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β-Adrenoceptor-$G_{\alpha s}$ Coupling Decreases with Age in Rat Aorta

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SUMMARY

β-Adrenoceptor (βAR) responsiveness, receptor density, receptor-G protein coupling, and the possible role of membrane fluidity in receptor-G protein coupling were investigated in the rat aorta with age. The βAR agonist isoproterenol (ISO) produced relaxation of KCl-induced aortic contractions by 97%, 21%, and 0% in aortae from 1-, 6-, and 24-month-old Fischer 344 rats, respectively. Forskolin completely relaxed the contractions at all ages. βAR density was determined in aortic membranes by saturation binding of ^{125}I-cyanopindolol (^{125}I-CYP). βAR density was 76, 52, and 47 fmol/mg of protein in 1-, 6-, and 24-month-old rats, respectively. To investigate βAR coupling to G proteins, displacement by ISO of ^{125}I-CYP binding was determined in aortic membranes in the presence and absence of the GTP analog guanosine-5′-(βγ-imido)triphosphate [Gpp(NH)p] (0.1 mM). The effect of Gpp(NH)p on the ISO displacement curve for ^{125}I-CYP binding was greatest in 1-month-old rats and decreased markedly with age. In 1-month-old aorta, in the absence of Gpp(NH)p the ISO displacement curve was biphasic and two affinity constants were determined (K_H = 0.061 μM and K_L = 2.4 μM). In the presence of Gpp(NH)p the ISO displacement curve was monophasic (K_d = 0.72 μM). In 6-month-old aorta, whereas an effect of Gpp(NH)p on the ISO displacement curve could still be observed [in the absence of Gpp(NH)p, K_H = 0.2 μM and K_L = 3.5 μM; in the presence of Gpp(NH)p, K_d = 0.83 μM], the affinity constant for high affinity agonist binding and the percentage of receptors with high affinity for agonist were decreased significantly. In 24-month-old aorta there was no effect of Gpp(NH)p on the ISO displacement curve and a single affinity constant was detected [0.7 μM and 0.8 μM in the presence and absence of Gpp(NH)p, respectively]. The presence of two affinity constants for ISO in 1- and 6-month-old aorta in the absence of Gpp(NH)p and single affinity constants in the presence of Gpp(NH)p presumably represent the G protein-coupled and uncoupled states of the βARs, which are not observed in 24-month-old aorta. The ability of the βAR to form the high affinity nucleotide-sensitive complex with the agonist was restored by treatment of the membranes with *cis*-vaccenic acid, which increases the fluidity of the membrane. The agonist-independent basal interactions of βARs with G proteins were examined using an immunoprecipitation approach. Aortic membranes were solubilized and then immunoprecipitated using specific antisera directed against the individual G protein α subunits ($G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha o}$, and $G_{\alpha q}$). βARs in the immunoprecipitate were then determined by measurement of the specific binding of ^{125}I-CYP. In solubilized preparations from 1-month-old aorta, βAR binding was immunoprecipitated by $G_{\alpha s}$-specific antiserum but not by $G_{\alpha i}$-, $G_{\alpha o}$-, or $G_{\alpha q}$-specific antiserum. $G_{\alpha s}$-specific antiserum did not immunoprecipitate βAR binding in solubilized preparations from 6- or 24-month-old aorta. These results indicate that βARs are coupled to $G_{\alpha s}$ in aortae from 1-month-old rats but that coupling is decreased or absent under basal and agonist-stimulated conditions in aortae from 6- and 24-month-old rats. These studies show that the decline in aortic βAR responsiveness with age is accompanied by decreased βAR density and by a loss of βAR-$G_{\alpha s}$ coupling, which may be due to a decrease in membrane fluidity.

Adrenergic neurotransmitters are important regulators of blood pressure. Whereas α-adrenoceptor stimulation increases blood vessel contraction and peripheral resistance, βAR stimulation causes vessel relaxation and decreased resistance. The balance between stimulation of these receptors has a major role in maintenance of blood pressure. Age-related alterations in vascular responsiveness to adrenergic neurotransmitters have been widely reported (1, 2). In several studies, decreased βAR responsiveness has been noted in blood vessels as a function of age (3–5). The loss of responsiveness to βAR stimulation may result in decreased relaxation of vascular tissues and increased peripheral resistance. Stimulation of βARs activates $G_{\alpha s}$, leading to activation of adenylyl cyclase and production of cAMP, which is largely responsible for βAR-mediated effects. Decreased vascular relaxation and cAMP production with age, in response to βAR agonists but not forskolin, acetylcholine, or nitroglycerin, have been reported (6, 7). Thus, it appears that blood vessels from older animals are capable of relaxation and production of cAMP when stimulated by means other than βAR agonists.

