Altered autonomic control in conscious transgenic rabbits with overexpressed cardiac Gsα

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Nishizawa T, Shen YT, Rossi F, Hong C, Robbins J, Ishikawa Y, Sadoshima J, Vatner DE, Vatner SF. Altered autonomic control in conscious transgenic rabbits with overexpressed cardiac Gsα. Am J Physiol Heart Circ Physiol 292: H971–H975, 2007; doi:10.1152/ajpheart.00791.2006.—Both enhanced sympathetic drive and altered autonomic control are involved in the pathogenesis of heart failure. The goal of the present study was to determine the extent to which chronically enhanced sympathetic drive, in the absence of heart failure, alters reflex autonomic control in conscious, transgenic (TG) rabbits with overexpressed cardiac Gsα. Nine TG rabbits and seven wild-type (WT) littermates were instrumented with a left ventricular (LV) pressure micromanometer and arterial catheters and studied in the conscious state. Compared with WT rabbits, LV function was enhanced in TG rabbits, as reflected by increased levels of LV dP/dt (5,600 ± 413 vs. 3,933 ± 161 mmHg/s). Baseline heart rate was also higher (P < 0.05) in conscious TG (247 ± 10 beats/min) than in WT (207 ± 10 beats/min) rabbits and was higher in TG after muscarinic blockade (281 ± 9 vs. 259 ± 8 beats/min) or combined β-adrenergic receptor and muscarinic blockade (251 ± 6 vs. 225 ± 9 beats/min). Bradycardia was blunted (P < 0.05), whether induced by intravenous phenylephrine (arterial baroreflex), by cigarette smoke inhalation (nasopharyngeal reflex), or by veratrine administration (Bezold-Jarisch reflex). With veratrine administration, the bradycardia in TG rabbits was reported previously (11). Briefly, the transgene was injected into fertilized oocytes of New Zealand White rabbits. The TG rabbits was reported previously (11). Briefly, the transgene was injected into fertilized oocytes of New Zealand White rabbits. The transgene consisted of the β-myosin heavy-chain promoter linked to a Gsα cDNA coding. The rabbits mated after reaching early adulthood (5–6 mo). TG and wild-type (WT) rabbits were born at nearly a 1:1 ratio, and no premature death was observed.

Preparation of Gsα TG rabbit. The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, the National Research Council (1996), and the studies were approved by the New Jersey Medical School Institutional Animal Care and Use Committee. The generation of cardiac-specific, Gsα-overexpressed TG rabbits was reported previously (11). Briefly, the transgene was injected into fertilized oocytes of New Zealand White rabbits. The transgene consisted of the β-myosin heavy-chain promoter linked to a Gsα cDNA coding. The rabbits mated after reaching early adulthood (5–6 mo). TG and wild-type (WT) rabbits were born at nearly a 1:1 ratio, and no premature death was observed.

Both enhanced sympathetic drive and altered autonomic control are involved in the pathogenesis of heart failure (HF) (3, 15). Most of the prior work on autonomic control in HF has concentrated on understanding the depressed regulation of high-pressure arterial baroreflexes (6, 14, 16). Less is known regarding other reflexes; indeed, it has been proposed that low-pressure reflexes, e.g., the Bezold-Jarisch reflex, are not depressed in HF (4). It is also recognized that elevated sympathetic tone, per se, can elicit altered autonomic control (8, 13). However, there are relatively few models of chronically elevated sympathetic drive, except for transgenic (TG) mouse models, many of which develop cardiomyopathy (5, 7, 9, 15).

Understanding whether altered reflex control can be elicited by chronically enhanced sympathetic drive in the absence of cardiac decompensation could be important for understanding the pathogenesis of HF. A limitation of prior studies examining enhanced sympathetic drive induced by exercise or intravenous sympathomimetic amines is that all vascular beds, including the brain, are affected. The TG rabbit model does not have this limitation.

The major goal of the present investigation was to examine three different reflex pathways (arterial baroreflex, Bezold-Jarisch reflex, and nasopharyngeal reflex) on cardiac function, focusing primarily on heart rate (HR) regulation, in a situation of chronically enhanced sympathetic drive, in the absence of cardiac decompensation or HF. This study was conducted in conscious TG rabbits, where the enhanced sympathetic drive was derived from overexpressed Gsα in the heart. The rabbits with overexpressed cardiac Gsα (11), in contrast to the mouse model (12), do not display cardiomyopathy even after 16 mo of elevated HR and contractility, potentially due to an accompanying increase in cardiac Giα.

