Renal Sympathetic Nerve Activity and Arterial Pressure Responses to Changes in Postural Position of Conscious Dogs (42993)

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Abstract. **Experiments were conducted in conscious dogs to determine the relationships between postural position, arterial pressure, and renal sympathetic nerve activity. Observations of the changes in arterial pressure and renal nerve activity were made when animals spontaneously changed postural position from lying to sitting, sitting to standing, standing to sitting, and sitting to lying. Rising to sit from lying down increased arterial pressure from 109 ± 5 to 125 ± 3 mm Hg and increased renal nerve activity by 96 ± 58 μV/sec (61% of control). Movement from the sitting to standing position decreased renal nerve activity by 90 ± 39 μV/sec (48% of control) without changing mean arterial pressure. Sitting down from standing also did not change arterial pressure, whereas renal nerve activity increased by 56 ± 17 μV/sec (33% of control). Returning to the lying position (from sitting) decreased arterial pressure, and this hypotension was associated with significant reductions in renal nerve activity. These results indicate that nonuniform changes in sympathetic outflow from the central nervous system must occur to various vascular beds during changes in postural position of conscious dogs. Thus, renal sympathetic outflow may or may not reflect changes in nerve traffic which contribute to alterations in arterial pressure.** [P.S.E.B.M. 1989, Vol 192]

The use of chronic, unanesthetized animals has become more prevalent in recent years with the development of various techniques allowing for the measurement of several physiologic parameters in the conscious state. These developments include methods for blood flow measurement to different vascular beds (by electromagnetic and doppler flow technology), improved methods for chronic vascular and bladder cannulation, and, more recently, techniques for the recording of sympathetic nerve activity.

The recording of sympathetic nerve activity by chronic electrode implantation has been conducted in several species including rats (1), rabbits (2, 3), cats (4), dogs (5), and humans (6). In the experimental animal models, renal sympathetic nerve activity has been the neural pathway chosen as an index of sympathetic outflow by most investigators. Changes in renal sympathetic outflow, however, may not always be indicative of sympathetic outflow to all vascular beds. Since changes in sympathetic outflow from the central nervous system, in response to a given stimulus may not always occur in uniform patterns (7, 8), correlations between sympathetic nerve activity to a given organ with specific changes in function of that organ become essential to the integral understanding of the neural control of physiologic functions.

The present study was conducted to evaluate the relationship between arterial pressure and renal nerve activity of conscious, unrestrained dogs during spontaneous changes in postural position. These observations were made not only to determine whether renal nerve activity is significantly influenced by the position of animals but also to determine whether reflex changes in arterial pressure and renal nerve activity were correlated in quadrupeds as would be normally expected given the spatial relationship of their cardiovascular system.

Materials and Methods

Mongrel dogs of either sex (n = 14) weighing 14–22 kg initially were anesthetized with sodium thiamylal (25 mg/kg, iv) and intubated with a cuffed endotracheal tube. Surgical plane anesthesia was maintained with halothane (1–2%) and the animals were artificially ven-

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tilated with 100% oxygen. All surgical procedures were conducted utilizing sterile techniques.

The femoral artery and femoral vein were exposed, and chronic indwelling catheters (Tygon tubing) were inserted into the aorta and vena cava, respectively, and exteriorized to the scapular region of the back of the neck. The left or right kidney was exposed by a retroperitoneal flank incision. A renal nerve bundle leaving the aorticorenal ganglion and entering the kidney near the hilus was carefully dissected free from surrounding connective tissue for a distance of approximately 2 cm. Teflon-coated bipolar platinum electrodes were coiled along the renal nerve bundle and secured into place with silicone (Wacker Sil-Gel 604). A stainless steel ground wire was attached to the connective tissue near the aorta and the electrodes were secured to the strap muscles of the back. The electrode leads then were exteriorized subcutaneously to the back of the neck and the incision was sutured closed. The catheters and electrode leads were placed in the pocket of a nylon mesh jacket worn by each dog (Mach Designs, Rockford, IL).

All dogs were alert and awake within 3 hr after completion of surgery and each animal was allowed at least 24 hr to recover before observations were made. All observations were made over the next 1–3 days. Dogs were maintained on a normal sodium intake (40 mEq/day) administered in their low sodium food (H/D, Rivianna Foods). Water was allowed *ad libitum* throughout the study.