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ABBREVIATIONS: βAR, β-adrenoceptor; ISO, isoproterenol; CYP, cyanopindolol; Gpp(NH)p, guanosine-5′-(βγ-imido)triphosphate; PBS, phosphate-buffered saline.

This suggests that the age-related decrease in βAR responsiveness is related to a specific decrease in receptor signaling (βAR, $G_{\alpha s}$ protein, and/or βAR-$G_{\alpha s}$-cyclase coupling).

Decreased responsiveness to βAR stimulation with age has also been reported in other organs, including brain (8), lung (9), and heart (10). Proposed mechanisms for the decreased responses include changes at both the receptor and post-receptor levels (2, 11). Although some studies found decreased βAR density in rat adipose tissue (12) and several brain areas (13, 14), other studies found no change in lymphocytes (15), heart (16), and lung (17). Therefore, decreased βAR density may help to account for decreased βAR responsiveness in a tissue-dependent manner.

The agonist affinity and the high affinity state of the hormone-receptor-G protein complex decrease with age in several tissues, including heart (18), lung (9), lymphocytes (19), and parotid gland (20). In vascular smooth muscle, the proportion of βARs in the high affinity state for agonists has not been established, despite a pronounced decline in βAR responsiveness during aging. This study examines βAR expression, βAR-G protein coupling, and the possible role of membrane fluidity in βAR-G protein coupling in aortic membranes from 1-, 6-, and 24-month-old Fischer 344 rats.

Experimental Procedures

Animals. Male Fischer 344 rats (1, 6, and 24 months of age) were obtained from Harlan Sprague Dawley (Indianapolis, IN), where they are bred and maintained under the auspices of the National Institute on Aging. Upon receipt at this institution, animals were maintained for 1–2 weeks under barrier conditions comparable to those under which they were raised.

Aortic relaxation. Rats were decapitated and the aortae were removed and placed in an ice-cold physiological salt solution (120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 25 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 11 mM glucose, 0.024 mM EDTA). Vessels were cleaned of fat and connective tissue and cut into rings 3 mm wide. Aortic ring segments were mounted at 37° in 15-ml organ baths, using stainless steel hooks connected by fine gold chain at the bottom to a stationary glass rod attached to the bath and at the top to a Grass model FT.03 force-displacement transducer, and were bubbled continuously with 95% O_2/5% CO_2. Responses were recorded on a Grass model 7 polygraph. Rings were equilibrated for 1 hr at a resting tension of 1.5 g. Rings were contracted with a depolarizing solution that had the same composition as described above except that 60 mM NaCl was replaced isotonically with KCl. After contraction reached a plateau, concentration-response curves for relaxation were determined by cumulatively increasing the concentration of ISO or forskolin. All experiments with ISO were conducted in the presence of 1 μM prazosin, a selective α_1-adrenergic receptor antagonist. Relaxation responses are expressed as percentage of maximum contraction.

Binding studies. Aortae were isolated as described above, placed in PBS (20 mM NaH_2PO_4/Na_2HPO_4 buffer, pH 7.6, containing 154 mM NaCl), homogenized using a glass/glass homogenizer, and centrifuged at 500 × *g* for 10 min at 4°. The supernatant was centrifuged at 100,000 × *g* for 60 min at 4°. The resulting pellet was resuspended, rehomogenized, and then recentrifuged under the same conditions. The final pellet was resuspended in PBS and used for binding assays. Protein content was measured according to the method of Bradford (21).

βAR density was measured by saturation binding of ^{125}I-CYP (2200 Ci/mmol; NEN). The membranes were incubated with different concentrations (nine to 14) of ^{125}I-CYP for 60 min at 25°. The incubation volume was 0.2 ml of PBS containing 0.01 mg/ml bovine serum albumin (20–30 μg of membrane protein/tube). For competition binding experiments, membranes were incubated with ^{125}I-CYP (40–50 pM) in the presence or absence of 14 or 15 different concentrations of ISO, with or without Gpp(NH)p (0.1 mM), for 60 min at 25°. The incubation volume for competition assays was 0.2 ml of PBS containing 0.01 mg/ml bovine serum albumin and 5 mM $MgCl_2$. Reactions were terminated by rapid filtration using a Brandel cell harvester and Whatman GF/C filters. Filters were washed four times with 4 ml of ice-cold PBS. The filter-bound radioactivity was determined in a Beckman γ counter. Nonspecific binding was defined as binding in the presence of 1 mM ISO or 1 μM propranolol, with identical results. Assays were conducted in duplicate.

***Cis*-vaccenic acid treatment.** Aortic membranes were treated with *cis*-vaccenic acid as described previously (22, 23). Briefly, *cis*-vaccenic acid (Sigma) was dissolved at a concentration of 100 mM in absolute ethanol. Aortic membranes were incubated with 1 mM *cis*-vaccenic acid or an equal volume of ethanol for 1 hr at 4°, with shaking. The final ethanol concentration did not exceed 1% (v/v), and there was no significant difference in the binding results obtained using membranes treated with ethanol, compared with untreated membranes.

