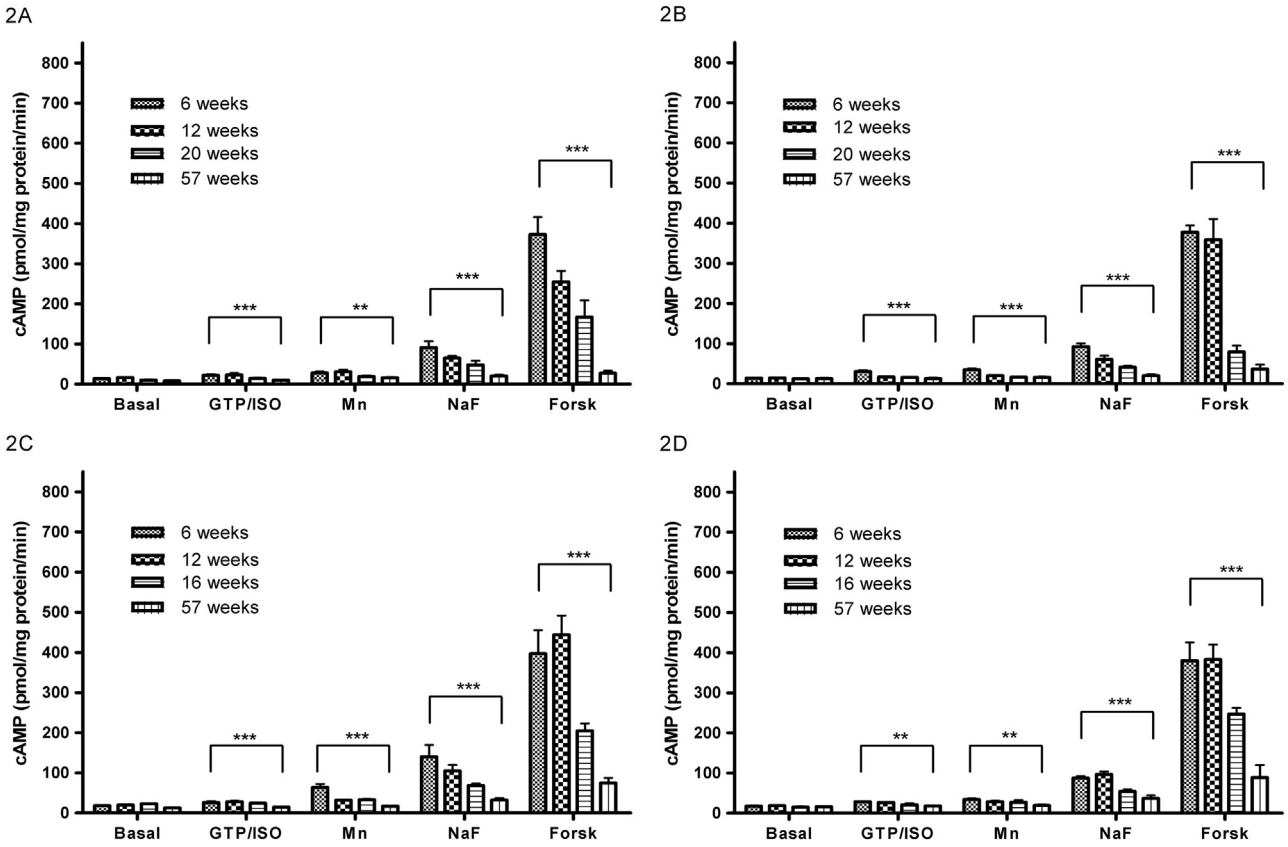

Fig. 2. Age-dependent decrease in cAMP level in membranes of right and left atria of male (A, B) and female (C, D) turkey poults. Data are given as means ± S.E.M. of n=5 experiments (minimum of 5 different animals per age group and sex). Statistical significant decrease of cAMP level with age $^{**}P < 0.01$; $^{***}P < 0.001$ (One-way ANOVA with *post hoc* for linear trend).

birds (83% reduction).

4. Discussion

Recently, we have shown that in cardiac chambers of the current animal model only the β_1-adrenoceptor subtype is expressed and uniformly distributed in all heart membranes (Hoffmann et al., 2015). In this study, we describe age- and sex-associated changes in β_1-adrenoceptor density and the cAMP production at post-receptor level in four cardiac chambers of the meat-type B.U.T. Big 6 turkeys during the fast growing period. The main findings were: first, with age there was gradual decline in β-adrenoceptor density in female and male turkey heart chambers without altered receptor affinity to the radioligand; second, this decrease was specific for the β_1-adrenoceptor subpopulation since displacement studies with subtype-selective antagonists delivered no shift in subtype distribution; and third, we observed a significant inverse correlation between age and cAMP level in response to all stimulating agents.

