AGE (2014) 36:9675
DOI 10.1007/s11357-014-9675-7

Long-term α_{1B}-adrenergic receptor activation shortens lifespan, while α_{1A}-adrenergic receptor stimulation prolongs lifespan in association with decreased cancer incidence

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Received: 2 January 2014 / Accepted: 18 June 2014 / Published online: 4 July 2014
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Abstract The α_1-adrenergic receptor (α_1AR) subtypes, α_{1A}AR and α_{1B}AR, have differential effects in the heart and central nervous system. Long-term stimulation of the α_{1A}AR subtype prolongs lifespan and provides cardio- and neuro-protective effects. We examined the lifespan of constitutively active mutant (CAM)-α_{1B}AR mice and the incidence of cancer in mice expressing the CAM form of either the α_{1A}AR (CAM-α_{1A}AR mice) or α_{1B}AR. CAM-α_{1B}AR mice have a significantly shortened lifespan when compared with wild-type (WT) animals; however, the effect was sex dependent. Female CAM-α_{1B}AR mice lived significantly shorter lives, while the median lifespan of male CAM-α_{1B}AR mice was not different when compared with that of WT animals. There was no difference in the incidence of cancer in either sex of CAM-α_{1B}AR mice. The incidence of cancer was significantly decreased in CAM-α_{1A}AR mice when compared with that in WT, and no sex-dependent effects were observed. Further study is warranted on cancer incidence after activation of each α_1AR subtype and the effect of sex on lifespan following activation of the α_{1B}AR. The implications of a decrease in cancer incidence following long-term α_{1A}AR stimulation could lead to improved treatments for cancer.

Keywords α_1-Adrenergic receptor · Aging · Longevity · Cancer · Norepinephrine

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Introduction

Epinephrine and norepinephrine are catecholamines that act as chemical messengers. They are synthesized in the adrenal medulla and in adrenergic neurons in the brain, respectively, as well as in postganglionic neurons in the sympathetic nervous system. In the periphery, both epinephrine and norepinephrine help regulate heart rate and blood vessel constriction and modulate the force of heart contraction and physiological arousal. When stressed, the body releases both chemicals to mediate the fight-or-flight response. All of these effects are a result of epinephrine or norepinephrine binding to adrenergic receptors (ARs), of which there are three families: α_1, α_2, and β.

Our lab has found differential effects of activating either the α_{1A} or α_{1B}AR subtype. Long-term stimulation of the α_{1A}AR, either pharmacologically or through transgenic manipulation, increases adult neurogenesis, reduces depression and anxiety-like behavior, and enhances learning and memory in the mouse (Gupta et al.

2009; Doze et al. 2009, 2011). Mice with a constitutively active mutant (CAM) form of the α_{1A}AR (CAM-α_{1A}AR) also live significantly longer lives when compared with wild-type (WT) animals of the same background (Doze et al. 2011). In contrast, chronic activation of the α_{1B}AR increases depression-like behavior (Doze et al. 2009). In transgenic models of increased α_{1B}AR activity, a maladaptive cardiac hypertrophy was either present at baseline or developed over time concomitantly with other cardiac and cardiac-related deficits (reviewed in Perez and Doze 2011). Chronic α_{1B}AR activity also leads to age-related apoptotic neurodegeneration, a synucleinopathy with Parkinson-like movement deficits similar to human multiple system atrophy (Zuscik et al. 2000; Papay et al. 2002). The neurodegeneration begins in areas of the brain with a high density of α_{1B}ARs and leads to a substantial loss of dopaminergic neurons in the substantia nigra. The α_1AR-selective antagonist terazosin protects against α-synuclein aggregates, neurodegeneration, and partially rescues the movement deficits. Terazosin treatment also ameliorated the α_{1B}AR activation's detrimental effect on early mortality; however, animals were only followed up to 70 weeks of age. Taken together, these results suggest the α_1AR subtypes mediate neurogenesis and neurodegeneration which is in line with their role in proliferation in the peripheral nervous system.

