Carotid Sinus Baroreceptor Reflex in Dogs With Experimental Heart Failure

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We have previously demonstrated a decrease in baroreceptor discharge sensitivity in dogs with experimental heart failure. In the present study, we determined the sensitivity of the carotid sinus baroreceptor reflex in dogs with pacing-induced heart failure. The carotid sinus baroreceptor reflex sensitivity was determined by pressurizing one carotid sinus with all other baroreceptor and cardiopulmonary receptor inputs removed. The data were analyzed by plotting carotid sinus pressure–mean arterial pressure curves and carotid sinus pressure–renal sympathetic nerve activity curves in the two groups of dogs. The peak arterial pressure during carotid hypotension was significantly depressed in dogs with heart failure compared with normal dogs (107.1±5.7 versus 139.8±7.0 mm Hg, p<0.001). Mean arterial pressure range, renal sympathetic nerve activity range, and peak slope were significantly decreased in the heart-failure group. To determine if this depression was completely due to depression of baroreceptor discharge per se, or to alterations in central or end-organ responsiveness, similar experiments were performed by stimulating the carotid sinus nerve and evaluating frequency, voltage, and duration of stimulation on the resultant mean arterial pressure and renal sympathetic nerve activity. As was the case with carotid sinus pressurization, electrical stimulation caused a significantly smaller change in mean arterial pressure in heart-failure dogs compared with the normal dogs. However, there was no significant difference between normal and heart-failure dogs for the renal sympathetic nerve activity–electrical stimulation curves. These data strongly suggest that the depressed carotid sinus baroreceptor reflex in heart failure is not solely the result of depressed baroreceptor responsiveness but may be related to poor end-organ responses and normal central control of renal sympathetic outflow. (Circulation Research 1991;68:1294–1301)

Depressed arterial baroreceptor reflex control of heart rate and peripheral resistance have been shown in heart failure both in human and animal studies.1–5 The site or sites within the baroreflex arc that are responsible for the depressed baroreflex in heart failure are not clearly identified. Several studies6–8 have indicated that the efferent vagus and sympathetic nerves do not function normally in heart failure. Previous studies from our laboratory9,10 have indicated that both carotid and aortic baroreceptor discharge sensitivity is depressed in a model of high-output heart failure. Recently, we demonstrated11 that in the more clinically relevant low-output heart-failure model the carotid sinus baroreceptor discharge sensitivity is significantly attenuated. Moreover, aortic baroreceptor discharge sensitivity is also blunted in this model of low-output congestive heart failure.12

It is not known to what extent the blunted baroreflex seen in heart failure13 is mediated by changes in central integration, efferent and/or afferent nerve endings, neurotransmitter release, or end-organ responsiveness. In the present study, one carotid sinus was isolated and pressurized, or the carotid sinus nerve was electrically stimulated with varying frequencies, voltages, or durations while recording mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA). This experiment was designed to determine if the depression of the arterial baroreflex in chronic heart failure is mediated exclusively by a depressed baroreceptor mechanism or by changes in the central and/or efferent components of the baroreflex arc.

Materials and Methods

The experiments were carried out on 35 adult mongrel dogs of either sex. The control group consisted of 18 normal dogs (mean weight, 22.7±1.3 kg). The control group consisted of normal uninstru-
mented dogs. The experimental group consisted of 17 heart failure dogs (mean weight, 23.1±1.4 kg.). The heart-failure model used in these experiments was that of low-output congestive heart failure, which was originally described by Whipple et al.14 and expanded on by Coleman et al.15 The surgical preparation has been described previously.11,16 In brief, a left thoracotomy was performed with standard sterile surgical technique. A screw-type epicardial pacing lead (model 6197-35T, Medtronic, Inc., Minneapolis, Minn.) and a pair of ground wires were implanted on the left ventricle and left atrial appendage, respectively. The leads were brought out of the chest and tunneled beneath the skin to exit in the midscapular region. After surgery, the dog was placed on an antibiotic regimen and allowed to recover for 7–10 days before pacing was started. The pacing rate was set at approximately 250 beats/min for 4–6 weeks. The pacing dogs were weighed and examined daily until clinical signs of heart failure were evident, such as pulmonary edema, dyspnea, and ascites. When the dogs were determined to be in stable heart failure for several days, the acute experiments were performed.