The dogs were placed in a stainless steel cage and all experimental observations were made in a sound-isolated room. This room contained all neurological recording equipment. The walls and door were padded with high fidelity sound-absorbent material in order to produce a quiet environment free from external distractions. We have documented that this type of facility is required in order to conduct experiments utilizing sympathetic nerve recording in conscious animals and that the procedures utilized provide steady state sympathetic activity recordings for up to 3 days (unpublished observations). Arterial pressure was measured with a pressure transducer (Statham P23 id) placed in the dog's jacket and calibrated to atmospheric pressure equivalent to zero at the level of the dog's heart. Renal nerve activity was amplified using a Grass preamplifier (7P511) and high impedance probe (7HIP Grass Instruments, Quincy, MA). The neural signals were full wave rectified and the renal nerve activity was quantitated by integration of the total voltage. Before study of each experimental preparation, a threshold for integration of sympathetic outflow was adjusted after increasing arterial pressure a minimum of 30 mm Hg with an acute iv injection of norepinephrine so that the integrated renal nerve activity was equal to zero. At the conclusion of each experiment, the integrator was calibrated using a known input from a microvolt generator (Bioelectric Instruments, Inc.). All signals were recorded on a direct writing oscillograph (Grass polygraph).

Observations of the changes in arterial pressure and renal nerve activity were made in 14 conscious dogs when animals spontaneously changed postural position from lying to sitting, sitting to standing, standing to sitting, and sitting to lying. Values for arterial pressure and renal nerve activity were averaged for 2 min before and for 1 and 2 min after the dog changed postural position. In each dog, values for at least four position changes were obtained and averaged to provide each data point for that animal. In addition, basal arterial pressure, renal nerve activity, and signal to noise ratios of the raw electroneurogram were averaged for 20 min before and 20 min after each experiment on the day of electrode implantation and for 3 consecutive days after electrode placement. Peak signals were determined by direct measurement of random bursts of nerve activity and compared to the average noise level measured during maximum baroreceptor-induced inhibition of sympathetic outflow after acute elevation of arterial pressure (30 μg of norepinephrine iv). Signal to noise ratios then were calculated from a minimum of 25 randomly selected bursts of nerve activity. All data are expressed as mean ± SE among dogs. Statistical analysis of arterial pressure was made by comparing values for each minute after a positional change to the previous steady-state value by two-way analysis of variance. Mean differences were determined by the Student-Neuman-Keuls procedure. Absolute changes in renal nerve activity were determined to be different from zero by paired t test. The 0.05 level of probability was utilized as the criterion of significance.

Results

The relationship between pulsatile arterial pressure and renal nerve activity is shown in a conscious dog (lying position) in Figure 1. This figure depicts the typical bursting nature of renal nerve activity with the largest amplitude bursts being predominantly coincident with end diastolic pressure. The signal to noise ratio in all preparations ranged from 3 to 5:1 and remained constant over a period of 1–3 days.

The reflex responses of renal nerve activity following acute increases and decreases in arterial pressure are shown in Figure 2. Intravenous injection of norepinephrine (50 μg) markedly increased arterial pressure and reduced the integrated renal sympathetic nerve activity to values not different from zero. After arterial pressure returned to control values, renal nerve activity also returned to values not different from control. Intravenous injection of acetylcholine elicited an abrupt decrease in arterial pressure and reflex activation of renal nerve activity (Fig. 2). The rapid and large increase in renal nerve activity is most likely due to the abrupt and rapid fall in arterial pressure evoked by this relatively large dose of acetylcholine. This response was