Solubilization of aortic membranes. Aortic membranes were solubilized by a modification of a previously described procedure (24, 25). Briefly, aortic membranes were prepared as described above. They were then solubilized by gentle end-over-end shaking for 60 min at 4° in PBS containing 1.5% digitonin, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin. The sample was centrifuged at 100,000 × *g* for 60 min at 4° and the supernatant was used as the soluble membrane fraction. The pellet was also collected for determination of the insoluble βARs and G protein α subunits remaining in the membranes. After solubilization of aortic membranes, 40–50% of the initial βAR binding was detected in soluble form at all ages. The solubilized βARs were detected by measuring ^{125}I-CYP binding as described above, except that reactions were terminated by rapid filtration using Whatman GF/F filters that had been presoaked in 0.5% polyethylenimine.

Immunoprecipitation of G protein α subunits. Solubilized G protein α subunits were immunoprecipitated as described previously (26, 27). Soluble membrane protein (8–10 fmol of βAR; an equal amount of βARs for each age) was incubated with an appropriate dilution of G_α-specific antiserum overnight in a rotatory shaker at 4°. Nonimmune serum at the same dilution was used as a control. Appropriate dilution was determined when no further immunoprecipitation was observed at a higher concentration of the antiserum (the maximum concentration was 1/50). One hundred microliters of a 1:1 suspension of Protein A-Sepharose beads (CL-4B; Sigma), prewashed three times and diluted in PBS, were added to the samples and incubated overnight in a rotary shaker at 4°. The samples were centrifuged at 10,000 × *g* for 3 min and the pellet was resuspended in PBS and recentrifuged as described above. The immunoprecipitate was resuspended in PBS and βARs were detected by measurement of ^{125}I-CYP binding, as described above. In several samples, the immunoprecipitate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting to confirm the identity of the precipitated G protein α subunits. In several experiments, the immunoprecipitate was incubated with 0.1 mM Gpp(NH)p for 60 min at 25° and then centrifuged at 10,000 × *g* for 3 min, the pellet was resuspended in PBS, and βARs in the immunoprecipitate were determined using the radioligand binding assay described above.

Immunoblots. Aortic membranes, solubilized membranes, or membrane immunoprecipitates were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (28) and then transferred electrophoretically to nitrocellulose membranes. Immunoblotting was performed using the antisera RM/1 ($G_{\alpha s}$-specific), AS/7 ($G_{\alpha i}$-specific), GC/2 ($G_{\alpha o}$-specific), and QL ($G_{\alpha q/11}$-specific) (1/1000 dilutions; NEN) and enhanced chemiluminescence detection, as described previously (29). Briefly, nitrocellulose membranes were in-

cubated overnight at 4° in PBS containing 3% bovine serum albumin and 8% nonfat dry milk. Blots were washed several times with PBS and then incubated with antisera at room temperature for 1–2 hr, with shaking. Blots were then washed several times with PBS and incubated with horseradish peroxidase-labeled donkey anti-rabbit IgG (Amersham) for 1 hr at room temperature. Blots were washed several times with PBS, incubated with enhanced chemiluminescence Western blotting reagent (Amersham) for 1 min, and exposed to X-ray film for 15–45 sec.

Data analysis. Radioligand binding data were analyzed with the LIGAND program (30). A model for two binding sites was applied only when the LIGAND program fitted the data significantly better ($p < 0.05$), compared with the model for one binding site. The means ± standard errors from at least three different experiments are given. Differences were determined by analysis of variance and *post hoc* analysis for multiple comparisons. A p value of <0.05 was considered significant.

Results

Aortic relaxation. In 1-month-old aorta, ISO effectively relaxed KCl-induced contraction, but this relaxation declined markedly in 6-month-old aorta and was completely absent in 24-month-old aorta (Fig. 1). Forskolin completely relaxed KCl-induced aortic contraction at all ages, and there were no differences in the concentration-response curves between ages (Fig. 1).

