Breed-related differences in age-dependent down-regulation of the β_1-adrenoceptor and adenylate cyclase activity in atrial and ventricular myocardium of Cröllwitzer ("wild-type") turkeys

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ABSTRACT In conventional meat-type (British United Turkey (B.U.T.) Big 6) turkey hearts, it has been shown that all cardiac chambers exhibit down-regulation of the β_1-adrenoceptors (β_1-AR) and concomitantly cAMP accumulation with increasing age regardless of sex. In this study we proved the hypothesis that breed differences exist in age-dependent alterations in the β_1-AR system. Right (RA) and left (LA) atrial as well as right (RV) and left (LV) ventricular tissues were collected from male and female Cröllwitzer "wild-type" turkey poults of increasing age (6 wk, 12 wk, 16 wk, 21 wk). The β_1-AR density and function were quantified by (-)-[^{125}I]-iodocyanopindolol (ICYP) radioligand binding analysis in cell membranes from 4 cardiac chambers. Basal and stimulated cAMP production was determined as indicator of the receptor function. Wild-type turkeys showed significantly higher heart to body weight ratio than the meat-type B.U.T. Big 6 turkeys. In both sexes of Cröllwitzer turkey hearts, the β_1-AR density decreased with age but significance was reached in male cardiac chambers. The receptor affinity (K_D) and subtype distribution were not altered. Sex had no effect on age-related decrease in receptor density but had an effect on adenylate cyclase (AC) activity and subsequently cAMP production. In male Cröllwitzer turkey hearts of all ages, cAMP remained at same level, whereas this was even increased in female cardiac chambers. Thus, breed affected age-related receptor-, G-protein and AC-stimulated cAMP formation in normal ventricles and atria, with females exhibiting pronounced increase with age. This suggests that the receptor signaling in wild-type turkey hearts is not as blunted as in hearts of meat-type turkey poults in which stressful farming conditions and fast growing lead to receptor down-regulation.

Key words: Cröllwitzer turkey, age, sex, heart, β-adrenoceptor

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INTRODUCTION

The contractile function of the heart is regulated by the sympathetic nervous system through the cardiac β_1-adrenoceptor (β_1-AR) signal transduction pathways (Braunwald, 1965; Bristow, 2011). In all cardiac chambers of meat-type (British United Turkey [**B.U.T.**] Big 6) turkeys, the β_1-AR is the predominant subtype population expressed (Hoffmann et al., 2015). The chronic activation of β_1-ARs is also the main regulator of cardiac enlargement, which results in decreased function and enhanced cardiac hypertrophy (Brodde and Michel, 1999). From this point of view, one can speculate that blocking this receptor likely elucidates more of the clinical merits of β-blockers in treating and preventing heart failure and cardiac arrest in stressed turkeys; or alternatively, the ethical benefit of keeping animals in a stress-free and species-specific environment.

Major factors that are known to influence receptor expression in different mammalian species including man are stress and acute exposure to adrenergic stimulation (Kolmus et al., 2015), long-term administration of β-agonists (Abraham et al., 2002; McGraw and Liggett, 2005), exercise (Hossack and Bruce, 1982) and gender (Carroll et al., 1992; Krumholz et al., 1993; Aurigemma et al., 1995), which all aggravate the development of cardiac failure. The β_1-AR system is a dynamically changing system in response to normally occurring fluctuations such as plasma catecholamine levels mediated by, for example, sex and breed, generally resulting in altered receptor signal transduction pathways in cardiac tissues of different animal species (Stadler et al., 1990; Carroll et al., 1992; Roets and Burvenich, 1995). Interestingly during prenatal embryonic development in chicken, hypoxia seems to differentially regulate the β-AR sensitivity; i.e., hypoxia enhanced the receptor sensitivity and inhibited receptor

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down-regulation in the heart, whereas postnatally hypoxia alters the cardiac β-AR function (Lindgren and Altimiras, 2009, 2013). In cardiac chambers of fast-growing, stress-exposed male and female meat-type B.U.T. Big 6 turkeys, age-dependent down-regulation of the β_1-AR density and cAMP formation has been detected which might potentiate heart failure and cardiac arrest in these animals (Hoffmann et al., 2016). On the contrary, no data are yet available regarding the impact of turkey breed on the β-AR system in relation to age and sex.

Therefore, in the current investigation, we examined the β-AR density and adenylate cyclase activity (AC) in cardiac chamber membranes from Cröllwitzer turkeys, a wild-type breed of the domestic turkey, which is not selected for weight gain or meat production. We hypothesized that breed and consequently the corresponding husbandry conditions might differently influence the receptor function with age in the heart in contrast to B.U.T. Big 6 turkeys.

