Cardiac afferents play the dominant role in renal nerve inhibition elicited by volume expansion in the rabbit

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Badoer, Emilio, Viatcheslav Moguilevski, and Barry P. McGrath. Cardiac afferents play the dominant role in renal nerve inhibition elicited by volume expansion in the rabbit. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R383–R388, 1998.—In the rabbit, vagotomy combined with arterial baroreceptor denervation abolishes the renal sympathoinhibition elicited by volume expansion. However, the effect of removing cardiopulmonary afferents alone has not been investigated. The aim of the present study was to determine the role of the cardiac afferents in the renal sympathoinhibitory response elicited by volume expansion in the normal conscious rabbit. Four experimental groups were used in which rabbits were administered 1) volume expansion (Haemaccel, 1.9 ml/min for 60 min), 2) volume expansion + bolus intrapericardial procaine (20 mg) to block cardiac afferents, 3) volume expansion + intravenous procaine (20 mg bolus), and 4) intrapericardial procaine alone (20 mg bolus). Volume expansion did not significantly affect mean arterial pressure or heart rate but produced a profound fall in renal sympathetic nerve activity (~50%). Intrapericardial procaine administered 30 min after the start of volume expansion markedly reversed the renal sympathoinhibition to within 20% of the pre-volume expansion level, an effect that wore off over 25 min. In contrast, intravenous procaine lowered renal sympathetic nerve activity slightly further. The results suggest that cardiac afferents play the dominant role in the renal sympathoinhibition in response to volume expansion in the normal conscious rabbit.

renal sympathoinhibition; intrapericardial procaine

volume expansion elicits effects that include increases in urine flow, sodium excretion, cardiac output, and stroke volume and a decrease in total peripheral resistance; there may or may not be an observable change in blood pressure (5, 28). These effects are mediated by both hormonal and neural components. The hormonal changes include reductions in antidiuretic hormone, renin, and aldosterone and increases in atrial natriuretic peptides. The changes in total peripheral resistance involve changes in sympathetic nerve activity. The renal nerve activity appears to be particularly sensitive because a reduction in sympathetic outflow to the kidney is consistently observed following a volume load (5, 8, 30).

Sympathetic nerves to the kidney innervate both the afferent and efferent arterioles as well as the renal tubules (2, 26). Thus changes in the activity of the renal sympathetic nerves can alter renal blood flow (RBF) and induce changes in urine output, sodium excretion, and renin release. Direct or indirect activation of renal efferent nerves can elicit marked renal vasoconstriction resulting in decreased RBF and glomerular filtration rate (GFR). These effects may not be readily observed because of the potent autoregulatory ability of the kidney. However, the diuretic and natriuretic effects that result from renal nerve stimulation can be elicited with low-level sympathetic nerve stimulation, independent of measurable changes in RBF and GFR or renin release (3, 7, 23).

The marked decrease in renal sympathetic nerve activity (RSNA) observed in response to volume expansion may contribute to, but does not appear to be essential to, the increase in RBF and the decrease in renal vascular resistance observed (30). However, the reduction in RSNA is important for the natriuretic and diuretic response elicited by volume expansion. Renal denervation markedly impairs the natriuresis and diuresis induced by volume expansion in conscious dogs (30) and in rats fed a low-sodium diet (8). Impairment of the normal natriuretic and diuretic reflex responses could have important pathophysiological sequelae in situations where circulating volume is expanded. Continued retention of sodium and water in those situations is undesirable.

The afferent nerves involved in conveying information to the brain following changes in blood volume appear to originate entirely from the arterial baroreceptors and the cardiopulmonary baroreceptors. The reflex renal sympathoinhibitory response that accompanies volume expansion can be abolished by combined arterial baroreceptor denervation and vagotomy in almost all species examined to date (12, 13, 20, 36). In most of the studies that have contributed to this view, the experiments were performed in anesthetized animals. In more recent studies, combined vagotomy and sinoaortic denervation also prevented the volume expansion-induced sympathoinhibition in conscious animals (19, 30), confirming the findings of earlier reports in anesthetized preparations. Vagotomy has been used invariably in all studies to date to establish a role for the cardiopulmonary afferents in mediating the sympathoinhibition following a volume load. However, vagotomy has several disadvantages. 1) It is difficult to establish the origin of the afferents that are involved because vagal afferents arise not only from the heart but from other regions, e.g., the lungs. It is well known that lung inflation receptors influence sympathetic nerve activity (35, 37). 2) Surgically produced vagotomy is irreversible.