**General Experimental Preparation**

Each dog was anesthetized with sodium pentobarbital (30 mg/kg i.v.) and intubated. A femoral artery and vein were catheterized for acquisition of blood samples and anesthetic supplements and for pressure measurements. Before carotid sinus isolation and denervation, a transducer-tipped catheter (model PC-350, Millar Instruments, Inc., Houston) was inserted into the left ventricle via the contralateral femoral artery, and the left ventricular systolic pressure and left ventricular end-diastolic pressure were determined. The catheter was then withdrawn into the aortic arch, and systolic, diastolic, and pulse pressures and MAP were measured. Cardiac output was determined with a 5F Swan-Ganz catheter and a cardiac output computer (model 9510, Edwards Laboratories, Santa Ana, Calif.) using the thermodilution technique. Arterial blood gases were measured throughout the experiment and kept within normal limits. One tenth of the initial anesthetic dose was given each hour. Because the heart-failure dogs were more sensitive to anesthesia, a lower dose was used for supplementation (approximately half of that used in the normal dogs). The depth of anesthesia was well maintained throughout the experiment as judged by corneal and withdrawal reflexes. During the renal nerve recording experiments, neuromuscular blockade was accomplished by administration of pancuronium bromide (Pavulon, Organon, West Orange, N.J.) at a dose of 0.1 mg/kg. During neuromuscular blockade, anesthetic supplements were given as they were for nonblocked dogs.

**Preparation of Isolated Carotid Sinus**

Through a midline incision in the neck, the left carotid sinus area was exposed. The common carotid, external carotid, and lingual arteries were catheterized. All other branches of the carotid sinus region were ligated. Carotid sinus pressure (CSP) was measured from the external carotid catheter, the tip of which was located at the bifurcation of the internal and external carotid arteries. All innervations to the carotid sinus were sectioned except the carotid sinus nerve. Both vagi and the contralateral carotid sinus nerve were cut. The inflow and outflow perfusion catheters were placed in the common carotid artery and the lingual artery, respectively. The sinus was perfused with nonpulsatile pressure from a reservoir with an oxygenated Krebs-Henseleit solution at 38°C and pH 7.4; the solution contained (mM) NaCl 129, KCl 4.8, CaCl2 1.1, MgSO4 2.5, KH2PO4 1.2, NaHCO3 25, dextrose 5.5, and pyruvate 2.0. The CSP level was controlled by adjusting a regulator valve connected to a pressurized air source that pressurized the reservoir and by adjusting the outflow resistance distal to the pressure catheter using an adjustable tubing clamp.

**Carotid Sinus Nerve Stimulation**

The carotid sinus nerve was identified and dissected free. A pair of stimulating electrodes were placed on the carotid sinus nerve. A square-wave stimulator (model S88, Grass Instrument Co., Quincy, Mass.) was used to stimulate the nerve. The stimulation parameters used in these experiments were 5 V and 1-msec duration with varying frequencies from 1 to 60 Hz, or 40 Hz and 1-msec duration with varying voltages from 1 to 10 V, or 40 Hz and 5 V with varying duration from 0.025 to 2 msec. During stimulation of the carotid sinus nerve, the CSP was kept below the pressure threshold (≤50 mm Hg).