MATERIAL AND METHODS

Animals and Experimental Design

All Cröllwitzer turkeys were obtained from the same local turkey breeder at the age of 4 to 5 wk and were kept and fed in the bird housing unit of the Clinic for Birds and Reptiles of the Leipzig University. 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. A11/14). For the purpose of the present study, male and female wild-type (Cröllwitzer) turkeys in 3 age groups were used: 6-week-old (beginning of fattening period), 12-week-old (middle of fattening period) and 16-week-old for female/21-week-old for male turkeys (end of fattening period). Depending on the animal age, 5 to 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 tissues. At 6 and 12 wk 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. The remaining 5 female and 5 male birds remained in the barn and were euthanized as described above at the age of 16 wk (female) and 21 wk (male). 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 (allometric analysis), samples were frozen and stored at –80°C until use.

Preparation of Heart Crude Membranes

Frozen atrial and ventricular (left, right) tissues (400 mg) were thawed in 10 mL of the lysis buffer (1 mM $KHCO_3$). All procedures were carried out at 4°C. The tissues were minced with scissors and homogenized twice for 30 sec 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 4 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 to 6 mg/mL determined by the method of Lowry et al., 1951. Membrane preparations were stored at –80°C.

Receptor Binding Assays

Assays of total β-adrenoceptor (**β-AR**) binding were carried out in duplicates on washed crude membranes of all heart chambers of Cröllwitzer turkeys as recently described (Hoffmann et al., 2015). In brief, (-)-[^{125}I]-iodocyanopindolol (**ICYP**) was used to determine the receptor affinity and total number of β-ARs. The total assay volume amounted to 250 μL in which ICYP at 6 increasing concentrations ranging from 5 to 140 pM, 1 μM (±)-CGP-12,177 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) 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). Specific binding of ICYP was defined as the difference between total ICYP binding in absence and presence of 1 μM (±)-CGP-12,177 and was usually 70–80% at 40 pM of ICYP.

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

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

Table 1. Allometric parameters for male and female Cröllwitzer turkeys.

	6 wk		12 wk		21/16 wk	
	Male	Female	Male	Female	Male	Female
Body Weight (kg)	$0.57^{***} \pm 0.02$	0.46 ± 0.01	$1.76^{***} \pm 0.04$	1.33 ± 0.03	$4.67^{***} \pm 0.08$	2.02 ± 0.01
Heart Weight (g)	3.58 ± 0.08	2.77 ± 0.08	9.56 ± 0.27	6.8 ± 0.24	22.92 ± 0.71	10.31 ± 0.33
Heart Weight/Body Weight (%)	0.63	0.60	0.54	0.51	0.49	0.51
Left Ventricular Weight/Body Weight (%)	4.54	4.23	4.07	3.87	3.75	3.81
Left Ventricular Weight/Right Ventricular Weight	4.53	4.16	5.04	5.04	4.94	4.98

Data are given as means ± SE of n = 10 per age group and sex. In all age groups, male birds gained significantly more weight than female birds (unpaired t-test, $^{***}P < 0.001$)

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). 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 0.01–100 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.

Statistical Analysis

The receptor density of ICYP binding (B_{max}) and the equilibrium dissociation constant (K_D) were calculated from individual saturation curves using linear regression analysis according to Scatchard (1949) or nonlinear regression analysis of the binding data. All binding data were analyzed using an iterative, non-linear curve fitting computer program GraphPad Prism (GraphPad Software, San Diego, CA). Linear regression was used to determine a possible relationship between age and β-AR density. A goodness of fit (r^2) < 0.9 was considered acceptable.

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 differences in B_{max}, K_D and cAMP level for each cardiac chamber. Unpaired t-test was used to compare body weights of male and female birds within each age group. Significance was accepted at a level of *P-value* smaller than 0.05. Dose-response curves, EC_{50} values and all statistical calculations were also assessed by GraphPad Prism. F-test was used to compare 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.

RESULTS

Sex had measurable effect on body and heart weight, i.e., male birds gained significantly 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. Similarly, the ratio of left ventricular weight to right ventricular weight was lesser in older than in younger turkeys, whereas the ratio of left ventricular weight to body weight decreased with age.