The α_1ARs can mediate proliferation and cell growth in the periphery (Hoffman and Hu 2000; Michelotti et al. 2000). α_1AR activation stimulates DNA synthesis in human vascular smooth muscle cells through a PI3 kinase and MAPK pathway (Hu et al. 1996; Hoffman and Hu 2000). It also induces the expression of the proto-oncogenes *c-fos* and *c-jun* in arterial smooth muscle (Okazaki et al. 1994). In a Rat-1 fibroblast microarray, the α_1AR subtypes preferentially induced transcription of genes that regulate the cell cycle (Gonzalez-Cabrera et al. 2004). The microarray results suggested that α_{1A} and α_{1D}ARs halt the cell cycle at the G1-S checkpoint. α_{1B}AR expression led to cell cycle progression through the G1-S checkpoint by inducing transcription of cdk-6- and cyclin E-associated kinases. This cell cycle progression is evident in Rat-1 and NIH-3T3 fibroblast cells transfected with α_{1B}ARs which enhances focus formation after agonist stimulation. The cells are also tumorigenic when injected into nude mice, implicating the gene as a potential proto-oncogene (Allen et al. 1991; Gonzalez-Cabrera et al. 2004). That is the only study to date that has examined in vivo tumor formation mediated by the α_1AR subtypes. The role of the α_{1A}AR subtype in cancer incidence has not been examined prior to the current study.

Our hypothesis was that long-term transgenic activation of the α_{1A} and α_{1B}ARs would lead to a decrease and increase in the incidence of cancer, respectively. It was expected that chronic activation of the α_{1B}AR would lead to a shorter lifespan due to cancer and neurodegeneration. In this study, we followed transgenic mice overexpressing the α_{1A} or α_{1B}AR throughout their lifespan. We also completed necropsy on subsets of each group to assess cancer incidence. Surprisingly, we found that chronic α_{1B}AR activation had no significant effect on cancer but did reduce longevity, presumably secondary to increased neurodegeneration. Importantly, we observed that long-term α_{1A}AR stimulation was associated with a significantly lower incidence of cancer and longer lifespan.

Methods

Animals

This study used transgenic mice overexpressing a CAM form of either the α_{1A} or α_{1B}AR that was created on a B6CBA background. The CAM receptor genes were expressed under the endogenous promoters, and the animals have been previously characterized (Zuscik et al. 2000; Rorabaugh et al. 2005). The longevity part of the study included 235 mice, and the pathology study included 157 mice with some overlap. Animals were bred and provided with identifying ear tags at the Cleveland Clinic Foundation and transferred to the University of North Dakota. Both facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The experimental protocols employed in this study conform to the *Guide for the Care and Use of Laboratory Animals*, published by the US National Institutes of Health, and were approved by the Animal Care and Use Committee at both institutions.

Mice were housed in translucent, polycarbonate boxes of 17×28×13 cm, with one to five mice per box with one rodent clubhouse. Animals were maintained on a 12-h light-dark cycle with lights on at 0700. Harlan diet 8640 and water were provided ad libitum (Harlan,

Indianapolis, IN). The temperature was held constant at 22 °C and the humidity at 23–27 %.

Longevity

Animals were observed daily by facility personnel, but handled only for cage changes and clinical assessments. All staff members who observed and handled the mice were trained to detect and record signs of illness and notified the veterinarian and research team immediately regarding sick animals. The veterinarian and researchers examined the mice for severity of illness or impairment, and animals were euthanized if they were not likely to survive for another 48 h. The likelihood of survival was based on the occurrence of at least two of the following clinical signs set forth by The Jackson Laboratory (Yuan et al. 2009). The signs included failure to drink or eat, extreme weight loss over a short period of time, severe weakness based on responsiveness to touch, serious locomotor impairments, or tumors that had ulcerated or were bleeding (Ray et al. 2010). The date of death for each mouse was logged, and the number of days lived was calculated and used for analysis. The mean age at death for the euthanized mice was not significantly different from the mice which died spontaneously; therefore, euthanized mice were included in the analysis.