**Renal Sympathetic Nerve Recording**

A left flank incision was made, and a retroperitoneal dissection was used to expose the renal artery and nerves. The renal sympathetic nerves were identified, and a branch was carefully dissected free of the surrounding connective tissue. The nerve was immersed in a warm mineral oil bath and placed on a pair of platinum-iridium recording electrodes. The signal was amplified with a Grass DC preamplifier (model P18D) with low-frequency cutoff set at 30 or 100 Hz and high-frequency cutoff set at 1 or 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121N, Tektronix, Beaverton, Ore.) and connected to a neuronal spike analyzer (model N750, Mentor, Minneapolis, Minn.). A window discriminator was set just above the noise so that only the discharge signal was discriminated. The discriminator pulses were fed into a rate meter (Frederick Haer & Co., Brunswick, Maine) for quantification. The raw nerve activity, rate meter output, discriminator pulses, arterial pressure, and carotid sinus pressure were all recorded on an FM tape recorder (model D, A.R. Vetter Co., Rebersburg, Pa.) and on an electrostatic strip-chart recorder (model ES 1000B, Gould, Inc., Glen Burnie, Md.).
Experimental Protocol

The CSP was kept at 100 mm Hg for at least 30 minutes before construction of each CSP–MAP curve and CSP–RSNA curve. Then, the CSP was reduced to approximately 25 mm Hg; thereafter, the CSP was increased stepwise up to 300 mm Hg. Each step was 25 mm Hg and lasted approximately 20 seconds. At each step, MAP and RSNA (rate meter output) data were sampled during the last 5 seconds with an analog-to-digital converter (Tecmar Co., Solon, Ohio) in an IBM XT computer. Both arterial pressure and RSNA had reached a stable level at this time. The CSP was then set at <50 mm Hg, and the carotid sinus nerve was stimulated at varying frequencies, voltages, and durations while recording MAP and RSNA. Each stimulation was held until the output parameter (either MAP or RSNA) reached a new steady-state level. This usually took approximately 20 seconds. The last 5 seconds of data were computer-sampled.

Data Analysis

The CSP–MAP and CSP–RSNA relation data were fit to a sigmoid logistic function\(^\text{17}\) using a nonlinear regression program (SAS, PROC NGLIN)\(^\text{18}\) run on an IBM mainframe computer. Four parameters were derived from the equation

\[
\text{MAP} (\text{or RSNA}) = A_1/[1 + \exp(A_2(\text{CSP}-A_3))] + A_4
\]

where \(A_1\) is MAP or RSNA range, \(A_2\) is slope coefficient, \(A_3\) is the CSP at which the peak slope is located, and \(A_4\) is minimum MAP or RSNA. By using these four parameters, MAP range or RSNA range \((A_1)\) and peak slope \((A_2A_3/4)\) were derived. The slope at a given CSP was derived from the first derivative of this function.

For the CSP–RSNA relations, RSNA was normalized as a percent change from the baseline level that was recorded at CSP of 100 mm Hg. For the RSNA responses to electrical stimulation of the carotid sinus nerve, RSNA was determined as the percent change from baseline nerve traffic (CSP <50 mm Hg) before stimulation of the carotid sinus nerve.

All values are expressed as mean±SEM. Student’s t test was used to determine significant difference between normal and heart-failure groups. Differences were considered significant at \(p<0.05\).

Results

Hemodynamic Data

The hemodynamic data of anesthetized normal and heart-failure dogs are summarized in Table 1. The most marked differences between the two groups were left ventricular end-diastolic pressure and cardiac output. Left ventricular end-diastolic pressure was significantly elevated, and cardiac output was significantly decreased in the heart-failure group.

CSP–MAP and CSP–RSNA Relations in Normal and Heart-Failure Dogs

Figure 1 (upper panel) shows systemic arterial pressure responses to pressurizing the carotid sinus in a normal dog. As can be seen, MAP decreased during elevation of CSP in a stimulus–response fashion.