The data of total β-AR density (B_{max}) and affinity (K_D) are summarized in Table 2 (A and B). From saturation ICYP binding studies, a progressive decrease (15 to 20%) in B_{max} for 12-week-old male turkey hearts (∼52 fmol/mg protein, ± 2,5) compared with 6-week-old turkey hearts (∼59 fmol/mg protein, ± 2.7) could be identified (Table 2A). In 21-week-old male turkey hearts, B_{max} was up to 45% less than that of 6-wk-old turkey hearts. In all cardiac chambers of male Cröllwitzer turkeys, this age-dependent decrease in β-AR number was statistically significant. In contrast, in female Cröllwitzer turkeys, a slight but not statistical significant decrease in β-AR density with age could be observed in left and right atrial as well as in right ventricular membranes. In the left ventricle, there was no age-dependent change in receptor density. Figure 1A-D displays linear regression of receptor density with age for male and female Cröllwitzer turkeys. For male birds, goodness of fit (r^2) was > 0.9 in all cardiac chamber indicating a linear relationship between age and β-AR density for the investigated time frame. Results of linear regression were poor for female birds; merely, in

Table 2. β-adrenergic density (2A) and affinity (2B) measured in cardiac chamber membranes of Cröllwitzer turkeys with age.

	B_{max} (fmol/mg protein)							
	Male				Female			
Age	RA	LA	RV	LV	RA	LA	RV	LV
(2A)								
6 wk	63.3 ± 4.4	59.2 ± 6.6	60.5 ± 4.8	62.7 ± 5.5	43.5 ± 4.9	48.2 ± 4.7	48.4 ± 9.9	46.6 ± 4.3
12 wk	53.9 ± 3.5	47.0 ± 5.2	51.3 ± 3.9	50.8 ± 4.8	46.1 ± 3.4	42.0 ± 2.9	40.7 ± 5.3	53.3 ± 7.0
16/21 wk	37.1^{***} ± 3.8	33.9^{**} ± 3.2	38.9^{**} ± 4.7	34.5^{***} ± 2.8	38.7^{ns} ± 1.2	38.8^{ns} ± 2.6	42.3^{ns} ± 4.8	47.1^{ns} ± 2.0
(2B)								
	KD (pM)							
6 wk	24.3 ± 1.9	33.3 ± 3.2	23.1 ± 2.1	39.4 ± 5.8	31.0 ± 6.5	42.0 ± 8.5	31.1 ± 6.6	30.4 ± 3.2
12 wk	31.6 ± 6.0	17.6 ± 2.7	22.6 ± 4.9	27.5 ± 3.0	37.7 ± 9.2	25.2 ± 1.5	25.0 ± 2.5	37.9 ± 5.3
16/21 wk	19.7^{ns} ± 1.5	26.4^{ns} ± 3.1	20.1^{ns} ± 2.2	25.0^{ns} ± 1.5	20.3^{ns} ± 3.5	26.4^{ns} ± 3.1	20.9^{ns} ± 2.3	32.4^{ns} ± 1.4

B_{max} is given as means ± SE 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 ± SE of n = 5 experiments (minimum of 5 different animals per age group and sex).

Statistical significant decrease of β-adrenergic receptor density with age was obtained within each cardiac chamber for male poults. (One-way ANOVA with *post hoc* for linear trend, $^{**}P < 0.01$; $^{***}P < 0.001$). Statistic insignificant results ($P > 0.5$) are marked with "ns" (not significant).