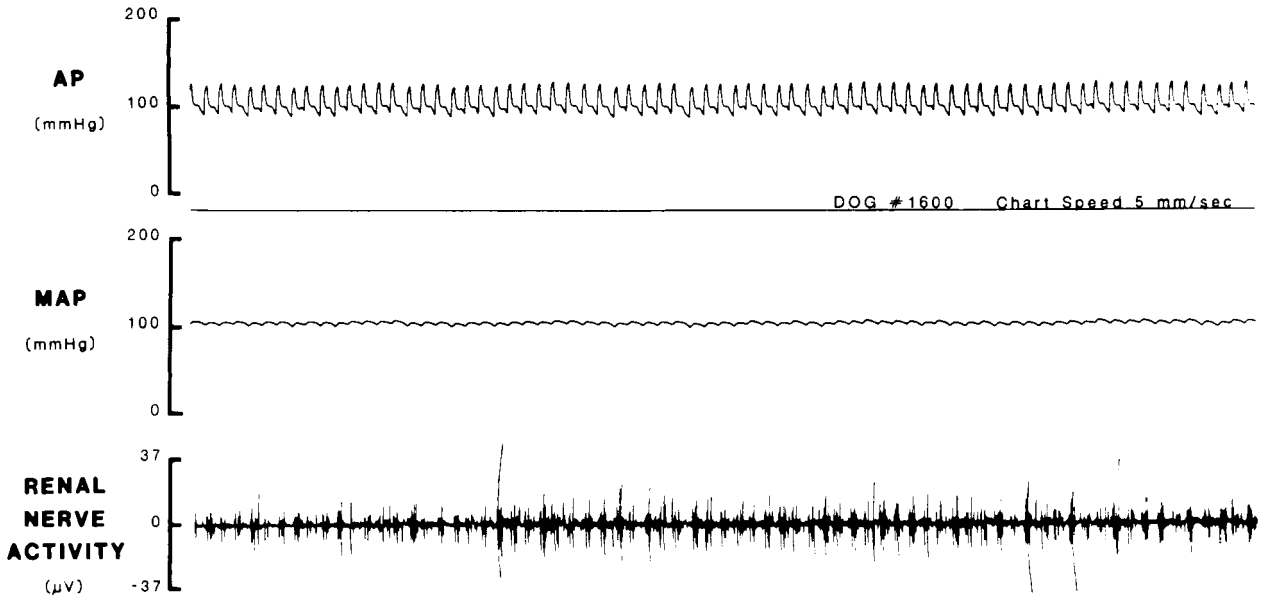

Figure 1. Original record depicting pulsatile arterial pressure (AP), mean arterial pressure (MAP), and the renal electroneurogram of a conscious dog.

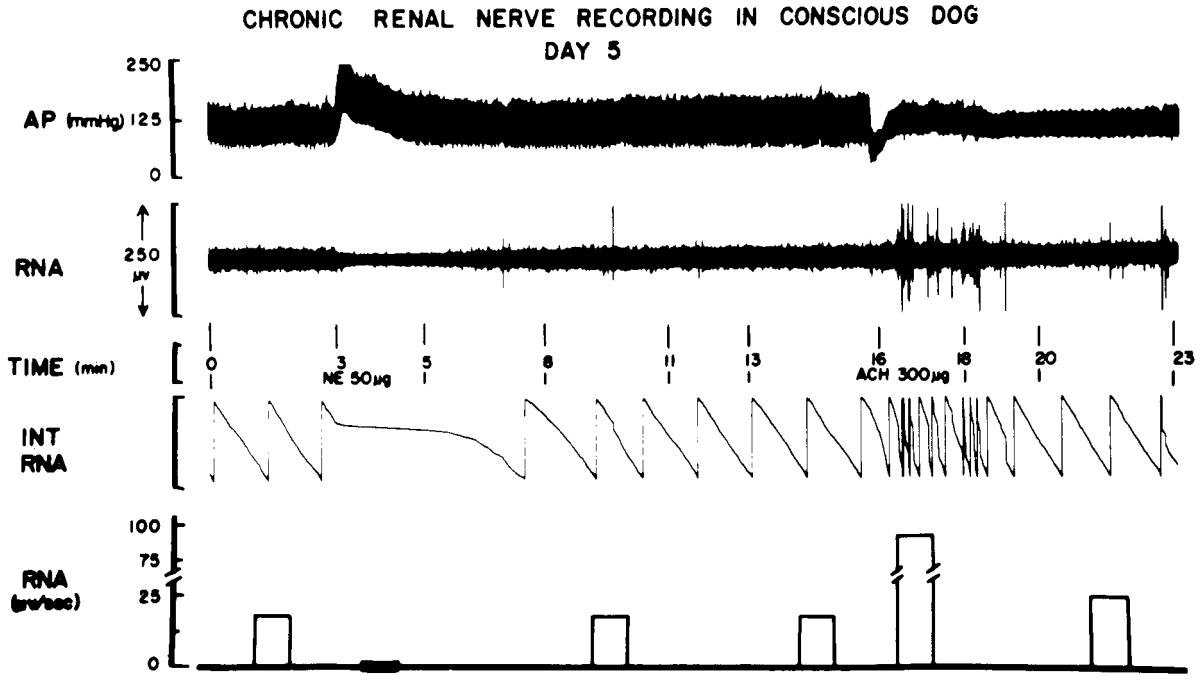

Figure 2. Original record describing baroreflex-induced changes in renal sympathetic nerve activity (RNA) in response to acute increases and decreases in arterial pressure (AP) evoked by iv injection of norepinephrine (NE) and acetylcholine (ACH), respectively, INT RNA = integrated renal nerve activity.