MATERIALS AND METHODS

Preparation of Gsα TG rabbit. The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, the National Research Council (1996), and the studies were approved by the New Jersey Medical School Institutional Animal Care and Use Committee. The generation of cardiac-specific, Gsα-overexpressed TG rabbits was reported previously (11). Briefly, the transgene was injected into fertilized oocytes of New Zealand White rabbits. The transgene consisted of the β-myosin heavy-chain promoter linked to a Gsα cDNA coding. The rabbits mated after reaching early adulthood (5–6 mo). TG and wild-type (WT) rabbits were born at nearly a 1:1 ratio, and no premature death was observed.

Surgical instrumentation and physiological measurements. Nine adult TG rabbits (8–12 mo) and seven age-matched WT rabbits were tranquilized with ketamine hydrochloride (2–3 mg/kg iv). General anesthesia was induced by administration of thiamylal sodium (5–10 mg/kg iv) and maintained with isoflurane (0.5–1.5 vol/100 ml in oxygen). The chest was open via the fourth left intercostal space by use of sterile surgical technique. A miniature solid-state pressure gauge (Konigsberg Instruments) was inserted into the left ventricle (LV) through the apex for measurements of LV pressure and LV dP/dt. A fluid catheter was implanted in the descending thoracic aorta to measure arterial pressure. The catheter and wire were externalized...
between the scapulae, the incision was closed in layers, and air was evacuated from the chest.

Arterial pressure was measured by strain gauge manometers connected to the fluid-filled catheters. The solid-state LV pressure gauge was cross-calibrated in vitro and in vivo with aortic pressure. LV dP/dt was obtained by electronically differentiating the LV pressure signal. ECG limb lead electrodes were attached to measure HR. Because of technical problems, LV pressure was not obtained in three TG and one WT rabbits.

Experiments were initiated at least 1 wk after recovery from surgical instrumentation. Conscious rabbits were placed in a sling, and hemodynamics were recorded on a digital recorder (PC216Ax; Sony Precision Technology) and played back onto a multiple-channel strip chart (Astro-Med). A venous catheter was inserted in the ear vein for drug administration. The rabbit was allowed to rest in the sling for 10–30 min. After stable HR and aortic pressure measurements were achieved, baseline hemodynamics and ECG were recorded for 10 min.

Blocking the autonomic nervous system was achieved by administration of atropine (0.2 mg/kg iv) and propranolol (1 mg/kg iv). On a separate day, arterial baroreflex sensitivity was examined, which was induced by administration of nitroprusside (80 μg/kg iv) and phenylephrine (50 μg/kg iv). In addition, the nasopharyngeal reflex and Bezold-Jarisch reflex were also tested on a different day. The nasopharyngeal reflex was induced by cigarette smoke inhalation (17, 18) (Fig. 1). The Bezold-Jarisch reflex was stimulated by administration of veratrine (40 μg/kg iv).

Data analysis. All data are reported as means ± SE. The data between the TG and WT rabbits were compared by Student’s group t-test. The responses to autonomic blockades were analyzed by one-way ANOVA for repeated measurements. A value of P < 0.05 was taken as the minimum level of significance.

RESULTS

Baseline values of HR, LV systolic pressure, and LV dP/dt in the presence and absence of autonomic blockade are shown in Table 1 in conscious TG and WT rabbits. Baseline HR was higher (P < 0.05) in conscious TG (247 ± 10 beats/min) than in WT rabbits (207 ± 10 beats/min). In the presence of muscarinic receptor blockade with atropine, HR was higher (P < 0.05) in the TG (281 ± 9 beats/min) than in the WT (259 ± 8 beats/min) rabbits. After combined muscarinic and β-adrenergic receptor blockades, the HR was still higher (P < 0.05) in the TG rabbits (251 ± 6 beats/min) than in WT rabbits (225 ± 9 beats/min). The baseline value of LV systolic pressure in the TG rabbits (92 ± 2 mmHg) was greater (P < 0.05) than in the WT rabbits (80 ± 2 mmHg). LV dP/dt was also greater (P <
0.05) in the TG than in the WT rabbits in the absence and presence of muscarinic blockade and combined muscarinic and β-adrenergic receptor blockades (Table 1).

Arterial baroreflex sensitivity, assessed by pharmacologically manipulating arterial pressure via the administration of phenylephrine, was depressed in TG rabbits; i.e., the slope of the relationship between systolic arterial pressure and HR was depressed (P < 0.05) (Fig. 2). The effects of nasopharyngeal stimulation induced by cigarette smoke inhalation in the TG and WT rabbits are shown in Figs. 1 and 3. Arterial pressure responses were depressed (P < 0.01) in the TG rabbits. Figure 4 shows the systolic arterial pressure vs. HR relationship to stimulation of the Bezold-Jarisch reflex, induced by administration of veratrine. The y-intercept of the relationship was reduced (P < 0.05) (Fig. 2). The effects of nasopharyngeal stimulation by cigarette smoke inhalation in conscious TG and WT rabbits. Data are expressed as means ± SE. Note that smoke inhalation did not induce a significant change in systolic arterial pressure (SAP) in both groups, but HR and LV dP/dt responses were significantly reduced (*P < 0.01) in TG compared with WT rabbits.