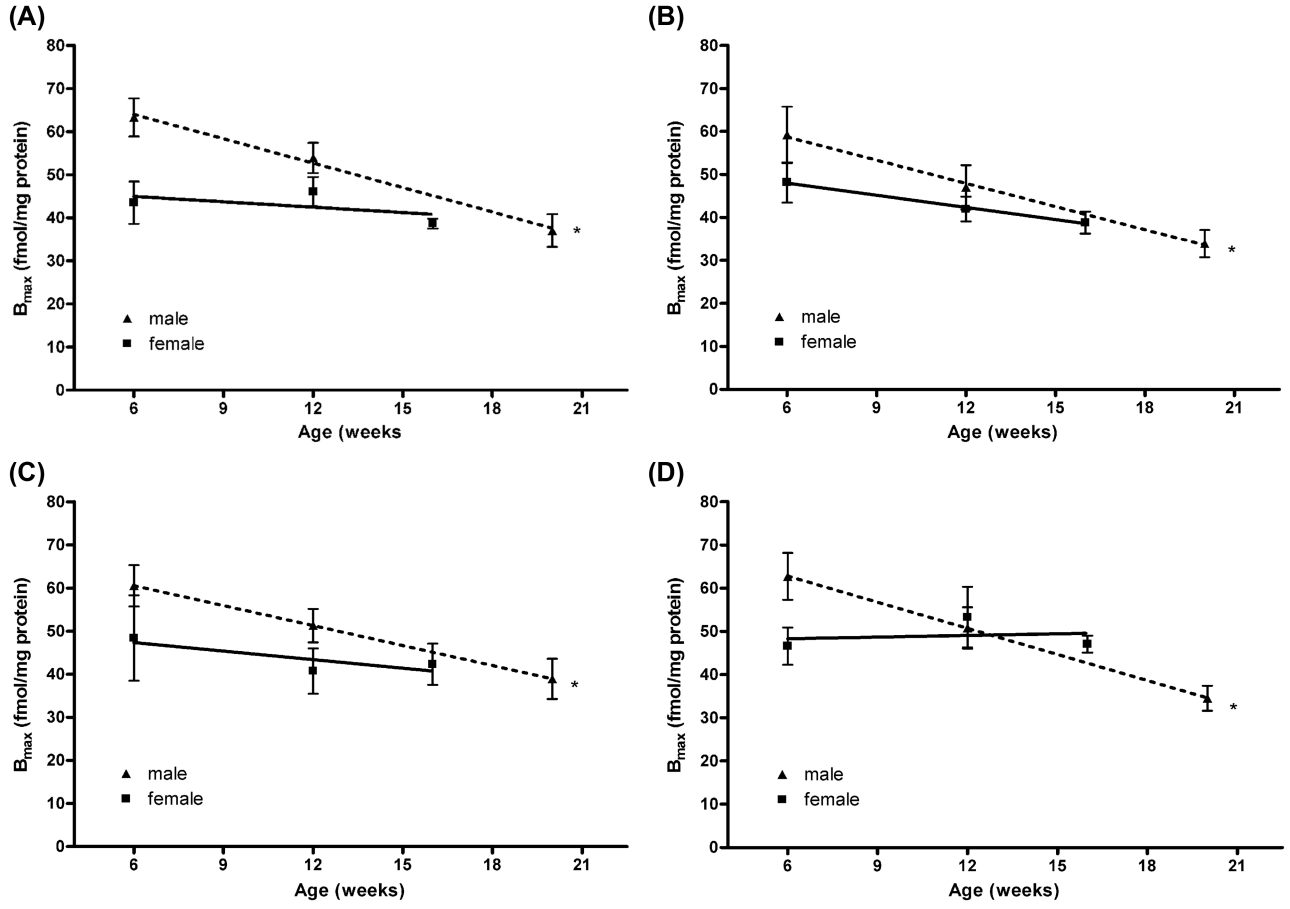

Figure 1. Linear regression of receptor density with age for male and female Cröllwitzer turkeys in right atrium (A), left atrium (B), right ventricle (C) and left ventricle (D) of male (Δ) and female (■) Cröllwitzer turkey poults. Data are given as means ± SE of n = 5 experiments (minimum of 5 different animals per age group and sex). $r^2 > 0.9$ for all cardiac chamber of male birds and left atrium of female birds. *Statistical significant decrease of β-adrenergic receptor density with age was obtained within each cardiac chamber for male poults. (One-way ANOVA with *post hoc* for linear trend, $P < 0.01$).

the left atrium of female birds, r^2 was > 0.9. Regarding the receptor density of cardiac chambers within one age group, we could not observe any difference between 4 cardiac chambers. The receptor affinity to the ligand (K_D) (∼ 32 pM 6 wk vs. ∼ 24 pM 21 wk old) between cardiac chambers was similar and was unaffected by sex and age of the Cröllwitzer turkeys (Table 2B). Competition binding studies using the β_1-AR selective antagonist CGP 20712A and β_2-AR selective antagonist ICI 118.551 detected similar population of the β_1-subtype