Pathology

Mice were removed from their cages as soon as possible after death and frozen at −20 °C until pathological analysis. Animals sacrificed at a younger age were included in the analysis to increase statistical power. At necropsy, the mice were visually inspected and the exterior palpated to assess skin condition and any outward signs of abscess, disease, or tumors. The abdomen and thoracic cavities were opened and organs inspected and removed. Tumors were digitally photographed in situ and, after removal with a measurement scale, clearly visible. Samples of the heart, lungs, spleen, kidney, liver, and intestine were immersion-fixed in neutral buffered 10 % formalin for at least 24 h. Samples were dehydrated in graded ethanol, cleared in xylene, infiltrated, and paraffin embedded. Serial sections were cut at 3–5 μm, stained with hematoxylin and eosin, and evaluated for cancerous cells on a Carl Zeiss Axioskop 50 microscope (Zeiss, Germany). Classification of tumors was based on the current World Health Organization Classification of Tumors with modifications, as needed, for mouse tissue. The primary diagnosis for each animal was used for analysis; tumor burden was not assessed.

Statistical analysis

Survival was analyzed using Kaplan-Meier survival curves and the log-rank (Mantel-Cox) test with GraphPad Prism 5.04 (San Diego, CA). The median lifespan was the point at which the fractional survival of each curve equaled 50 %. Cancer incidence was analyzed using a χ^2 test on raw data, but is presented as percentages for clarity. Mice still alive at the end of the study were interval-censored. An unpaired *t* test was used for comparison of maximal lifespan. Significance levels were set at $p<0.05$.

Results

Chronic activation of the α_{1B}AR leads to decreased body weight starting during middle age, while long-term α_{1A}AR stimulation does not affect adult weight

We weighed cohorts of mice at various ages to rule out increased lifespan due to caloric restriction because acute activation of the α_{1A}AR can suppress appetite (Davies and Wellman 1992; Morien et al. 1993). Just after weaning, CAM-α_{1A}AR ($p<0.05$) and CAM-α_{1B}AR mice ($p<0.001$) weighed significantly less than WT mice (Fig. 1). Between the second month and the end of the eighth month of age, there were no significant differences in weight between groups. However, CAM-α_{1B}AR animals weighed significantly less when compared with WT mice starting at approximately 9 months of age. The 21–24-month age range had a low number of CAM-α_{1B}AR animals, and no significant differences in weight were observed.

CAM-α_{1A}AR mice have an increased lifespan, while CAM-α_{1B}AR mice have shorter lives

We previously reported that CAM-α_{1A}AR mice live significantly longer when compared with WT animals (Doze et al. 2011). The CAM-α_{1A}AR longevity data included here is the previously published data and is included only for comparison. There was no significant difference between lifespans of previously published

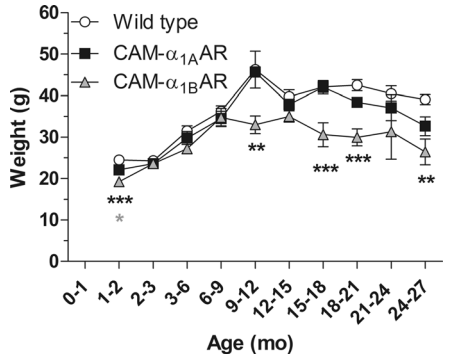

Fig. 1 Weight across the lifespan. CAM-α_{1B}AR mice weighed significantly less than WT mice at most time points after 9 months of age (*p<0.05, **p<0.01, ***p<0.001)

WT animals and WT mice that have died since; therefore, both groups are included here to provide statistical power (Fig. 2, Table 1). Within the WT group, there was no significant difference between female (711 days) and male mice (721 days, χ^2=2.300, df=1, p=0.129). The median lifespan of CAM-α_{1B}AR mice (637 days) was significantly shorter than that of WT animals (719 days, χ^2=7.194, df=1, p=0.007), a decrease of approximately 11 % (Fig. 2, Table 1). There was no significant difference in the lifespan between the male CAM-α_{1B}AR mice (652 days) and male WT animals (721 days, χ^2=0.251, df=1, p=0.616). Female CAM-α_{1B}AR mice (619 days) lived significantly shorter lives than female WT animals (711 days, χ^2=9.415, df=1, p=0.002).