typical for these hypotensive stimuli; however, more gradual reductions of arterial pressure result in diminished neural responses.

In order to verify the stability of the nerve recordings in the conscious state, average renal sympathetic outflow, the signal to noise ratio of the raw electroneurogram, and the mean arterial pressures of these 14 dogs were averaged on the day of electrode implantation and for 3 consecutive days after electrode placement (Fig. 3). Measurements were obtained over a 20-min time interval at the beginning and end of each experiment. After the completion of surgical implantation, renal nerve activity averaged 105 ± 38 μV/sec while the animals remained under a surgical plane of anesthesia and 152 ± 110 μV/sec 3–5 hr after regaining consciousness (Fig. 3). The average renal nerve activity remained unchanged for 3 consecutive days after electrode implantation. This constant renal nerve activity

was associated with a stable mean arterial pressure and a constant signal to noise ratio during this same time period (Fig. 3).

Figure 4 depicts an original record from one experiment which indicates the changes in renal sympathetic nerve activity and arterial pressure of one conscious dog moving from the lying position to the sitting position and then to the standing position. In the lying position renal sympathetic nerve activity averaged 104.5 μV/sec at an arterial pressure of 102 mm Hg. Upon moving to the sitting position, there was a transient pressure response lasting less than 1 min, and this pressure response was associated with an abrupt increase in renal sympathetic nerve activity averaging 201.1 μV/sec over a 4-min time period. When the dog moved to the standing position, renal sympathetic nerve activity decreased to an average value of 155.6 μV/sec over the next 5-min period.

Figure 5 describes the changes in arterial pressure and concomitant changes in renal nerve activity following shifts in postural position of conscious dogs. Steady-state values for renal nerve activity are shown in the lying, sitting, and standing postures. After rising to the sitting from the lying position, mean arterial pressure increased from 109 ± 5 mm Hg to 125 ± 7 and 125 ± 3 mm Hg at 1 and 2 min, respectively. This pressor response was associated with increased renal nerve traffic by 38 ± 16 and 96 ± 58 μV/sec, respectively.

Movement from the sitting position to the standing position resulted in a significant reduction of renal nerve activity (by 89 ± 36 and 90 ± 39 μv/sec, respectively) without changing mean arterial pressure. Similarly, when animals returned to sitting from the standing position, mean arterial pressure remained unchanged but renal nerve activity increased by 39 ± 13 and 56 ± 17 μV/sec (Fig. 5).

Lying down from the sitting position by dogs resulted in opposite responses to sitting up from the lying position (Fig. 5). Upon lying down, mean arterial pressure decreased from 117 ± 7 mm Hg to 101 ± 6 and 104 ± 5 mm Hg over a 2-min time interval. This marked hypotension was associated with significant reductions in renal nerve activity by 128 ± 31 μV/sec, respectively. The absolute values for both mean arterial pressure and renal nerve activity after returning to the lying position from sitting up were not different from those steady-state values of animals lying down prior to assuming a sitting posture.