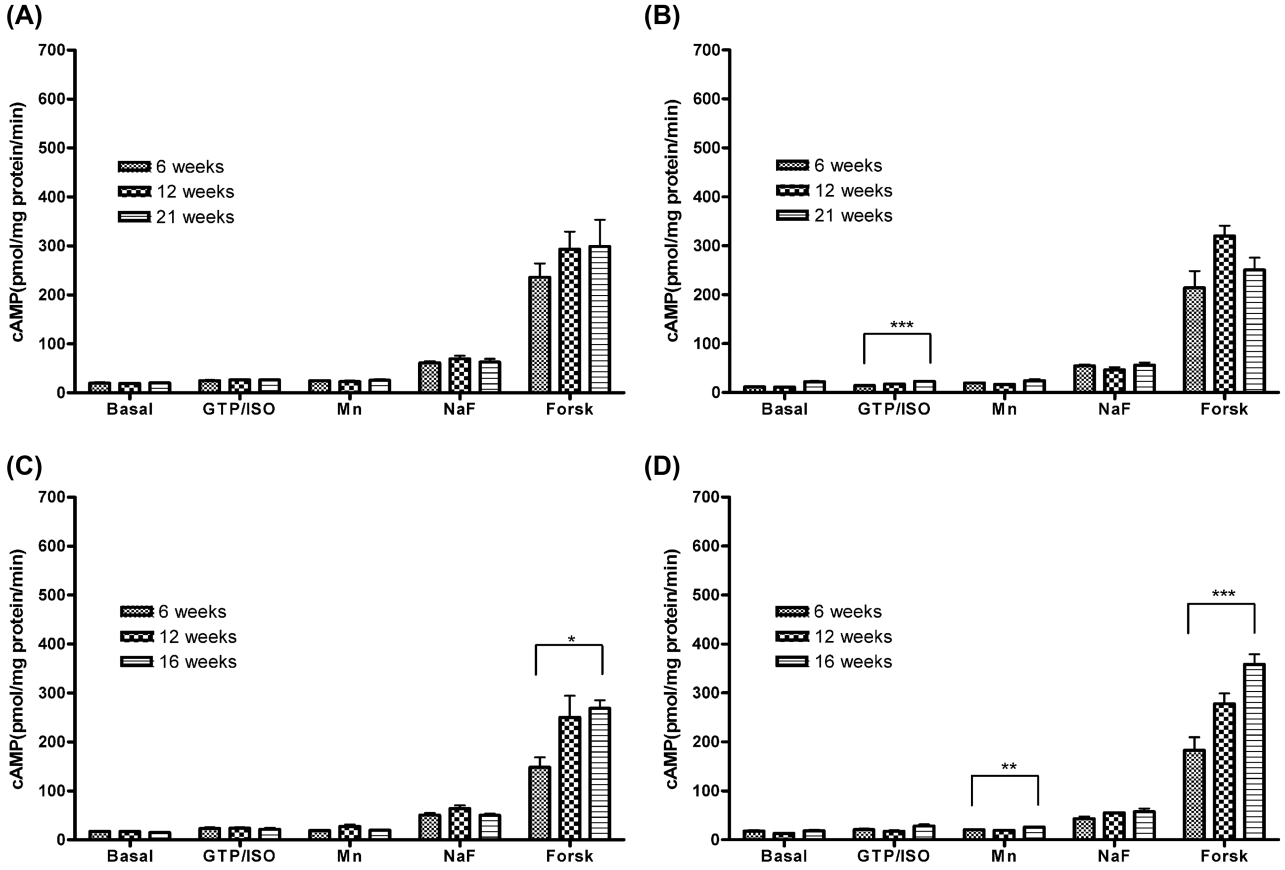

Figure 2. Age-dependent decrease in adenylate cyclase activity in crude membranes of right and left atria of male (A, B) and female (C, D) turkey poults. Data are given as means ± SE of n = 5 experiments (minimum of 5 different animals per age group and sex).

in all cardiac chambers of all age groups and sexes without affecting the subtype distribution. The selective β_1-AR antagonist CGP 20712A inhibited ICYP binding with more than 25-fold potency than β_2-AR antagonist ICI 118.551, providing a homogeneous population of β_1-subtype also in Cröllwitzer turkey hearts as recently described (Hoffmann et al., 2015).

In both male and female Cröllwitzer turkey poults of all age groups, the basal cAMP level remained similar in all cardiac chamber membranes (∼18 pmol/mg protein/min 6-week-old turkey hearts vs. ∼ 21 pmol/mg protein/min 21-week-old turkey hearts) (Figures 2A-D and 3A-D). The isoproterenol- and GTP-stimulated cAMP production was higher in cardiac membranes of all age groups than the basal cAMP level; however, it did not reach statistical significance. The direct β-AR activation by the nonselective β-AR agonist isoproterenol combined with GTP and the direct AC activation by Mn^{2+} resulted in a similar pattern of AC activation but the net effect was less than NaF- and forskolin-stimulated AC activity (Figures 2A-D and 3A-D). Accordingly, cAMP formation was activated the most in the presence of forskolin followed by NaF and isoproterenol + GTP. The Mn^{2+}-stimulated cAMP level was similar with that of isoproterenol in the presence of GTP.

No age-dependent differences could be observed in cAMP accumulation in all 4 cardiac chambers of male turkey poults; hence, cAMP remained at the same level for all tested substances during the whole fattening period (Figures 2A-D and 3A-D). On the contrary, in female Cröllwitzer poults, even an age-dependent increase in cAMP level could be observed in all cardiac chambers and for the majority of substances tested, whereby forskolin stimulated the highest AC activity and cAMP production with up to 300% in the right ventricle (Figure 3C). 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.