Maximal lifespan is an index of slowed aging and is calculated by comparing the ages of the 10 % longest living mice in each group. The maximal lifespan of CAM-α_{1B}AR mice was significantly shorter than that of WT mice (p=0.025). Female CAM-α_{1B}AR mice had a significantly shorter maximal lifespan than female WT animals (p=0.009). However, the maximal lifespan of male CAM-α_{1B}AR mice was significantly increased when compared with that of male WT mice (p=0.004).

The sigmoidal shape of all three survival curves suggests deaths were not due to a terminal infectious agent or a disease causing rapid death (Van Zwieten et al. 1981). The CAM-α_{1B}AR curve begins to deviate from the WT curve at approximately 9–10 months (270–300 days) of age suggesting normal development to that point. The longest living CAM-α_{1B}AR animals lived to similar ages as WT animals, likely due to incomplete penetrance of the transgene in those animals. Similarly, the CAM-α_{1A}AR curve shifts closer to WT at the end of life stage.

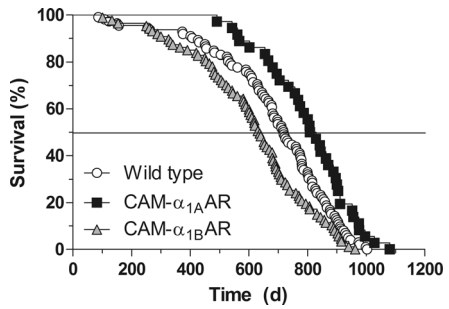

Fig. 2 Kaplan-Meier survival plots of CAM-α_{1B}AR (n=87), WT (n=112), and CAM-α_{1A}AR (n=36) mice. CAM-α_{1B}AR mice have a shorter lifespan than WT mice (p=0.007). As previously published, CAM-α_{1A}AR animals live significantly longer than WT mice (p=0.004) (Doze et al. 2011)

Chronic α_{1B}AR stimulation has no significant effect on the incidence of cancer, while activating α_{1A}ARs decreases cancer incidence

The overall cancer incidence between CAM-α_{1B}AR (n=34) and WT mice (n=70) did not differ (χ^2=0.1242, df 1, p=0.7245; Fig. 3). In contrast, CAM-α_{1A}AR mice (n=53) had a significantly lower overall incidence of cancer than WT animals (n=70) (χ^2=17.83, df 1, p<0.0001). There was no significant difference in cancer incidence between male and female animals for any of the groups.

The most common diagnoses among WT mice were epithelial and hematological cancers (Table 2). CAM-α_{1A}AR and CAM-α_{1B}AR animals had a similar incidence for the type of cancers as WT mice. Epithelial cancers included adenocarcinoma of the lung, renal cell carcinoma, hepatocellular carcinoma, and neuroendocrine carcinoma. Hematological cancers included lymphoma and leukemia involving various organs. The only mesenchymal cancer was spindle cell sarcoma. Noncancerous epithelial lesions included hepatocellular and small intestine adenomas and one case of hepatocellular hyperplasia. The only noncancerous hematological finding was lymphoid hyperplasia. Mesenchymal tumors included hemangioma and a noncancerous fibroma. Nontumor lesions included pathologies such as pulmonary edema, chronic inflammation, glomerular disease, cardiomyopathy, and heart thrombi. Representative images of common diagnoses are shown in Fig. 4.

Table 1 Lifespan data of CAM-α_{1B}AR, wild-type, and CAM-α_{1A}AR mice

Genotype	Median (days)	Mean (days)	S.D. (days)	S.E.M. (days)	95 % CI (days)	90th percentile (days)	Deaths (*n*)
CAM-α_{1B}AR	637	617	200	21	575–660	890	87
Male	652	635	204	29	576–694	907	48
Female	619	595	196	31	532–659	882	39
Wild type	719	689	203	19	651–727	912	112
Male	721	674	195	28	618–729	875	50
Female	711	701	210	27	648–754	932	62
CAM-α_{1A}AR	819	806	146	24	757–856	982	36
Male	822	821	150	35	746–895	999	18
Female	819	792	145	34	720–864	928	18