Binding studies. βAR density was 76 ± 7, 52 ± 4, and 47 ± 4 fmol/mg of protein in 1-, 6-, and 24-month-old aorta, respectively, and was significantly higher in 1-month-old aorta, compared with the other ages (Fig. 2). The affinity constant ($-\log K_d$) for ^{125}I-CYP was 10.5 ± 0.5, 10.6 ± 0.2, and 10.5 ± 0.1, respectively, and was not different among the ages. The effect of Gpp(NH)p on the agonist displacement curves for ISO was greatest in 1-month-old rats and decreased markedly with age (Fig. 3A). In 1-month-old aorta, in the absence of Gpp(NH)p two binding sites were detected by ISO, whereas in the presence of Gpp(NH)p the displacement curve was monophasic and a single site was detected (Fig. 3A; Table 1). In 6-month-old aorta, there was an effect of Gpp(NH)p on the displacement curve for ISO and two sites were detected with ISO in the absence of Gpp(NH)p, whereas in the presence of Gpp(NH)p one site was found (Fig. 4A; Table 1). There was no effect of Gpp(NH)p on the displacement curve for ISO in 24-month-old aorta and a single site was detected (Fig. 5A; Table 1). The affinity constant for ISO in the presence of Gpp(NH)p was not different among the ages (Table 1). In the absence of Gpp(NH)p, the high affinity constant was significantly lower (4-fold) in 1-month-old aorta than in 6-month-old aorta (Table 1). The distribution of high and low agonist affinity binding sites for βARs also changed with age, such that by 24 months all of the receptors were in the low affinity state (Table 1). Based on the number of βARs and the percentage of high agonist affinity sites, the absolute number of high affinity sites was estimated and found to decline markedly, from 49 fmol/mg of protein at 1 month of age to 21 fmol/mg of protein at 6 months and to 0 at 24 months.

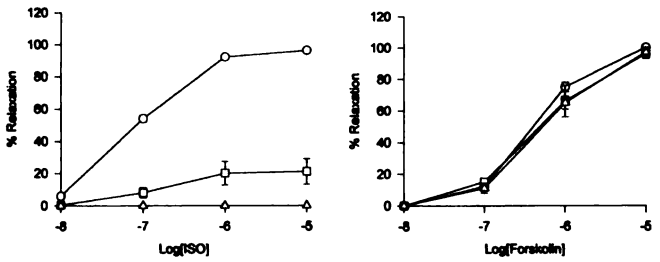

Fig. 1. ISO- and forskolin-stimulated relaxation of aortic ring segments from 1- (○), 6- (□), and 24-month-old (△) rats. Data represent means and standard errors determined from five or six animals in each group.

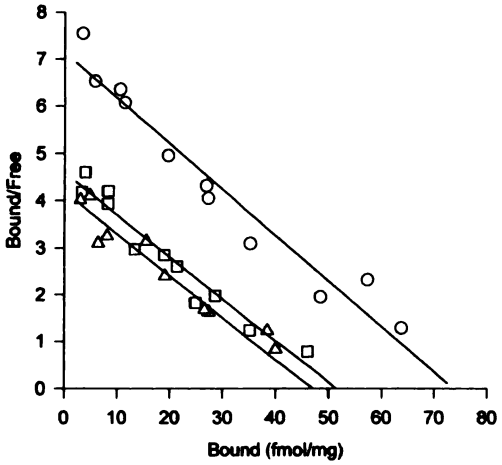

Fig. 2. Saturation binding of ^{125}I-CYP in aortic membranes from 1- (○), 6- (□), and 24-month-old (△) rats. Results are representative of three independent experiments.

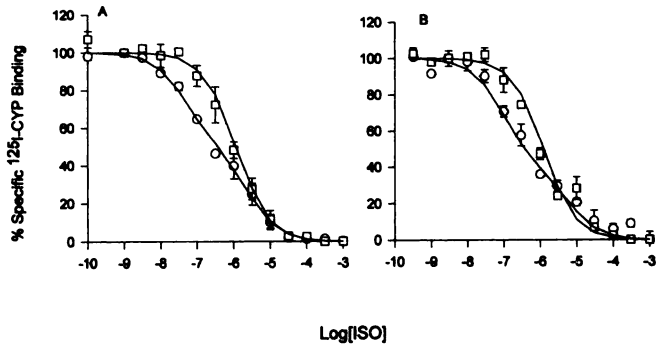

Fig. 3. Inhibition by ISO of ^{125}I-CYP binding to control (A) and *cis*-vaccenic acid-treated (B) aortic membranes from 1-month-old rats. Aortic membranes were incubated with ^{125}I-CYP and various concentrations of ISO in the absence (○) or presence (□) of 0.1 mM Gpp(NH)p. Data represent means and standard errors of determinations from three or four separate experiments.

Effects of *cis*-vaccenic acid treatment. After treatment with *cis*-vaccenic acid, the affinity constant for high affinity agonist binding (0.06–0.03 μM) and the percentage of high affinity binding sites (64–65%) did not change in 1-month-old aorta (Fig. 3B; Tables 1 and 2). In contrast, *cis*-vaccenic acid decreased the high affinity constants in 6-month-old (0.03 μM) and 24-month-old (0.05 μM) aorta to values comparable to those in the 1-month-old aorta (0.03 μM) (Figs. 3B, 4B, and 5B; Tables 1 and 2). Treatment with *cis*-vaccenic acid increased the percentage of high affinity binding sites from 40 to 58% in 6-month-old aorta and from 0 to 45% in 24-month-old aorta (Fig. 4B; Tables 1 and 2).