Activation of arterial baroreceptors and cardiopulmonary baroreceptors elicits reductions in RSNA. Interestingly, there is a negative interaction between the two so that removal of one input enhances the effect of the other (4, 25, 27). Thus studies in which arterial baroreceptor denervation was performed prior to volume expansion may overestimate the importance of the role of the cardiopulmonary baroreceptors in eliciting the renal sympathoinhibition.
The aim of the present study was to examine the role of the cardiac afferents in the renal sympathoinhibitory response elicited following volume expansion in conscious animals with intact arterial baroreceptor inputs. We have used intrapericardial procaine to block the cardiac afferents in the conscious rabbit, a species commonly used in cardiovascular research. These approaches have several advantages over the studies performed to date. First, intrapericardial procaine blocks afferents confined to the heart or major veins within the pericardial sac. Furthermore, it is a reversible blockade. The second advantage is the absence of general anesthesia, which overcomes the nonspecific actions of anesthesia such as depression of normal reflex function.

METHODS

New Zealand White rabbits (2.5–3.2 kg) were used in this study. All major surgical procedures were performed while the rabbits were under general anesthesia (either 1) 2–2.5% halothane in oxygen-enriched air, after induction with Brietal sodium (25–50 mg iv) and endotracheal intubation or 2) ketamine (35 mg/kg im) and xylazine (5 mg/kg im), with additional ketamine (17 mg/kg im) administered every 30 min. A minimum of 14 days was allowed between major surgical procedures.

Major Surgical Procedures

Intrapericardial Catheter. In this procedure, the animals were artificially resired prior to a left thoracotomy. Small pledgets of gauze, soaked in saline, were used to prevent damage to the lungs and to remove them from the field of view. A silicone rubber catheter, filled with normal saline, was inserted 25 mm through a small puncture in the pericardial sac near the apex of the heart so that it lay adjacent to the heart ventricles. The catheter was secured to the pericardial sac with the aid of a small piece of nylon fabric that was sutured to the pericardial sac and overlay the small puncture hole. The lungs were reinflated, and the thorax was closed using temporary underwater drainage. The free end of the catheter was blocked and buried subcutaneously between the scapulae.

Renal nerve electrode. The left kidney was exposed via a left lumbar incision. The sympathetic nerve going to the kidney was cleared from the surrounding tissues and placed onto bipolar spiral electrodes that were sutured to the adventitial wall of the renal artery. Wacker Sil-Gel was used to cover the electrode and nerve. The free ends of the electrode wires were buried subcutaneously on the back, and the wound was closed. The rabbits were used 3–4 days later.

Minor Surgical Procedures

On the day of the experiment, under local anesthesia (0.5% lignocaine), an ear artery and vein were catheterized, and the free ends of the intrapericardial catheter and electrode wires were exposed.

Experimental Protocol

After completion of the minor surgical procedures, at least 1 h was allowed to elapse before the experiment was started. The experimental design consisted of four experimental groups. In group 1 (control group), six rabbits were volume expanded with Haemaccel (1.9 ml/min for 60 min; Gehring). Three of the rabbits were also administered saline intrapericardially (0.4 ml) 30 min after start of the volume expansion. Monitoring Cardiovascular Variables

Blood pressure was monitored using the indwelling arterial catheter connected to a pressure transducer. The signal was recorded using a MacLab data acquisition system (AD Instruments). Mean arterial pressure (MAP) and heart rate (HR) were determined electronically using the blood pressure signal.

Raw RSNA was amplified using a low-noise differential amplifier (BMA 200, CWE, or ENG models 187B and 133, Baker Institute, Melbourne, Australia), filtered (bandpass 100–3,000 Hz), rectified, and integrated at 0.5-s intervals. The threshold was set visually to cut out background nerve activity during quiet periods between bursts. The average integrated RSNA over 1–2 min was calculated and expressed as a percent of the resting level prior to volume expansion. MAP, HR, and RSNA were monitored before and for 90 min after start of the volume expansion.

Statistical Analysis

Resting cardiovascular variables were compared using unpaired t-tests. To determine the effects of procaine on the volume expansion-induced responses, the MAP and integrated RSNA at 5 and 10 min postprocaine were averaged and expressed as changes from the levels immediately before the drug's administration. For HR, the changes 15- to 25-min postprocaine were averaged. These changes corresponded to the maximum changes observed after procaine administration. The data were compared with the control group using Student's unpaired t-test. The effects of intrapericardial procaine alone were compared with the predrug level using Student's paired t-test. The Bonferroni procedure was used to compensate for multiple comparisons.