Discussion

It is well documented that caloric restriction increases lifespan in many species including rodents (reviewed in Masoro 2005). Injection of α_1AR agonists systemically or into the paraventricular nucleus of the hypothalamus can cause appetite suppression in acute studies (Davies and Wellman 1992; Morien et al. 1993). In our lab, long-term treatment (8–9 weeks) with cirazoline, an α_{1A}AR-selective agonist, has not reduced food or water intake nor reduced body weight (Doze, Goldenstein, and Collette, unpublished data). To our knowledge, food intake has not been studied in CAM-α_{1A}AR or CAM-α_{1B}AR mice. However, because even modest caloric restriction can reduce body weight, we weighed cohorts of mice to determine whether transgenic activation of either α_1AR subtype decreased body weight with a subsequent increase in lifespan (Colman et al. 2009). There was no difference in body weight between CAM-α_{1A}AR and WT mice except one time point after weaning. Body weight of the CAM-α_{1B}AR animals was significantly lower when compared with that of WT mice after weaning and again after approximately 9 months of age. However, the lifespan of CAM-α_{1B}AR mice was decreased rather than increased, so the effect was not positive on lifespan. Food intake was not assessed during the longevity study, so we cannot definitively conclude that the α_{1A}AR-stimulated lifespan increase was not through a caloric reduction mechanism, but it seems unlikely because the CAM-α_{1A}AR animals were of normal weight.

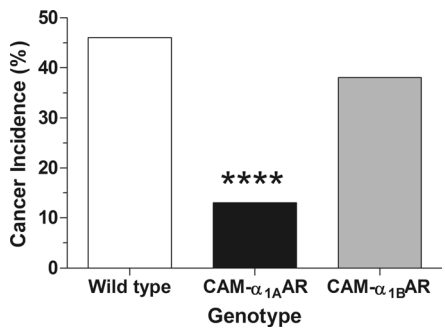

Fig. 3 Cancer incidence. CAM-α_{1A}AR (n=53) mice had a significantly lower incidence of cancer when compared with WT animals (n=70, p<0.0001). The incidence of cancer in CAM-α_{1B}AR mice did not differ from that of WT mice (n=34, p=0.7245). [CAM-α_{1A}AR lifespan curve reprinted with permission]

Previous studies of increased lifespan in mouse found a 15–80 % extension, depending on the intervention (Brown-Borg et al. 1996; Blüher et al. 2003). Shortened lifespan is not reported as often, but in certain transgenic models, death in utero or within the early postnatal period is not uncommon if the mutated gene is involved in critical developmental processes (Thyagarajan et al. 2003). In our study, CAM-α_{1B}AR mice had an 11 % decrease in lifespan when compared with WT animals, but the cause of the decrease is still unknown. Some genetic models with shortened lifespan showed accelerated aging or increased cancer incidence with subsequent lifespan decreases of 25–50 % (Rudolph et al. 1999; Keyes et al. 2005). We observed signs of accelerated aging including reduced body weight, alopecia, and spine curvature in our model, but did not examine other factors such as bone density or cellular senescence (de Boer et al. 2002; Sun et al. 2004). Cancer incidence was similar to WT mice. Another factor which must be considered in mouse models of aging is the background strain because the baseline lifespan is variable.

Table 2 Pathology of WT, CAM-α_{1A}AR, and CAM-α_{1B}AR mice at death

	B6CBA WT (n=70)	CAM-α_{1A}AR (n=53)	CAM-α_{1B}AR (n=34)
Cancerous	*45.7 %* (*32*)	*13.2 %* (*7*)	*38.2 %* (*13*)
Epithelial	25.7 % (18)	5.7 % (3)	23.5 % (8)
Hematological	18.6 % (13)	7.5 % (4)	8.8 % (3)
Mesenchymal	1.4 % (1)	0.0 % (0)	5.9 % (2)
Noncancerous	*54.3 %* (*38*)	*86.8 %* (*46*)	*61.8 %* (*21*)
Benign tumor			
Epithelial	7.1 % (5)	3.8 % (2)	0.0 % (0)
Hematological	2.9 % (2)	3.8 % (2)	11.8 % (4)
Mesenchymal	0.0 % (0)	3.8 % (2)	2.9 % (1)
Nontumor lesions	35.7 % (25)	49.1 % (26)	35.3 % (12)
No abnormal findings	8.6 % (6)	26.4 % (14)	11.8 % (4)

There have not been any studies yet on the lifespan of the B6CBA mouse, which is a cross between the C57BL/6 and CBA strains. The median age of C57BL/6 mice varies depending upon the environment but ranges from 682 to 930 days (Ikeno et al. 2005; Selman and Withers 2011; reviewed in Nadon et al. 2008). In a study of 31 inbred strains at Jackson Labs, C57BL/6J mice had median ages of 866 days for males and 901 days for females (Yuan et al. 2009). In the same study, CBA/J mice had median ages of 679 days for males and 644 days for females. Our WT B6CBA animals had a median age of 719 days, which is between the two strains, but slightly closer to the CBA/J median age.