Using radioligand binding studies, the present study demonstrated an age-related successive decrease up to 70% in total β-adrenoceptor density in crude membranes of left and right ventricle as well as atria of female and male turkeys with no change in receptor affinity to the ligand. No prior systematic analysis of aging effects on cardiac chamber β-adrenoceptor density and affinity has been performed in this animal species. In cardiac tissues of different species, studies have found variable results, whereby several investigations demonstrated no age-related altered β-adrenoceptor density and affinity which contrast our data of the present study (Abrass and Scarpace, 1981; Bazan et al., 1994; Roth et al., 1998; Tumer et al., 1987). In agreement with our finding, studies have found an age-related decline in B_{max} in human (White et al., 1994), chicken (Lindgren and Altimiras, 2009), mice (Chen et al., 1979) and rat (Chevalier et al., 1991; Tobise et al., 1994; Whitsett and Darovec-Beckerman, 1981) myocardium as well as in rat cardiac myocytes (Xiao et al., 1998), also without altered receptor affinity. The receptor decline in all cardiac chambers in our study was prominently apparent, even in ages between 3 and 6 months of growing meat-type B.U.T. Big 6 turkeys and is surprisingly more dramatic (2–3-fold higher) than receptor alterations observed in older hearts of different mammalian species including man described above.

Presumably, this striking discrepancy with regard to the receptor density parallels the use of different animal species or the presence of underlying age-related stress. The latter factor applies worth for meat-type turkeys with fast-growing behavior. Rapid growth and consequently crowding might obviously cause stress which finally activates the sympathetic-adrenal medullary system and the hypothalamus-pituitary-adrenal cortex axis, resulting in β-adrenoceptor stimulation (Santos and Spadari-Bratfisch, 2006). Thus, with this regard, there is a plausible explanation for β-adrenoceptor down-regulation in turkey hearts with age. This appears to be associated with increased catecholamine concentration (norepinephrine and epinephrine) which are generally associated with receptor desensitization and eventual down-regulation (Harden, 1983; Sibley and Lefkowitz, 1985) when the receptor is exposed to them for longer time courses. Even if we did not measure the catecholamine levels in turkey plasma or myocardium, and this remains to be the limitation of the current study, numerous studies have explored that chronic stimulation of β-adrenoceptors by catecholamines attenuate the receptor responses