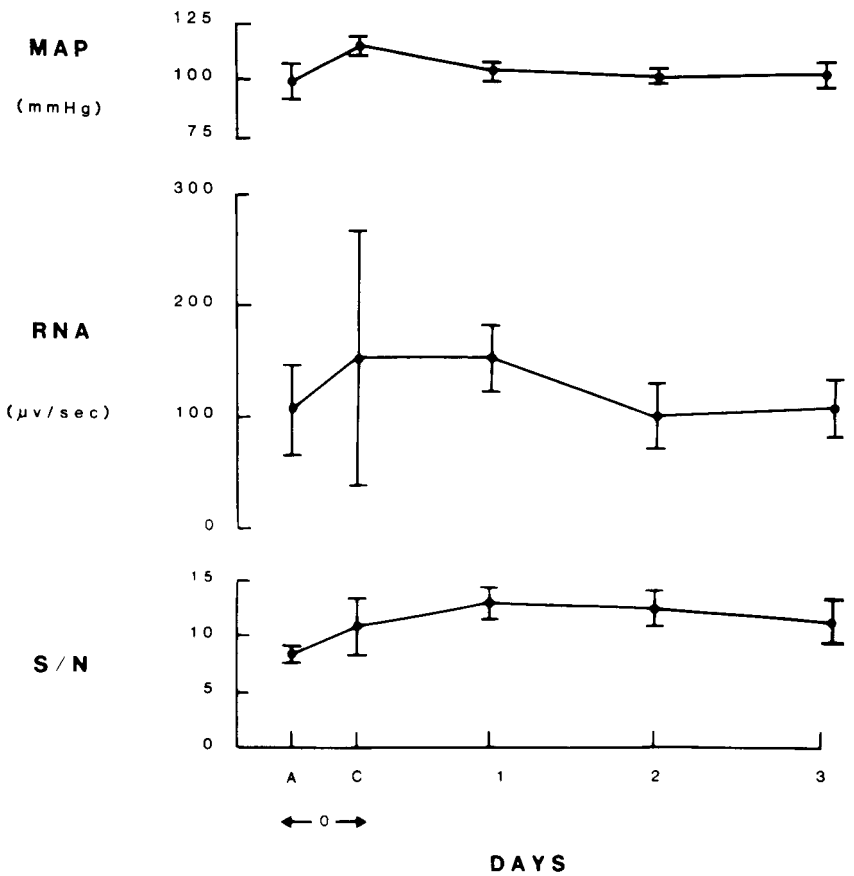

Figure 3. Average values of basal mean arterial pressure (MAP), renal nerve activity (RNA), and signal to noise ratio (S/N) on the day of electrode implantation (0) and for 3 consecutive days after electrode placement. A represents values obtained under halothane anesthesia and C represents values 3–5 hr after each animal regained consciousness.