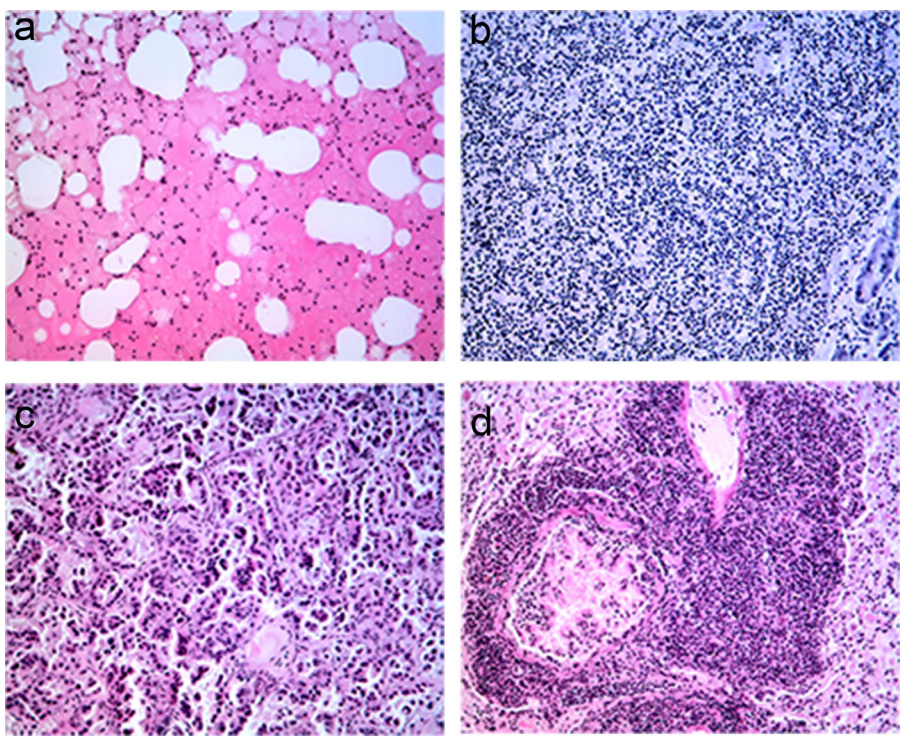

Fig. 4 Representative photomicrographs of the most common pathological findings at death. **a** Pulmonary edema in a CAM-α_{1A}AR mouse. **b** Lymphoma in a WT mouse. **c** Adenocarcinoma in a WT mouse. **d** Lymphoma of the lung in a CAM-α_{1B}AR mouse

Prior to this work, survival had been assessed in CAM-α_{1B}AR mice, then called T1 mice, for up to 70 weeks (Papay et al. 2002). However, no previous studies followed all animals until the natural date of death. In the current study, the survival of CAM-α_{1B}AR animals began to deviate from that of WT mice at approximately 9 months of age, the timing of which coincides with the pathological appearance of neurodegeneration and the age at which body weight begins to differ (Papay et al. 2002). The α_1AR antagonist terazosin protects these mice against the weight loss and neurodegeneration. Terazosin also increased survival rates in the T1 mice but the animals were not followed until natural death, so the effect of terazosin on full lifespan is unknown. The combination of that study and the current results suggests that the effects of α_{1B}AR over-activation can cause a shortened lifespan. The α-synucleinopathy induced by chronic activation of α_{1B}ARs is similar to multiple system atrophy (Papp et al. 1989; Zuscik et al. 2000). Multiple system atrophy can lead to early death in humans by sudden cardiopulmonary arrest or pneumonia (Papapetropoulos et al. 2007). In our study, there was no increased incidence of either event in CAM-α_{1B}AR mice. The mechanism by which α_{1B}AR activation can decrease lifespan is still unknown.