When comparing all cAMP stimulating agents, receptor- and G-protein-stimulating substances triggered the cAMP production generally to a lesser extent; therefore, we further sought to provide concentration-dependent data of cAMP production by activating the catalytic units of the AC by forskolin. Forskolin highly stimulated the cAMP production in all cardiac membranes of female and male Cröllwitzer turkeys, whereby statistical significance could be observed only in female turkeys with increasing age (Figures 2A-D and 3A-D). A similar pattern of activation of cAMP level was obtained, when membranes were incubated with

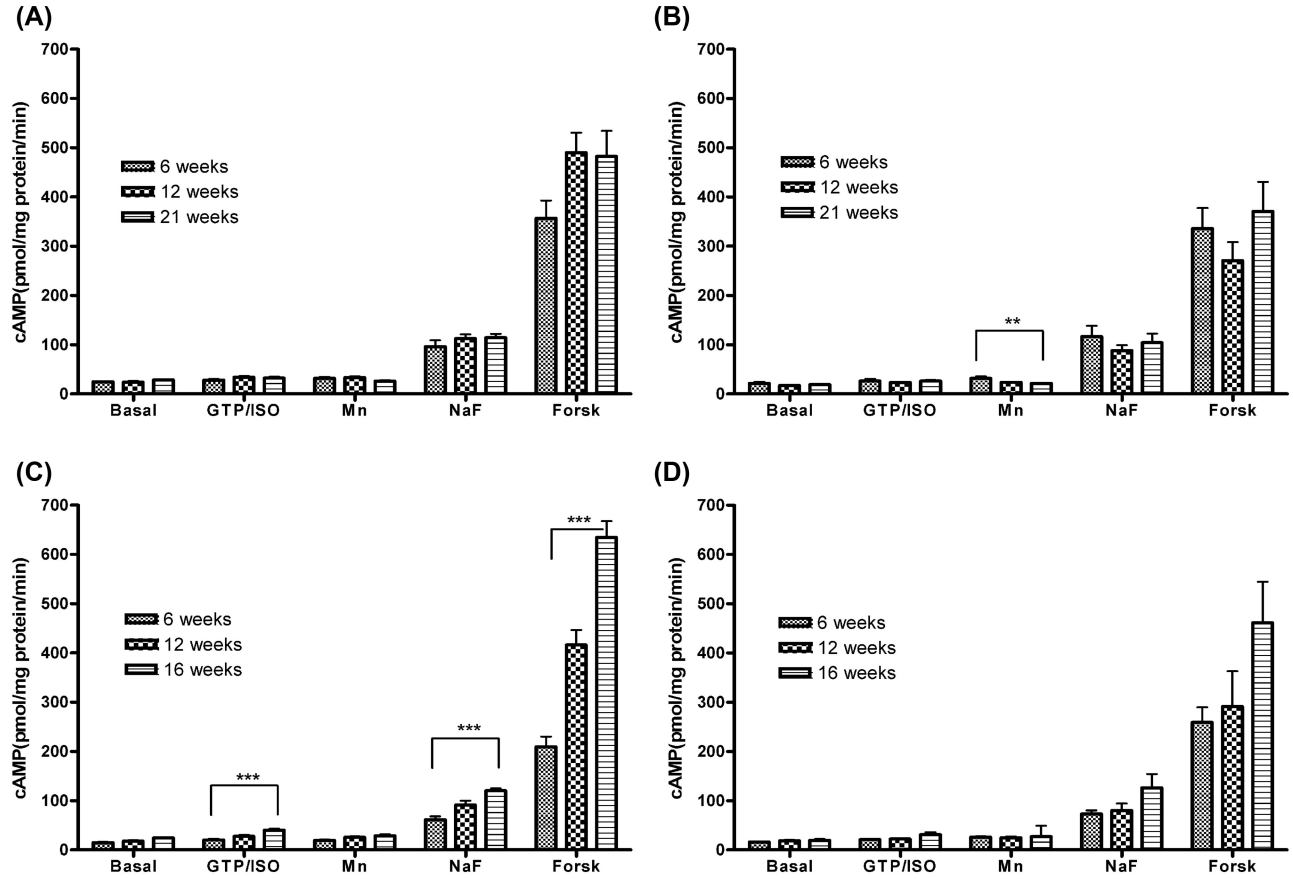

Figure 3. Age-dependent decease in adenylate cyclase activity in crude membranes of right and left ventricle of male (A, B) and female (C, D) turkey poults. Data are given as means ± SE of n = 5 experiments (5 different animals per age group and sex). One-way ANOVA with *post hoc* for linear trend $^{**}P < 0.01$; $^{***}P < 0.001$.