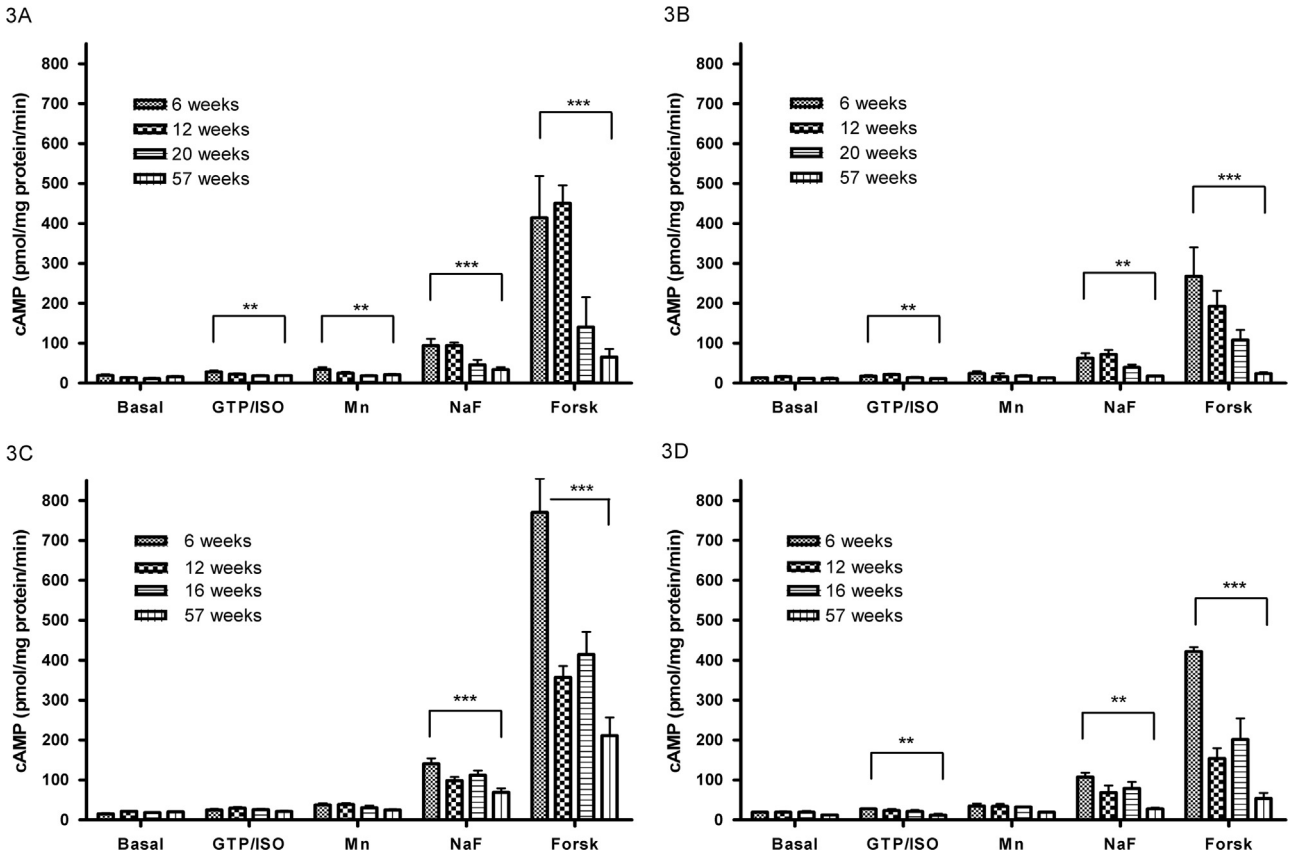

Fig. 3. Age-dependent decease in cAMP level in membranes of right and left ventricle of male (A, B) and female (C, D) turkey poults. Data are given as means ± S.E.M. of n = 5 experiments (5 different animals per age group and sex). Statistical significant decrease of cAMP level with age $^{**}P < 0.01$; $^{***}P < 0.001$ (One-way ANOVA with *post hoc* for linear trend).

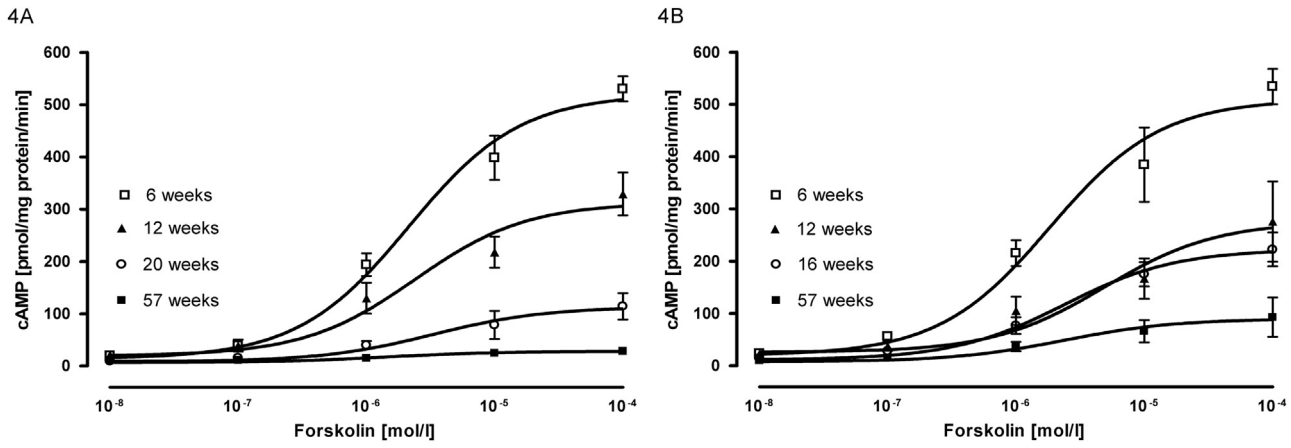

Fig. 4. Representative concentration-response curves of forskolin-stimulated cAMP level in left ventricular membranes of male (A) and female (B) turkey poults. Data are given as means ± S.E.M. of n = 3 experiments (3 different animals per age group).

(Sarsero and Molenaar, 1995). In humans, aging was associated with enhanced sympathetic activity with gradual increase in norepinephrine concentration (Seals and Esler, 2000; Ziegler et al., 1976); indeed, only less than a handful of studies have addressed β-adrenoceptor changes in normal myocardium (Davies et al., 1996; White et al., 1994).