The sex differences in median and maximal lifespan in CAM-α_{1B}AR mice were an unexpected result, particularly because cancer incidence was not different for either sex. However, we did not examine cancer progression over time, which could have clarified whether cancer in the female mice was more aggressive with a quicker latency to death. We have not found sex differences in other studies using the CAM-α_{1B}AR mice (Doze et al. 2009). There is a dearth of data on sex differences in general in the scientific literature and only one published study on sex differences in relation to the adrenergic receptors (Novakova et al. 2010). In it, female mice had higher basal α_1AR receptor densities in the lung and immobilization stress decreased the level of all three α_1AR subtypes. In male mice, only the α_{1A}AR level was decreased following stress. In the current study, shortened lifespan in the female CAM-α_{1B}AR animals may have involved hormonal changes mediated by the α_{1B}AR. There are noradrenergic innervations to the breast and ovarian follicles, where norepinephrine release can increase the levels of estradiol and progesterone and a decrease of sympathetic input can slow tumor growth (Romeo et al. 1991; Piccinato et al. 2012). Furthermore, the norepinephrine reuptake inhibitor desipramine promotes breast cancer progression with α_2AR activation; however, phenylephrine, a non-selective α_1AR agonist, did not have an effect (Szpunar et al. 2013). It is possible that the α_{1B}AR is involved in hormone-related cancer progression, but further work should be done to clarify if there is a role for the receptor.

The decrease in lifespan in the CAM-α_{1B}AR mice is unlikely due to heart failure, which is not present in this mouse model. While animal studies suggest that chronic activation of the α_{1B}AR subtype may be "bad" or maladaptive for the heart, the amount of dysfunction does not lead to heart failure if overexpression is more physiologically relevant or the heart is not stress-induced. CAM-α_{1B}AR mice also have a mild cardiac hypertrophy, decreased cardiac output, some diastolic dysfunction, and inflammation. These conditions did not, however, progress to heart failure on their own (Zuscik et al. 2001; Yun et al. 2003). Other groups have demonstrated that myocyte-targeted CAM-α_{1B}AR also induces a mild hypertrophy when the receptor is only overexpressed 3-fold or less, as it is in our mouse model, but only progressed to heart failure after blood pressure overload (Milano et al. 1994). Therefore, chronic stimulation of the α_{1B}AR leads to hypertrophy and some cardiac dysfunction, but only induces heart failure when the receptor is artificially overloaded. As this aging study only used nonstressed mice, it is unlikely that CAM-α_{1B}AR mice die younger because of heart failure. Furthermore, in the present study, only one heart-related diagnosis was made in CAM-α_{1B}AR mice, a heart thrombus.

On the other hand, previous studies have suggested the CAM-α_{1A}AR mice are cardio-protected, which may contribute to its increased longevity (reviewed in Perez and Doze 2011). While CAM-α_{1A}AR mice also display cardiac hypertrophy as in CAM-α_{1B}AR mice, α_{1A}AR activation results in positive adaptation of the heart to protect against ischemic damage through preconditioning via cardiac protective IL-6 and JAK/STAT pathways or by preventing apoptosis due to increased glucose uptake (Rorabaugh et al. 2005; Papay et al. 2013, Perez, unpublished data). In the current study, the only heart abnormality found in CAM-α_{1A}AR mice was a case of endocarditis/myocarditis.