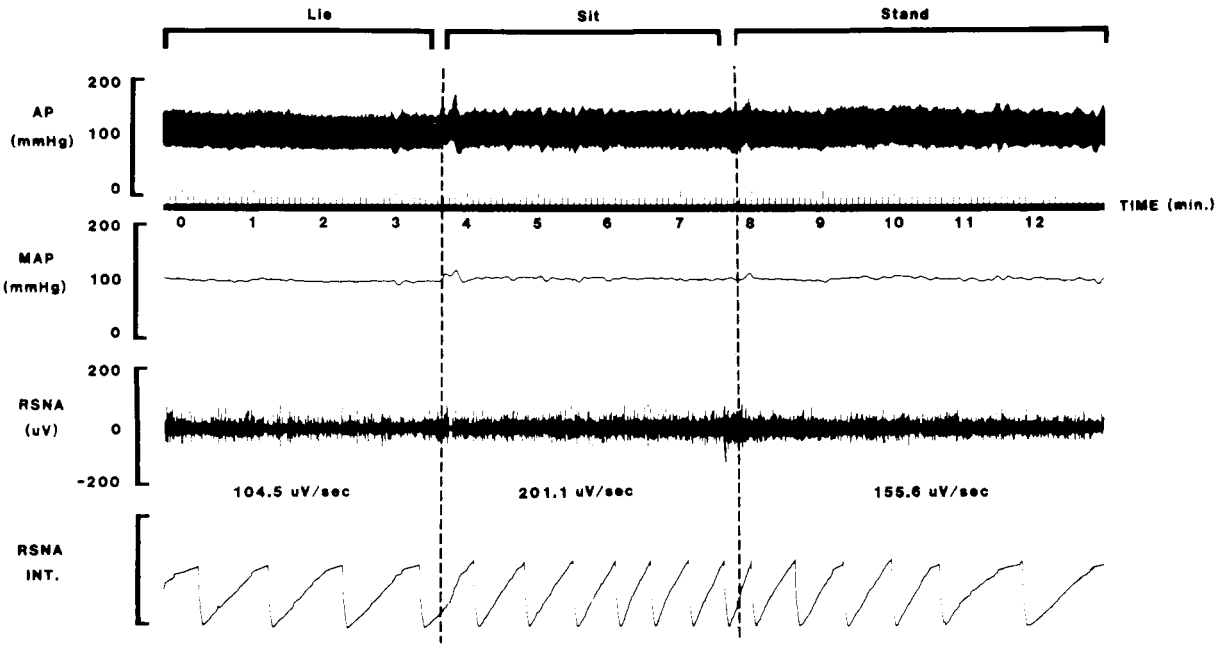

Figure 4. Original record depicting the changes in arterial pressure (AP), mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and integrated renal sympathetic nerve activity (RSNA INT). The figure shows the changes in each of these parameters when the animal was allowed to move spontaneously from the lying to the sitting and to the standing postural positions.

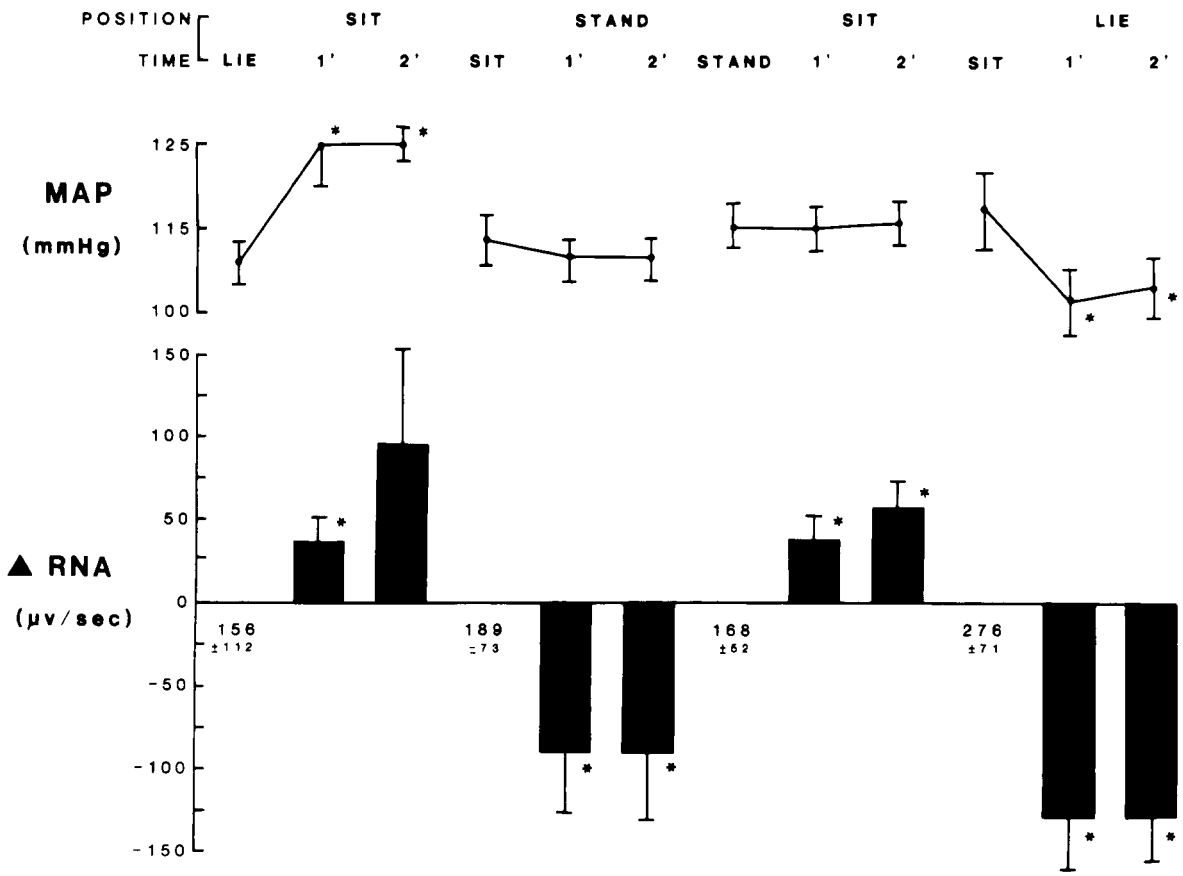

Figure 5. Changes in mean arterial pressure (MAP) and renal nerve activity (RNA) in response to changes in postural position from lying to sitting, sitting to standing, standing to sitting, and sitting to lying. Control values of renal nerve activity (± SEM) are shown next to each histogram. * Significantly different from control ($P < 0.05$).