**DISCUSSION**

It has been recognized for some time that the arterial baroreflex is depressed in HF (4, 6, 14). Because the pathogenesis of HF involves chronically enhanced sympathetic tone, it is conceivable that the mechanism, which can also result in depressed arterial baroreflex sensitivity in the absence of HF (8, 13), may be involved in mediating the altered reflex responses observed in HF. The TG rabbit with overexpressed cardiac Gso is a novel model to test the hypothesis because this model demonstrates chronically elevated levels of HR and LV contractility, mediated by enhanced Gso and adenylyl cyclase activity, but does not progress to HF (11). Furthermore, the increased sympathetic activity is specific to the heart, which is difficult to accomplish with pharmacological interventions or exercise-induced increases in sympathetic activity, which affect all vascular beds, including the brain. This also implies that peripheral vascular resistance is not altered in this model, which may explain why arterial pressure changes were not altered with the interventions.

The TG rabbit affords several advantages compared with the TG mouse. First, it is large enough to accommodate chronic instrumentation to measure cardiac function in the conscious state, as was done in this investigation. Second, the larger size of rabbits permits examination of other reflex pathways in addition to the arterial baroreflex, which was also an important component of the current investigation. Third, baseline levels...
of HR and LV dP/dt approximate more closely values in large mammals and humans than do those measurements in mice, which are considerably higher (10). For example, HR is generally <100 beats/min in large mammals but is over 200 beats/min in rabbits and 600 beats/min in mice. LV dP/dt is roughly 3,000 mmHg/s in conscious large mammals vs. almost 4,000 mmHg/s in this study in conscious rabbits, but it is roughly 15,000 mmHg/s in conscious mice (10). Lastly, there are several species differences between mice and larger mammals and humans, not shared by rabbits, with one of the most prominent differences regarding Ca$^{2+}$ regulation of contraction (2). There are several major differences between the rabbit and mouse models of overexpressed cardiac Gsα. Most importantly, the mouse begins to develop cardiomyopathy by 8–10 mo of age (1, 9), whereas the rabbit appears relatively resistant to the development of cardiomyopathy (11). This finding, albeit important, may not be that surprising, recognizing that there are over 100 models of cardiomyopathy described in TG mice. Apparently, this species is particularly sensitive to development of cardiomyopathy.

There are three other major differences between the two models. First, the rabbit with overexpressed cardiac Gsα also has overexpressed Giα, which could exert a restraining influence on the heart (11). Second, the mouse with overexpressed Gsα demonstrates even greater sensitivity to isoproterenol stimulation at higher doses (9), whereas the rabbit shows little difference in inotropic and chronotropic response to isoproterenol at the higher doses (11). Third, and potentially most importantly, the α-myosin heavy chain is predominant in the mouse and the β-myosin heavy chain in the rabbit. Accordingly, the promoters used for producing the TG models are quite different.

It was not surprising to find clear depression of arterial baroreflex sensitivity because this has been observed in other models of enhanced sympathetic tone (8, 13), as well as in mice with overexpressed cardiac Gsα (12). However, the concurrent depression of low-pressure reflexes (Bezold-Jarisch) and the trigeminal (nasopharyngeal) reflex pathway was not expected. Prior work in experimental models of HF demonstrates preservation of low-pressure reflexes despite de-
pression of the arterial baroreflex (4). Our data, which examined the Bezold-Jarisch reflex on a beat-by-beat basis, demonstrate clear depression of this reflex in the TG rabbits. Of course, as mentioned above, the rabbits used in the present study were not in HF, which suggests that the regulation of this reflex differs in the presence of chronically elevated sympathetic tone, depending on whether HF is also present. We also observed significantly greater negative chronotropic and inotropic responses in TG rabbits after smoke inhalation, which stimulates the powerful nasopharyngeal reflex (17, 18). Thus our results demonstrate depression of all reflex pathways studied in TG rabbits with chronically elevated β-adrenergic receptor signaling, as well as HR and LV contractility. It is also important to consider altered baseline levels as an explanation for these differences. However, baseline levels of HR were higher in the TG rabbits, which should permit even greater bradycardia. Because the decrease in HR for all of these reflex pathways is predominantly vagal, we examined whether direct electrical stimulation of vagal efferents could be responsible for the observed results. Actually, efferent vagal stimulation induced greater absolute decreases in HR, but percent decreases were similar in TG rabbits, which could be secondary to the elevated baseline HR in TG rabbits. The results of these experiments suggest that the mechanism of the depressed reflex control, secondary to chronically elevated sympathetic tone, rests in the central nervous system or in other afferent pathways.

GRANTS

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