Immunoprecipitation. Immunoblot analyses revealed single bands for $G_{\alpha o}$ (39 kDa), $G_{\alpha i}$ (41 kDa), and $G_{\alpha q}$ (42 kDa) and two bands for $G_{\alpha s}$ (45 and 52 kDa) in solubilized aortic membrane preparations from 1-, 6-, and 24-month-old rats (Fig. 6). In 1-month-old aorta, a 1/200 dilution of $G_{\alpha s}$-specific

TABLE 1

ISO affinity constants in the presence or absence of Gpp(NH)p, in 1-, 6-, and 24-month-old aortae

K_L and K_H are affinity constants obtained from a two-binding site analysis, and K_d is the affinity constant obtained from one-binding site analysis. R_H and R_L are the percentages of receptors in the high and low affinity binding states, respectively.

	K_H	K_L	K_d	R_H	R_L
	μM	μM	μM	%	%
1-month-old					
−Gpp(NH)p	0.06 ± 0.02	2.4 ± 1.3		64 ± 5	36 ± 3
+Gpp(NH)p			0.7 ± 0.2		
6-month-old					
−Gpp(NH)p	0.2 ± 0.05[a]	3.5 ± 3		40 ± 4[b]	60 ± 6[c]
+Gpp(NH)p			0.8 ± 0.2		
24-month-old					
−Gpp(NH)p			0.8 ± 0.15	0	100
+Gpp(NH)p			0.7 ± 0.3		

[a] Significantly higher K_H value, compared with 1-month-old aorta ($p < 0.05$).
[b] Significantly lower R_H, compared with 1-month-old aorta ($p < 0.05$).
[c] Significantly higher R_L, compared with 1-month-old aorta ($p < 0.05$).

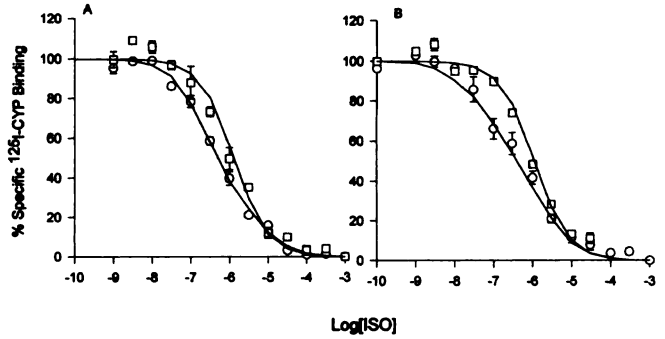

Fig. 4. Inhibition by ISO of ^{125}I-CYP binding to control (A) and *cis*-vaccenic acid-treated (B) aortic membranes from 6-month-old rats. Aortic membranes were incubated with ^{125}I-CYP and various concentrations of ISO in the absence (○) or presence (□) of 0.1 mM Gpp(NH)p. Data represent means and standard errors of determinations from three or four separate experiments.

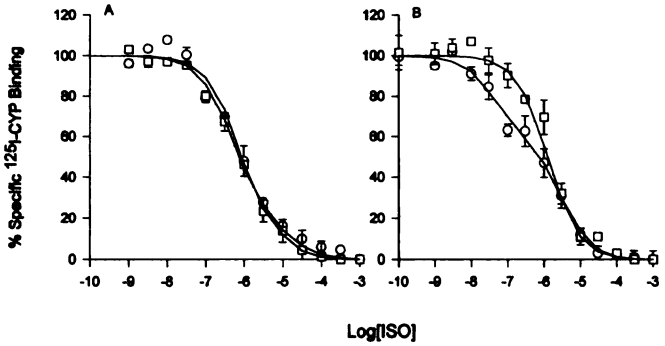

Fig. 5. Inhibition by ISO of ^{125}I-CYP binding to control (A) and *cis*-vaccenic acid-treated (B) aortic membranes from 24-month-old rats. Aortic membranes were incubated with ^{125}I-CYP and various concentrations of ISO in the absence (○) or presence (□) of 0.1 mM Gpp(NH)p. Data represent means and standard errors of determinations from three or four separate experiments.

antiserum was sufficient to immunoprecipitate βARs, whereas dilutions of up to 1/50 of $G_{\alpha o}$-, $G_{\alpha q/11}$-, or $G_{\alpha i}$-specific antiserum did not precipitate βARs (Fig. 7). None of the α subunit-specific antisera, including anti-$G_{\alpha s}$, immunoprecipitated βARs from 6- or 24-month-old solubilized aorta (Fig. 7), despite immunoprecipitation of the $G_{\alpha s}$ protein with anti-$G_{\alpha s}$ (data not shown). In 1-month-old aorta, 30 ± 5% of the soluble βARs were immunoprecipitated. To confirm the specificity of the immunoprecipitation, the precipitate obtained from 1-month-old aorta using $G_{\alpha s}$-specific antiserum was subjected to immunoblot analysis. Two bands were detected with $G_{\alpha s}$-specific antiserum (45 and 52 kDa), whereas no immunoreactive bands were detected by $G_{\alpha o}$-, $G_{\alpha q/11}$-, or $G_{\alpha i}$-specific antiserum in the anti-$G_{\alpha s}$ precipitate (Fig. 8).