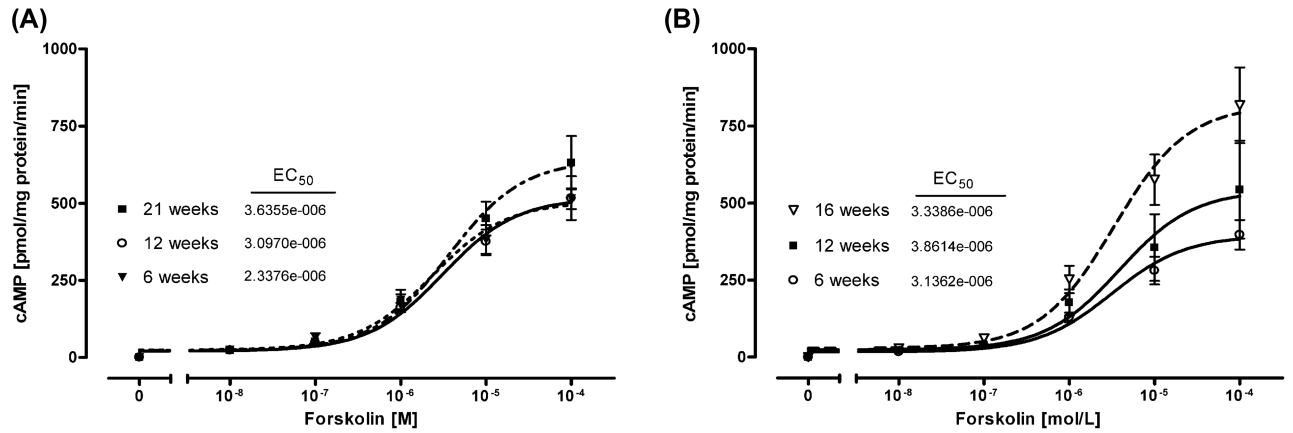

Figure 4. Representative concentration-response curves of forskolin-stimulated adenylate cyclase activity in left ventricular crude membranes of male (A) and female (B) turkey poults. Data are given as means ± SE of n = 3 experiments (3 different animals per age group).

increasing concentrations of forskolin. Dose-response curves of cAMP activation by forskolin were assessed representatively in left ventricular membranes of male (Figure 4A) and female (Figure 4B) turkey poults. The half-maximal forskolin concentration (EC_{50}) was similar in all cardiac chambers of all ages, indicating the same AC sensitivity for forskolin. However, dose-response curves in older female turkey hearts were steeper and higher than in younger turkey hearts. This gradual age-related increase in maximum forskolin-stimulated cAMP production was only seen in female turkey hearts.

DISCUSSION

The age-related reduction in cardiac β-adrenoceptor (β-AR) responsiveness in the meat-type turkeys (B.U.T. Big 6) with increasing age has been well

demonstrated. In particular, an age-associated reduction in β_1-AR density has been demonstrated in membranes of all cardiac chambers (atria, ventricles) and has been attributed to receptor down-regulation and decreased receptor coupling to AC (Hoffmann et al., 2016). As the β_1-AR subtype is the dominant receptor population in turkey hearts (Hoffmann et al., 2015), the decreased cardiac receptor response appears to be related mainly to down-regulation of this receptor subtype. The present study was undertaken to determine breed differences with respect to age- and sex-dependent changes in cardiac β_1-AR number and cAMP production, i.e., in wild-type Crölllwitzer turkeys. Main findings of the current study were: first, the β_1-AR density decreased in heart chambers of both male and female wild-type turkeys with age without altered affinity of the radioligand to the receptor and receptor subtype distribution; second, interestingly, sex had an effect on age-associated AC activity and subsequent cAMP formation; i.e., the cAMP level was not changed in male Crölllwitzer turkey hearts, whereas this was increased in female cardiac chambers.

In this study, in both sexes there was a considerable increase in body and heart weight of wild-type Crölllwitzer turkeys with increasing age and corresponded with those clinically observed. Male turkeys gained, indeed, more body and heart weight than female turkeys, but sex had no effect on age-dependent decrease in heart-to-body-weight ratio. The ratio of left ventricular to right ventricular weight was unchanged while the ratio of left ventricular weight to body weight decreased with age. Interestingly, in contrast to our recent data (Hoffmann et al., 2016), the relative heart weight of the Crölllwitzer turkeys of both sexes is greater than that of the meat-type turkeys (B.U.T. Big 6), indicating fast-growing behavior of the latter species with pronounced carcass gain and common features of deteriorated cardiac performance and compensation. This is the first and sole report to illustrate the marked difference between the 2 turkey breed lines with regard to heart morphometry in relation to body weight with age.

In morphologically normal Crölllwitzer turkey hearts, we found an age-related decline in β-AR density but not in subtype distribution. Indeed, specifically, in heart chamber membranes of male Crölllwitzer turkeys, there was a significant decrease in β-AR density with age between 20% (12-week-old) to 45% (21-week-old) (cf. Table 2A). In our previous study, in 57-week-old B.U.T. Big 6 turkey hearts, the decrease in β-AR density was up to 70% (Hoffmann et al., 2016), and this decrease was about 2-fold greater than the B_{max} of cardiac membranes of 21-wk old Crölllwitzer and B.U.T. Big 6 turkeys. Otherwise, there was no age-matched difference in β-AR density between B.U.T. Big 6 and Crölllwitzer turkey hearts. The dramatic β-AR down-regulation observed in "older" meat-type B.U.T. Big 6 turkey hearts suggests that increased susceptibility to stress in fast-growing turkey strains blunts highly the β-AR signal transduction system (Hoffmann et al., 2016).