RESULTS

Effect of Volume Expansion on MAP, HR, and RSNA

Volume expansion did not significantly affect MAP and HR, as seen in Fig. 1. However, the stimulus produced a marked renal sympathoinhibition. There was approximately a 50% reduction in RSNA, which reached a plateau at ~30 min after the start of the volume expansion (Fig. 1). The renal sympathoinhibition remained at that plateau level for the duration of the infusion and only began to return to control near the end of the 90-min observation period (Fig. 1).
Effect of Intrapericardial Procaine on Responses to Volume Expansion

Intrapericardial administration of procaine 30 min after the start of the volume expansion did not significantly affect MAP or HR compared with the control group of rabbits (Fig. 1). However, there was a dramatic difference in the RSNA response. Volume expansion resulted in a fall in RSNA of ~44% prior to procaine administration, which was similar to that seen in the control group (Fig. 1). Injection of procaine resulted in an immediate reversal of the renal sympathoinhibition to within 20% of the pre-volume expansion level (P < 0.0005, Fig. 1). The maximum effect was observed within 5–10 min of procaine administration. The effect of procaine was short-lasting and wore off over 25 min (Fig. 1). An example of the raw and integrated RSNA in one rabbit is shown in Fig. 2.

Effect of Intrapericardial Procaine Alone

Procaine alone administered intrapericardially into the conscious rabbit had no significant effect on the resting RSNA and HR (Fig. 3). However, there was a small but statistically significant increase in MAP at 10 min after procaine administration (Fig. 3).

Effect of Intravenous Procaine on Responses to Volume Expansion

Administration of procaine intravenously 30 min after the start of volume expansion had no significant effect on MAP and HR (Fig. 4). The change in the renal sympathoinhibition following the intravenous procaine administration was not significantly different from the control group (Fig. 4).

DISCUSSION

The present study is the first to examine the importance of cardiac afferents in the reflex renal sympathoinhibition elicited by volume expansion in the conscious normal animal. We found that even in the presence of intact arterial baroreceptors, there was a marked reversal of the renal sympathoinhibition following blockade...
of cardiac afferents with a single bolus injection of procaine administered into the intrapericardial sac. This effect was not observed after intravenous procaine, indicating that spillage from the intrapericardial space into the blood supply could not account for the effect. The results suggest that the cardiac afferents are extremely important in mediating the reflex inhibition of the renal sympathetic nerve elicited by volume expansion in the conscious normal rabbit.

In previous studies, vagotomy prevented the effect of volume expansion on RSNA (5, 12, 13, 17, 19, 20, 36). Although those findings support a role for cardiopulmonary afferents in the renal sympathoinhibition induced by volume expansion, the methodology used prevented any conclusion regarding the site of origin of the afferents. Vagotomy removes the influence of afferents from many sources including the lungs. Sensory inputs from the lungs are well known to influence sympathetic nerve activity (27, 35). Furthermore, acute vagotomy in the anesthetized or conscious animal can alter respiratory function (15, 21), and this, in turn, affect sympathetic nerve activity. It is also noteworthy that rabbits do not survive for long periods after vagotomy. An additional problem associated with most of the previous studies is the use of an anesthetized preparation. Anesthesia is well known to depress reflex responses.

One further difference between the present study and those reported in the rabbit previously, and indeed in most other species, is the presence of intact arterial baroreceptor afferents in our study. Removal of the arterial baroreceptor input allows an enhancement of the responses mediated by cardiac afferents (4, 25, 27). Thus the role of vagal cardiopulmonary afferents may be overestimated in those earlier studies. In the anesthetized dog and monkey, in which the effects of vagotomy alone have been assessed, the data indicate that approximately two-thirds of the volume expansion-induced renal sympathoinhibition could be attributed to the vagal afferents. The arterial baroreceptor afferents contributed the remainder (13, 36). In the present study in the conscious rabbit, the reduction in renal nerve activity in response to volume expansion did not normalize completely after intrapericardial procaine, suggesting there was a small contribution of the arterial baroreceptors. It may be argued that we have underestimated the effects of the cardiac afferents because removal of the cardiopulmonary influence is reported to enhance the arterial baroreceptor function (25, 33). This may be the case; however, this is further complicated by the observations that volume expansion may depress arterial baroreceptor reflex function (4). In an earlier study in the same species, in which systemic vascular resistance was measured in response to volume expansion (25), an indirect measurement of total nerve activity, the cardiac afferents were also found to be more powerful inputs and, as in our study, the contribution from the arterial baroreceptor afferents was small (22, 37).
Our finding that intrapericardial but not intravenous procaine reversed the renal sympathoinhibition following volume expansion extends previous studies by suggesting that the sensors mediating the renal sympathoinhibition are located in the heart or great veins lying within the pericardial sac. The heart contains afferents that travel in the vagus as well as those that travel together with the sympathetic nerves (18, 24, 31, 37). There is little evidence supporting a contribution of cardiac sympathetic afferents in the renal sympathoinhibitory response elicited by volume expansion (5, 12, 13, 17, 19, 20, 30, 34, 36, 38).