Discussion

The present results and previous studies have clearly established that βAR-mediated vascular relaxation is greatly reduced as a function of age (3, 4, 7). Decreased βAR responsiveness with age could be due to changes in the receptor, the G protein, or the downstream signaling elements. However, these results and previous studies (6, 31) have shown that maximal relaxant effects of forskolin and dibutyryl-cAMP do not change with age. Furthermore, whereas ISO-stimulated accumulation of cAMP decreases with age, forskolin-stimulated accumulation of cAMP does not decrease (6, 29), and blood vessel relaxation in response to agents that act independently of cAMP does not change with age (6, 7). These results implicate the receptors, the G proteins, or the receptor-G protein-effector interactions in the decline in βAR responsiveness. In the present investigation we examined βAR density and βAR-G protein coupling in the aorta as a function of age.

Decreased receptor density is associated with desensitization of agonist-stimulated responses (32). The present results show that aortic βAR density declines between 1 and 6 months of age, with no further decrease at 24 months. Several authors have reported that there is no change in βAR density in the rat heart between 6 and 24 months of age (16, 33), which is similar to the present findings in the aorta. Age-related decreases in βAR densities have been reported in heart (34), adipocytes (12), and brain (13) in several studies using 3- and 24-month-old rats. The results of the present studies, together with those previous reports, indicate that βAR density does decrease with age in several organs. Decreased receptor density may help to account for the decrease in βAR responsiveness. However, additional alterations must take place to account for the complete loss of βAR-mediated relaxation in the 24-month-old aorta despite the continued presence of 62% of the βARs.

Agonist binding to βARs is thought to complete the formation of a hormone-βAR-G_s protein complex. The coupled receptors display high affinity binding of agonist, and the for-

TABLE 2

After treatment with *cis*-vaccenic acid, ISO affinity constants in the presence or absence of Gpp(NH)p, in 1-, 6-, and 24-month-old aortae

K_L and K_H are affinity constants obtained from a two-binding site analysis, and K_d is the affinity constant obtained from one-binding site analysis. R_H and R_L are the percentages of receptors in the high and low affinity binding states, respectively.

	K_H	K_L	K_d	R_H	R_L
	μM	μM	μM	%	%
1-month-old					
−Gpp(NH)p	0.03 ± 0.02	2.3 ± 2.1		64 ± 6	35 ± 4
+Gpp(NH)p			2.1 ± 1.8		
6-month-old					
−Gpp(NH)p	0.03 ± 0.05	4 ± 3		58 ± 4	42 ± 6
+Gpp(NH)p			1 ± 0.4		
24-month-old					
−Gpp(NH)p	0.05 ± 0.03	2 ± 1		45 ± 5[a]	54 ± 5[b]
+Gpp(NH)p			2 ± 1.2		

[a] Significantly lower R_H, compared with 1-month-old aorta ($p < 0.05$).
[b] Significantly higher R_L, compared with 1-month-old aorta ($p < 0.05$).

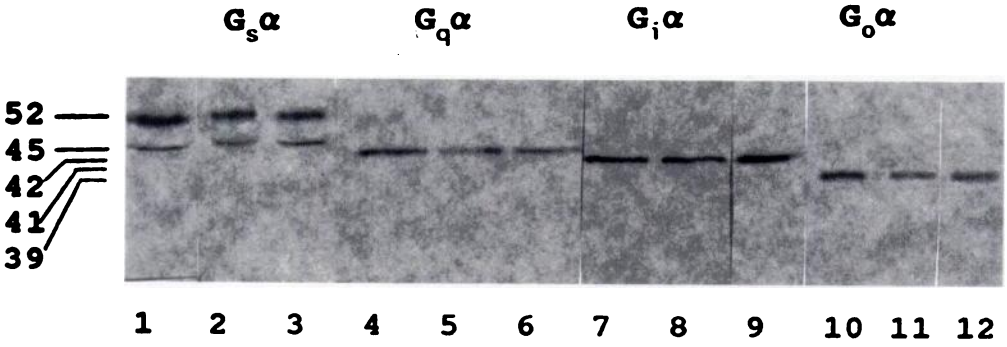

Fig. 6. Western blot analyses of $G_{\alpha s}$, $G_{\alpha q}$, $G_{\alpha i}$, and $G_{\alpha o}$ in solubilized aortic membranes from 1- (*lanes 1, 4, 7,* and *10*, respectively), 6- (*lanes 2, 5, 8,* and *11*), and 24-month-old (*lanes 3, 6, 9,* and *12*) rats.