![Figure 1. Tracings showing arterial blood pressure (ABP) responses to pressurizing of carotid sinus (upper panel) or electrical stimulation of the carotid sinus nerve with varying frequencies (lower panel) in a normal dog. CSP, carotid sinus pressure.](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.68.5.1296/-/DC1)
MAP Responses to Electrical Stimulation of the Carotid Sinus Nerve in Normal and Heart-Failure Dogs

Figure 2 shows mean data of the CSP–MAP relation in normal and heart-failure dogs. MAP < 175 mm Hg was significantly higher in the normal dogs than in the heart-failure group, and the slope of the CSP–MAP curves between 175 and 275 mm Hg was significantly steeper than that in the heart-failure dogs. In the heart-failure dogs, MAP was 18.6 ± 2.7 mm Hg compared with 32.0 ± 5.2 mm Hg in the normal dogs (p < 0.01); the peak slope of the CSP–MAP curve was 0.21 ± 0.05 mm Hg/mm Hg in the heart-failure dogs compared with 0.43 ± 0.10 mm Hg/mm Hg in the normal dogs (p < 0.05).

Figure 3 shows mean data for the CSP–RSNA relation in the normal and heart-failure dogs. During unloadling of the carotid sinus baroreceptors, RSNA was not increased in the heart-failure dogs as it was in the normal dogs. In the heart-failure dogs, RSNA was 50.8 ± 10.8% of control compared with 98.5 ± 18.5% of control in the normal dogs (p < 0.05). The slope of the CSP–RSNA curves between 150 and 200 mm Hg was significantly lower in the heart-failure group.

RSNA Responses to Electrical Stimulation of Carotid Sinus Nerve

Figure 4 shows mean data of electrical stimulation of the carotid sinus nerve on the MAP in normal and heart-failure dogs. Hypotensive responses to electrical stimulation of the carotid sinus nerve were significantly blunted in the heart-failure dogs with varying frequencies > 30 Hz, with varying voltages > 3 V, or with varying durations > 0.05 msec. The slope relating the percent change in MAP to the change in stimulus intensity was significantly lower in the heart-failure group with varying frequencies between 1 and 30 Hz (−0.97 ± 0.08 versus −0.49 ± 0.10), with varying voltages between 1 and 4 V (−12.8 ± 1.2 versus −6.7 ± 1.4), or with varying durations between 0.05 and 0.25 msec (−12.2 ± 17 versus −56 ± 14) (see Figure 5). The correlation coefficients were > 0.90 for each regression. These data are qualitatively similar when expressed as absolute change instead of percent change.

Figure 6 shows effects of electrical stimulation of the carotid sinus nerve with varying frequencies on RSNA in a normal dog. As the frequency of stimulation is increased from 10 to 60 Hz, RSNA decreases as is clearly evident by the period of silence in the raw nerve activity and rate meter tracings. Arterial pressure falls in a stimulus–response fashion.

Figure 7 summarizes the mean data of RSNA responses to electrical stimulation of the carotid sinus nerve in normal and heart-failure dogs. These
curves are almost superimposable. There were no significant differences between the two groups with varying frequencies or voltages. The slopes of the stimulus intensity–RSNA relation were not significantly different between the two groups of dogs.

Discussion

Consistent with our previous study,11 the heart-failure dogs used in the present study had a significant elevation in left ventricular end-diastolic pressure and a significant reduction in cardiac output in the anesthetized state. There was no significant difference compared with normal dogs in other hemodynamic parameters, such as arterial blood pressure or heart rate. In conscious animals,16 however, there was a significant reduction in left ventricular systolic pressure, arterial systolic pressure, arterial diastolic pressure, and MAP and a significant tachycardia. These data suggest that anesthesia masks some of the hemodynamic differences between normal and heart-failure animals.

Blunted baroreflex control of the circulation in the heart-failure state has been studied for many years.1–5,12 However, the mechanisms for the abnormalities of the baroreflex in heart failure are not completely understood. Specific abnormalities in the various components of the baroreflex arc need to be understood to determine how the baroreflex controls the circulation in heart failure. In previous studies from this laboratory9,11 using both high- and low-cardiac output models of heart failure, it was shown that single-unit baroreceptor discharge sensitivity was depressed. Similar results have been obtained for aortic baroreceptors both in high-output10 and low-output12 heart-failure models. The reduction in single-unit afferent discharge sensitivity is not related to altered vessel compliance but may be related to an increase in Na⁺,K⁺-ATPase activity, since perfusion of the isolated carotid sinus with ouabain normalizes discharge sensitivity in dogs with heart failure.11