The significant decrease of the β-AR density that was observed in male Crölllwitzer turkey hearts was, however, not so evident in female Crölllwitzer turkey hearts. The mechanism for the significant decrease in total β-ARs with age in male Crölllwitzer turkey hearts compared with female is not known. One plausible explanation for the decreased receptor density is an aging effect that could be delayed based on size (females are smaller) and/or presumably sex differences (sex hormones) (Adams et al., 1996).

Our data are only in part in agreement with human data describing that sex affects age-related β-AR down-regulation in normal ventricles; indeed, with females exhibiting more profound decline with increasing age reaching a plateau at the age of 40, suggesting loss of myocardial performance (Lindenfeld et al., 2016). However, it is not possible to evaluate from our present data whether the not significant change in β-AR density in female Crölllwitzer turkey hearts is a result of sex hormone effects.

Moreover, to relate the β-AR properties to cAMP response, AC activity was measured in crude membranes of all cardiac chambers in the absence and presence of isoproterenol +GTP, NaF, Mn^{2+} and forskolin. Upon direct receptor- or AC- stimulation, no age-dependent differences could be observed in cAMP accumulation in all 4 cardiac chambers of male turkey poults. On the contrary, in female poults, even an age-dependent increase in cAMP level could be demonstrated in all cardiac chambers and for the most substances tested.

This is in marked contrast to the AC data recently published for B.U.T. Big 6 turkey hearts of both sexes and age-related (Hoffmann et al., 2016) and also in disagreement with previous studies which showed that there is an age-related attenuation of the AC activity in rat myocardium (O'Connor et al., 1981; Scarpace, 1990; Scarpace et al., 1994; Tobise et al., 1994). Our data from male Crölllwitzer turkey hearts are, indeed, in concordance to other several studies that found also no changes in AC activity consequently in cAMP level (Whitsett and Darovec-Beckerman, 1981; Hatjis, 1986). Therefore, it is possible that not all receptors of the wild-type strains are coupled to AC with increasing age, indicating that a significant down-regulation of the β_1-AR in cardiac tissues of male Crölllwitzer turkeys is not accompanied by an impairment in the production of cAMP, especially, in response to forskolin.

On the other hand, we could observe rather a clear and significant age-dependent increase in AC activity in female Crölllwitzer turkey hearts (mainly in right and left atria as well as right ventricle) when stimulating the catalytic unit of the AC with forskolin, suggesting an effect of sex on receptor responsiveness. First, our data in all cardiac chambers of female Crölllwitzer turkeys suggest that the enhanced cAMP level might normally be coupled, despite a not significant decline in the β-AR density. Second, as mentioned above, it is possible that the suggested enhanced β-AR responsiveness is linked to sex hormones interactions in

female wild-type turkeys. Also here, the high cAMP level could also mean an augmented cardiac function during maturation. However, the cAMP data found in female wild-type turkey hearts contrast the data found in elderly women, where the receptor sensitivity is blunted to β-agonist stimulation associated with increased sympathetic nerve activity and receptor downregulation in aged women (Narkiewicz et al., 2005). Nevertheless, from our result we cannot provide information why the less age-dependent decline in receptor expression in female wild-type turkey hearts did not rather contribute to decreased AC responsiveness; or alternatively, the high expression of the AC subtype might be the cause of enhanced cAMP level. Also, in contrast to the present data, in both male and female meat-type turkeys (B.U.T. Big 6), the successive decrease in receptor number was accompanied with reduced AC activity and subsequently cAMP production in all cardiac chambers with age which was emphasized to be related with fast-growing behavior and stress situations that activate the sympatho-adrenal system, resulting in permanent β_1-AR stimulation (Santos and Spadari-Bratfisch, 2006).

The study has a number of potential limitations: we did not have access to older turkey hearts in this study. Age-related cardiac remodeling might have occurred in these animal models; indeed, ultrastructural analysis has not been carried out. Since cardiac remodeling is a prognostic negative factor also in aging, a better understanding of the mechanism through which gender or breed regulates cardiac remodeling could open the way to innovative animal husbandry and therapy strategies.

In conclusion, we report that age-related downregulation and receptor responsiveness in wild-type turkey cardiac chambers are quantitatively different in male vs. female, and these differences might explain some of sex differences in myocardial disease presentations in turkey populations. Those age-related changes are gender-specific and related perhaps to the husbandry of this model bird species.

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