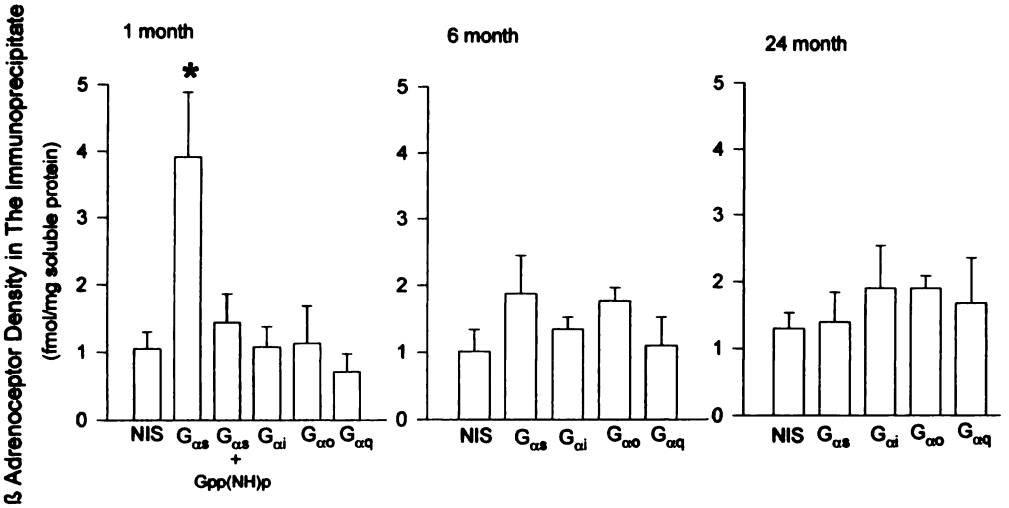

Fig. 7. Immunoprecipitation of βAR, by antisera to $G_{\alpha s}$, $G_{\alpha o}$, $G_{\alpha q}$, and $G_{\alpha i}$ and by nonimmune serum (*NIS*), from solubilized aortic membranes from 1-, 6-, and 24-month-old rats. Values are the means and standard errors of six or seven separate experiments. *, Significant difference, compared with the other groups (analysis of variance and Newman Keuls test, $p < 0.05$).

mation of the ternary complex enhances the binding of guanine nucleotides and subsequent stimulation of adenylyl cyclase activity (35, 36). Uncoupled receptors are less able to form the active ternary complex. The hormone-βAR-G_s complex and high and low affinity states of the βAR can be determined from agonist competition curves. The high affinity state of the βAR for ISO binding progressively decreased between 1 and 6 months of age and was lost by 24 months of age. There was a progressive decrease with age in the ability of Gpp(NH)p to shift agonist binding from high to low affinity. The high affinity binding constant was 4 times lower in 1-month-old aorta, compared with 6-month-old aorta, and in the 1-month-old aorta the percentage of high affinity sites was 65%, compared with 40% in the 6-month-old aorta. The high affinity constant for agonist binding and the percentage of high affinity binding sites are both important measures of agonist-stimulated production of the ternary complex (37, 38). In the presence of GTP or GTP analogs, G proteins and receptors dissociate, leaving the receptors in a low affinity state for agonist binding (35, 36). The progressive loss of this effect, the progressive decrease in the percentage of high affinity agonist binding sites, and the decrease in the affinity constant for high affinity agonist binding between 1 and 6 months of age indicate that βARs become uncoupled. Each may contribute to the progressive decline in βAR-mediated aortic relaxation. Several laboratories have reported age-related shifts in the proportion of high and low affinity βAR binding sites in leukocytes (39), lung (9), and heart (40).

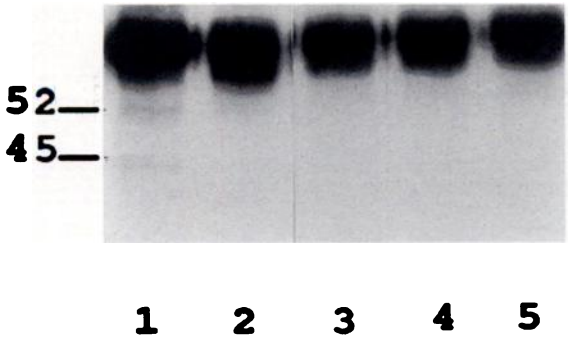

Fig. 8. Immunoprecipitation of G protein α subunits by $G_{\alpha s}$-specific antiserum in solubilized aortic membranes from 1-month-old rats. *Lane 1*, $G_{\alpha s}$; *lane 2*, nonimmune serum; *lane 3*, $G_{\alpha q}$; *lane 4*, $G_{\alpha i}$; *lane 5*, $G_{\alpha o}$.