The physiological importance of cardiac afferents in cardiovascular regulation is still the subject of intense debate (24, 32). Our present observation that intrapericardial procaine had no effect on the resting basal renal nerve activity, suggests that there is little tonic influence of the cardiac afferents. In arterial baroreceptor-denervated rabbits, cutting the vagus has been reported to produce an immediate rise in RSNA, suggesting that the cardiopulmonary afferents may have a tonic influence on the resting sympathetic nerve activity that is masked by the arterial baroreceptor input (5). However, those findings were observed in the presence of anesthesia, and it will be interesting to investigate whether cardiac afferents have a tonic influence on sympathetic nerve activity in the conscious arterial baroreceptor-denervated rabbit.

Cardiac afferents are continually responding to the changing pressures that occur during the cardiac cycle (18). The responses initiated by these afferents can be modulated by circulating hormones (17, 19) and interactions with other cardiovascular sensory inputs (4, 25, 27). Thus teasing out the physiological importance of cardiac afferents from the complex interwoven cardiovascular “system” has been understandably difficult. However, the results of the present study, together with earlier reports suggest that the cardiac afferents may become particularly important in conditions in which circulating volume is perturbed such as in congestive heart failure.

The evidence, to date, implies that any impairment of the sympathoinhibitory function of the cardiac afferents could have very important consequences for sympathetic function in heart failure. It has been well documented that the cardiopulmonary reflex function is attenuated in models of heart failure (6, 9, 10, 16, 39). In humans in which lower body negative pressure has been used to assess cardiopulmonary reflex function, the sympathetic reflex response is also attenuated in heart failure (29). The recent work by DiBona and Sawin (10) suggests that the vasomotor reflexes initiated by the cardiac afferents are more greatly attenuated than those elicited by the arterial baroreceptors. Thus the evidence to date suggests that impaired sympathoinhibition in the face of an elevation in blood volume could contribute to the elevated sympathetic activity characteristic of heart failure. In this condition, an inappropriately elevated renal nerve activity is likely to contribute to the retention of sodium and water observed in this debilitating condition.

Volume expansion with Haemaccel at the rate and duration used in the present study has been shown previously to produce a sustained increase in right atrial pressure of 2–3 mmHg and to induce the release of atrial natriuretic peptide (1). No significant changes in blood pressure and heart rate were observed in that study. The Haemaccel infusion also resulted in changes to plasma electrolytes. Plasma sodium and osmolality were significantly elevated by the volume expansion (1). Electrolyte changes can influence nerve activity; however, the present results suggest that those changes do not contribute to the renal sympathoinhibition that was observed following volume expansion.

Experimental Considerations

Intrapericardial procaine has been used by several authors to block afferents (and efferents) arising from the heart. However, care does need to be exercised when using this technique. We were careful to determine the dose of procaine required to block cardiac afferents and that dose did not influence respiratory function. High doses of procaine can decrease PO2 and increase PCO2 (personal observations; 14), which could conceivably elicit a centrally mediated sympathoexcitation and confound our interpretation. The dose we used blocked the cardiovascular effects of activating the cardiac afferents with phenylbiguanide (100 µg) administered into the right atrium, but did not affect blood gases or respiratory rate, nor did it alter resting RSNA.

Escape of procaine from the intrapericardial space into the blood stream has been postulated to influence the sympathoinhibition that accompanies acute hypovolemia (14). For this reason, we also examined the effect of intravenous procaine and found that it did not attenuate the renal sympathoinhibition observed following volume expansion. Therefore, escape of the drug from the intrapericardial space could not account for the effects observed.

There are several advantages of using intrapericardial procaine instead of vagotomy. One major advantage is the reversibility of the intrapericardial procaine block. Another advantage is the relative selectivity of the procaine technique for afferents originating from structures in or immediately adjacent to the heart (and accessible from the intrapericardial sac). Vagotomy removes not only cardiac afferents but also those vagal afferents arising from other organs such as the lungs.

Perspectives

The present findings indicate that volume expansion in the conscious normal rabbit is a simple technique to activate predominantly the cardiac mechanoreceptor: renal sympathetic reflex. We have recently examined brain regions that are activated by volume expansion in the conscious rabbit. We identified sites within the medulla oblongata that are likely to be involved in the reflex responses (1). The present results offer further support for the view that the brain medullary regions highlighted in that study may participate in the central pathways mediating the cardiac mechanoreceptor-reflex sympathetic nerve responses.
REFERENCES