The decrease in lifespan in CAM-α_{1B}AR mice does not appear to be due to an increase in cancer incidence. In vitro, α_{1B}AR overexpression and activation results in focus formations (Allen et al. 1991). In addition, the cells form tumors when injected into immune-compromised mice. Due to the tumorigenic quality of transplanted α_{1B}AR-expressing cells, we hypothesized that CAM-α_{1B}AR mice would have an increased incidence of cancer. Surprisingly, there was no significant difference in cancer incidence between CAM-α_{1B}AR mice and WT animals. The α_{1B}AR can regulate the cell cycle through cdk-6- and cyclin E-associated kinases, but the α_{1B}AR effect on proliferation may be cell-type specific (Gonzalez-Cabrera et al. 2004). For example, in CHO cells transfected with the α_{1B}AR, the cell cycle was stopped when activated by phenylephrine (Shibata et al. 2003). In TRAMP cells which expressed mostly the α_{1B}AR subtype, doubling time was faster with decreasing α_{1B}AR and increasing α_{1A}AR and α_{1D}AR expression (Shi et al. 2007). However, the cancerous state is highly complex and does not solely depend on a dysregulation of the cell cycle. Ours is the first study to explore the effect of α_{1B}AR activation in cancer in an immunocompetent mouse strain.

In most cell types studied, the α_{1A}AR subtype increases the levels of the cdk inhibitor p27Kip1 and halts the cell cycle at the G1-S checkpoint (Shibata et al. 2003; Saeed et al. 2004; Gonzalez-Cabrera et al. 2004). In our study, there was a significant reduction in cancer incidence in CAM-α_{1A}AR mice, which could be due to this cell cycle stoppage. The cell type specificity of α_1AR subtype localization, downstream pathways, and subsequently physiological effects are imperative to the mechanism behind the reduction. For example, in human prostate cancer cells, the α_1AR-selective antagonist naftopidil stops growth through the same mechanism by which agonists halt the cycle, via p27Kip1 (Kanda et al. 2008). These contradictions are perplexing much as contradictory studies were prior to the delineation of the three α_1AR subtypes. The answers may lie in the regulators of G-protein signaling (RGS), reactive oxygen species, and cell type-specific downstream effects (Hu et al. 1999; Shi et al. 2006; Abramow-Newerly et al. 2006).

Previous studies have implicated other ARs in longevity function, but these studies indicated shortened lifespan due to disease onset rather than increased lifespan. β_2ARs promote aging and reduced lifespan mostly due to adverse effects on cardiac and pulmonary functions and its age-related impairment to regulate insulin secretion (Gao et al. 2003; Santulli et al. 2012; Santulli and Iaccarino 2013). However, it could also be due to polymorphisms in the receptor. For example, one study compared β_2AR polymorphisms and found two variants that were associated with increased longevity in the Han Chinese population (Zhao et al. 2012). These polymorphisms reduced translational efficiency and receptor expression in transfected cells, suggesting that decreased β_2AR function promoted longevity. In contrast, our results are the first to report that increasing AR activity would promote a longer lifespan, but specifically through the α_{1A}AR subtype.

Human longevity has continued to increase as sanitation, health care, and modern medicine have evolved. Ideally, increased longevity will also translate into improved health in later years. Cancer is one of the leading causes of death worldwide with approximately 50 % of cases occurring in persons older than 65 years of age. Current therapeutics are not ideal because they kill all dividing cells indiscriminately which can lead to uncomfortable and dangerous side effects. Preferential activation of the α_{1B}AR subtype has negative effects in the heart and brain and may increase proliferation in some cell types, while stimulating the α_{1A}AR shows positive effects in these areas. The current work is the first to show that chronic activation of the α_{1B}AR can shorten lifespan. Whether this is due to accelerated neurodegeneration and/or other disease process(es) is still unknown. Importantly, we found that long-term α_{1A}AR stimulation significantly decreases cancer incidence which may account in part for our previous finding that it increases lifespan. While more work is needed, it is clear that activating the α_1AR subtypes leads to differential effects. α_{1A}AR subtype-specific therapeutics may lead to improved cancer therapeutics with fewer adverse effects.

Acknowledgments This research was supported by an American Physiological Society Undergraduate Research Fellowship (M.J.L.), a University of North Dakota School of Medicine and Health Sciences Undergraduate Research Fellowship (H.M.A.), a National Science Foundation Graduate Research Fellowship DGE-0950693 (K.M.C.), a National Science Foundation Research Experiences for Undergraduates Site grant 0851869 (V.A.D.), an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20 GM103442 (D.A.S.), and a grant from the National Institute of Heart, Lung, and Blood R01-HL098279 (D.M.P.). The authors thank Dr. Holly Brown-Borg and Brianna Goldenstein for helpful comments on this manuscript.

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