Another direct and convincing demonstration was obtained in the immunoprecipitation studies. βARs coupled to $G_{\alpha s}$ could be co-precipitated from solubilized aortae of 1-month-old rats with a $G_{\alpha s}$-specific antiserum but not with a $G_{\alpha o}$-, $G_{\alpha q/11}$-, or $G_{\alpha i}$-specific antiserum. Thus, it appears that in young rats βARs are coupled to G_s, as expected, and not to G_o, $G_{q/11}$, or G_i. In 6- and 24-month-old rats βAR could not be co-precipitated from solubilized aortae using $G_{\alpha s}$-specific antiserum. This age effect is not due to a loss of $G_{\alpha s}$ protein, because the present and previous results indicate that $G_{\alpha s}$ levels, detected in aortic membranes with Western blots, are not altered during aging (29). Taken together, these results show that βARs become uncoupled from G_s proteins with age.

Precipitation of the βAR-$G_{\alpha s}$ complex measures basal βAR-$G_{\alpha s}$ interaction. The failure of βARs and $G_{\alpha s}$ to co-precipitate from solubilized 6- or 24-month-old aorta suggests a decrease or the absence of the βAR-$G_{\alpha s}$ complex under basal conditions. There is a decrease with age in both high affinity binding sites for ISO and basal βAR-$G_{\alpha s}$ interaction, and both are affected by Gpp(NH)p. The decreased basal coupling of βAR and $G_{\alpha s}$ may therefore underlie the age-related decrease in high affinity binding of ISO and the decrease in ISO-stimulated vascular relaxation. The results also suggest that basal βAR-$G_{\alpha s}$ coupling may be related to the ability of agonist to form the hormone-βAR-G_s complex, which ultimately elicits the physiological response. In 6-month-old aorta, significant βAR-$G_{\alpha s}$ co-precipitation was not detected, indicating decreased or abolished interaction between βAR and $G_{\alpha s}$ under basal conditions. At that age it was still possible to detect agonist-stimulated relaxation and high affinity agonist binding, although both were reduced, compared with 1-month-old aorta. This indicates that the basal interaction between βAR and $G_{\alpha s}$ is substantially decreased or abolished but agonist binding to βAR can still produce functional βAR-$G_{\alpha s}$ coupling in 6-month-old aorta.

Previous studies reported that aortic relaxation mediated by adenosine A_2 receptors, which are similar to βARs in that they are coupled to G_s and activate cAMP formation, also declines with age (16). Impairment of both βAR and A_2 adenosine receptor responses in the older aortae suggests that there may be a common mechanism, such as a change in receptor phosphorylation, a post-translational modification of $G_{\alpha s}$, or a change in membrane fluidity, that affects multiple receptors. Decreased membrane fluidity with age has been reported in aorta and several other tissues (41–44). Increased membrane fluidity enhances coupling of βARs to G proteins and facilitates βAR-mediated activation of adenylyl cyclase, whereas decreased membrane fluidity causes decreased βAR-G protein interaction and decreased βAR responsiveness (22, 23, 45, 46). To investigate the importance of decreased membrane fluidity in modulating the formation of the high affinity nucleotide-regulated complex of agonist and receptor, aortic membranes were treated with *cis*-vaccenic acid. Previous studies showed that *cis*-vaccenic acid can increase membrane fluidity and receptor-G protein coupling, indicating that a fluid membrane environment is a prerequisite for lateral diffusion in the membrane and successful coupling of the βAR (22, 23, 45, 46). In this study, *cis*-vaccenic acid completely restored the increased affinity constant for high affinity agonist binding in 6- and 24-month-old aorta to the level observed in 1-month-old aorta. Furthermore, the percentage of βARs in the high affinity state was completely restored in 6-month-old aorta and was partially restored in 24-month-old aorta. These results suggest that a change in the fluidity of the membrane environment may be a major factor in the decline in βAR-$G_{\alpha s}$ coupling and vascular relaxation with age.

In summary, these studies show that the decline in aortic βAR responsiveness with age is accompanied by decreased βAR density and a loss of βAR-$G_{\alpha s}$ coupling. Decreased coupling is evidenced both by the loss of high affinity agonist binding and by the absence of precoupled βAR-$G_{\alpha s}$ complexes. Decreased membrane fluidity may contribute to the decrease in βAR-$G_{\alpha s}$ coupling. Additional studies will be required to determine whether functional βAR-effector coupling can be restored by increasing the membrane fluidity, to determine whether other receptors are similarly affected, and to determine the nature of the changes in the membrane environment with age.

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