In the present study, the CSP–MAP relation (Figure 2) indicates that the baroreflex, which includes afferent, central, and efferent arms of the arc, was significantly blunted in the heart-failure dogs. The CSP–RSNA relation (Figure 3), which includes afferent, central, and efferent arms of the baroreflex without the involvement of the target tissue (vascular smooth muscle), indicates that the abnormal baroreflex in the heart-failure dogs resides either at the baroreceptor level or in the central nervous system. This, of course, does not necessarily mean that there
FIGURE 6. Tracings showing renal sympathetic nerve discharge and arterial blood pressure (ABP) responses to electrical stimulation of the carotid sinus nerve with varying frequencies in a normal dog. CSP, carotid sinus pressure.

FIGURE 7. Graphs showing mean data of changes in renal sympathetic nerve activity (RNA) in response to electrical stimulation of the carotid sinus nerve in normal and heart-failure dogs with increasing frequency (top panel) and voltage (bottom panel).

is no additional impairment of function in vascular smooth muscle in heart failure.

Although it seems clear that the baroreflex sensitivity is depressed in this model of heart failure, it should be pointed out that the reduced slope of the CSP-MAP relation at high CSPs in the heart-failure dogs may be due to the fact that the baseline pressure was lower than that in the normal dogs. Even though this difference was not statistically different (Table 1), this is most likely due to the large standard error of the mean in these dogs. Certainly, in conscious dogs with this model of heart failure, arterial pressure is lower. The minimum pressure elicited by increases in CSP was similar to that seen in the normal dogs. Therefore, the reduction in slope in this pressure range may be related to the starting pressures. On the other hand, the dogs with heart failure also failed to increase MAP during reductions in CSP. What is more revealing than the slopes of these curves is the absolute MAP that could be achieved when CSP was reduced in the dogs with heart failure. In the present experiments, the MAP responses to electrical stimulation of the carotid sinus nerve (Figure 4) indicate that the baroreflex is attenuated in heart failure even when the baroreceptors themselves are removed from the reflex arc. However, the RSNA responses to electrical stimulation of the carotid sinus nerve (Figure 7), which includes central and efferent components of the baroreflex, demonstrated that there was no difference between normal
and heart-failure dogs. This suggests that the central gain of baroreflex is normal in the heart-failure group. There is only one study describing the central gain of the baroreflex in heart failure. Using the same pacing model as in the present experiments, Dibner-Dunlap and Thames used the ratio of the percent change in RSNA to the percent change in aortic nerve activity as an index of baroreflex central gain and found no evidence for a central abnormality in the heart-failure dogs. Whereas the data presented by Dibner-Dunlap and Thames showed preserved baroreflex control of RSNA at a time when the baroreflex control of the heart rate was depressed, our data show blunted RSNA responses in heart failure. The following explanations may account for the differences in RSNA responsiveness between our study and theirs. First, Dibner-Dunlap and Thames used vasoactive drugs to alter systemic pressure, whereas we mechanically pressurized the carotid sinus. The primary effect of this difference would be the number of baroreceptors stimulated; however, it is also possible that vasoactive drugs may alter the compliance of the carotid sinus and thereby affect receptor discharge in a manner different from mechanical pressurization. Second, they activated all baroreceptors, but our study was restricted to one carotid sinus. In any event, the data using electrical stimulation of the carotid sinus nerve is consistent with the view that there is no central abnormality in baroreflex control of RSNA in heart failure.