Furthermore, parallel to decreased β-adrenoceptor density we observed for the first time that the cAMP accumulation in all cardiac chambers stimulated by β-adrenoceptor stimulating (isoproterenol), G-protein stimulating (GTP, NaF), or adenylate cyclase-activating (forskolin, Mn^{2+}) agents were markedly reduced with age, indicating a uniform decrease in cAMP level in the fast-growing turkey hearts of both sexes (Figs. 2A-D and 3A-D). In contrast to our findings, several studies found rather no changes in adenylate cyclase activity consequently in cAMP level (Hatjis, 1986; Whitsett and Darovec-Beckerman, 1981) but our data are in agreement with previous studies which showed that there is an age-related attenuation of the adenylate cyclase (O'Connor et al., 1981; Scarpace, 1990; Shu and Scarpace, 1994; Tobise et al., 1994). Hence, the cAMP levels in turkey hearts were reduced with age in the rank order of those stimulating agents: forskolin > NaF > isoproterenol + GTP = Mn^{2+}.

The findings with decreased isoproterenol-stimulated cAMP formation indicate the relationship between decreased cardiac β-

adrenoceptor density and receptor-stimulated decline in signal-transduction with age. Nevertheless, interestingly, the decrease in cAMP production was much larger than the decrease in maximal receptor density with age in B.U.T. BIG 6 turkeys of both sexes (left ventricle male: 90% vs. 45%), therefore, suggesting altered signaling transduction in addition to fewer receptors. The results with G-protein-mediated stimulation (NaF, GTP) of the cAMP formation show an abnormality of G_s protein in heart chambers with increasing age. This accounts for the uncoupling of the β_1-adrenoceptor from the adenylate cyclase and the aberrant age-related signaling pathways of the β-adrenoceptor system resulting in a reduction in cAMP accumulation in cardiac chamber tissues of fast-growing meat-type turkeys of the current study. Alterations in G_s proteins that lead to the loss or gain of functional activity are also documented in aged and diseased cardiac tissues, such as hypertension and heart failure in humans (Feldman et al., 1995; Scarpace, 1986; Siffert, 1996; White et al., 1994) and various animal models (Bazan et al., 1994; Roth et al., 1998). Despite the fact that we did not examine cardiac G-proteins directly, they might be possibly altered during fast growing period. Previous studies have shown that inhibitory G-protein (G_i) appears to be increased in the aging myocardium (Brodde et al., 1995; Kilts et al., 2002) and in some studies not to be changed (Shu and Scarpace, 1994; White et al., 1994). In turkey hearts, the greatest cAMP production was achieved when crude membranes were incubated with forskolin when compared to other agents, suggesting activation of the adenylate cyclase catalytic unit results in larger cAMP production than receptor stimulation and functional G_s coupling. This applies also for the forskolin-induced cAMP decrease with age. Our data suggest that the ability for post-receptor stimulation of cAMP production might be altered and plays an important role in the β_1-adrenoceptor signal transduction pathway in fast-growing turkey hearts with age.

It is now well established that activation of the β-adrenoceptors stimulates the adenylate cyclase through the participation of G-proteins and promotes consequently cAMP production in the myocardium (for references see Brodde et al., 2006 and the references therein). Basically, the increase in cAMP level triggers the signal transduction events that ultimately produce the positive inotropic effect in the heart. This β-adrenoceptor-mediated signaling mechanism is considered to provide critical support for the maintenance of cardiac function during development of the heart and heart diseases (Brodde et al., 2006; Ciccarelli et al., 2013). Sex differences in cardiac performance and cardiac remodeling have been reported in different types of heart diseases (Gardner et al., 2002; Mozaffarian et al., 2015). Turkeys should show high incidence of cardiovascular-associated diseases, particularly as reported for male turkey poults (Stenzel et al., 2008). However, we did not observe any sex-associated differences in status of the β-adrenoceptor system (neither in β-adrenoceptor density nor in cAMP level).

5. Conclusions

In summary, all turkey heart chambers exhibit an age- but no sex-associated decrease in atrial and ventricular β-adrenoceptor density related with the uncoupling of the adenylate cyclase resulting in decreased cAMP formation, without altered receptor affinity and receptor subtype distribution. In conclusion, increasing age with increasing body mass in relation to small hearts diminishes the receptor signal transduction pathway. This may consequently lead to heart failure and a high incidence of stress-associated death. Study limitations: in view of the role of catecholamines in regulating turkey heart β-adrenoceptor expression and function with age, future studies should be carried out.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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