In the present study, the MAP responses to electrical stimulation of the carotid sinus nerve (Figure 4) were compared with the RSNA responses to electrical stimulation of the carotid sinus nerve (Figures 6 and 7). The MAP responses, which include the central and target organ responses of the baroreflex, were significantly depressed, and the RSNA responses, which only include central responses of the baroreflex, were normal in the heart-failure group. These results suggest that there is a blunted target-organ response of the baroreflex in the heart-failure dogs. In this regard, a variety of vasoconstrictor and vasodilator substances, including norepinephrine, atrial natriuretic peptide, prostaglandins, vasopressin, and angiotensin, are elevated in heart failure. Although some evidence indicates that peripheral α-receptor function is not altered in heart failure, vasodilator capacity is clearly abnormal. On the other hand, in a recent study by Ferguson et al., it was clearly shown that patients with ventricular dysfunction had an impaired constrictor response to baroreflex activation. This finding could be due to any one of a variety of factors, which may include the baroreceptors themselves. It is also possible that end-organ responsiveness is normal in heart failure but that synthesis and/or release of norepinephrine is depressed in chronic heart failure.

**Limitations of the Study**

The data and conclusions presented in this study must be viewed with certain reservations in mind. It seems reasonable to outline these concerns. First, the normal and heart-failure dogs had similar body weights. This may seem odd, since the heart-failure group retained fluid and exhibited signs of edema and ascites. However, these dogs also lost lean body mass; this loss tended to reduce weight at the same time as fluid retention increased. Second, it is important to understand that the data presented here only apply to the baroreflex control of sympathetic nerve activity to the kidney. It is well known that sympathetic nerve activity in response to different stimuli is nonuniform: it often reacts differentially depending on the vascular bed that is innervated. Whether the central gain and the baroreflex control of lumbar, splenic, or adrenal sympathetic outflow behave in the same way as RSNA will have to await further experimentation. It is certainly possible that the impaired MAP response to carotid baroreceptor stimulation may be mediated by a depressed sympathetic response to vascular beds other than the kidney and an impaired cardiac output response.

Although not statistically significant, it appears that MAP was lower, at least in some of the heart-failure dogs, compared with the normal dogs. The following question arises: could this lower MAP have caused baroreflex resetting or a change in the gain of the baroreflex in the heart-failure dogs? This is unlikely for several reasons. First, the carotid sinus was isolated and perfused at 100 mm Hg in both groups of dogs for at least 30 minutes before any data were collected. Second, baroreflex resetting does not alter the gain but rather shifts the baroreflex curve along the pressure axis; this was not seen in the present experiments. It is generally well accepted that reductions in baroreflex gain can be seen with chronic hypertension. Since the heart-failure animals in these experiments were, if anything, hypotensive, it is difficult to attribute the reduced gain during carotid pressurization to the above phenomenon. Finally, the use of anesthetized dogs in the present study is somewhat problematic. Although we have clearly shown that the baroreflex is depressed in conscious dogs with pacing-induced heart failure, the role of central mechanisms in this depression has not been studied in awake animals with heart failure. The latter experiment would necessitate not only an isolated carotid sinus preparation but also electrical stimulation of the carotid sinus nerve in awake, chronically instrumented dogs. Although these experiments are theoretically feasible, they are difficult and will have to await future study.

In any experiments in which the carotid sinus nerve is electrically stimulated, the problem of combined baroreceptor and chemoreceptor stimulation arises. In the present study we undoubtedly stimulated some chemoreceptors at some of the stimulation parameters used (especially at low frequencies and voltages). Indeed, we did observe a transient tendency for arterial pressure to rise in some individual dogs of both groups at low stimulation frequencies. However, the overall response was clearly depressor. Although we cannot rule out a differential contribution of
chemoreceptor stimulation in heart-failure versus normal dogs, we feel that the predominant response observed in these experiments was mediated by carotid sinus baroreceptor stimulation.

In summary, there was a depression of the baroreflex control arterial pressure in dogs with heart failure in response to either pressurization of the carotid sinus or electrical stimulation of the carotid sinus nerve. Central baroreflex control of RSNA was normal in the heart-failure group. These data suggest that the depression of the baroreflex control of arterial pressure in the heart failure resides at the baroreceptor level, the vascular end organ, or the sympathetic terminal.

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References


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