Discussion

Sympathetic nerve activity passing to the kidney is now thought to importantly influence intrarenal tubular as well as vascular functions (9). In order to eliminate a potential variable in the control of renal nerve activity in the conscious state, we needed to systematically evaluate the effects of changes in postural position on renal nerve activity and arterial pressure. That is, if changing the postural position of dogs influences the overall renal sympathetic outflow, then the animal's postural position would need to be fixed in order to effectively study the control of the renal nerve traffic. The data presented in this study documents that the level of both arterial pressure and renal nerve activity are highly dependent on postural position.

When moving from the lying to the sitting position, there is a marked increase in both arterial pressure and renal nerve activity. Conversely, when dogs lie down from the sitting position, there is a significant hypotension accompanied by a decrease in renal nerve activity (Fig. 5). Since dogs are quadrupedal animals, it would appear that these changes in arterial pressure are resulting from baroreflex activation of sympathetic outflow in response to changes in arterial pressures. When dogs sit up from lying down, the afferent input resulting in these evoked sympathetic responses may originate from decreased arterial pressure at the aortic arch and carotid sinus and/or decreased vascular pressure in the atria (i.e., increased venous pooling) resulting in inhibition of high pressure baroreceptors and cardiopulmonary baroreceptors, respectively. These changes in pressure likely are evoked by the changing position of the dog's head and thorax in relation to the hindquarters.

In contrast to these unidirectional and simultaneous changes in arterial pressure and renal nerve activity when dogs sit up and lie down, movement from the sitting to standing position and standing to sitting position resulted in alterations of renal nerve activity without changing mean arterial pressure. Under these circumstances, renal nerve activity decreased upon rising to stand and, conversely, increased after sitting down from the standing posture. These changes in renal nerve activity also may be resulting from afferent input from both high and low pressure baroreceptors; however, the efferent sympathetic outflow under these circumstances must be relatively specific and directed toward vascular beds which do not result in altered arterial pressure. In other words, marked changes in renal sympathetic nerve activity may occur in the absence of changes in arterial pressure. These changes in renal sympathetic outflow, however, do not necessarily reflect neural patterns similar to those of other vascular beds as indicated by the lack of any changes in arterial pressure. Thus, when studying the neural influences on renal function of conscious dogs, one must be cognizant of the animal's postural position in order to correctly determine the effects of an independent variable within each experiment.

The changes in renal nerve activity which occur in response to alterations in postural position impose an important consideration when conducting experiments in conscious animals. These neurogenic responses indicate the necessity for conducting short-term studies on renal function with each animal placed in a fixed postural position. This objective can be accomplished by the use of a sling or by training the animal to lie quietly for the duration of the experiment. However, the results of the present study provide important insight into the level of basal renal nerve activity present in each of these postural stances.

Reduction of renal perfusion pressure by renal artery occlusion has been shown to activate renal afferent nerve activity (10, 11). In the present studies, where quantification of basal afferent nerve activity has been limited by threshold adjustment of the integrated signal, changes in renal perfusion pressure could lead to activation of renal afferent nerves. Significant activation of afferent nerve activity (and subsequent quantification of afferent activity), however, is unlikely. The changes in renal perfusion pressure which occur with altered postural position are extremely small compared with the abolition of renal perfusion pressure during renal artery occlusion observed in previous studies. Thus, quantified changes in afferent nerve activity in the present study are likely quite small due to threshold adjustment and the minimal changes in perfusion pressure evoked by changes in postural position (Fig. 5).

Receptors other than high and low pressure baroreceptors also may contribute to the afferent input to the central nervous system which results in these changes in renal sympathetic nerve activity in response to shifts in postural position of conscious dogs. These receptors include proprioceptors of the skeletal muscles (12, 13) and low pressure baroreceptors located in the abdominal viscera (14, 15) including the kidneys (10). Changes in postural position likely activate these receptors and their afferent nerve traffic via muscle contraction, movement of joints, and shifts in blood volume within the great veins of the thorax and abdomen. In addition, anticipation of these movements from higher brain centers also can result in input to the cardiovascular center of the medulla. Recently, Victor *et al.* (16) have reported that sympathetic outflow recorded in the adrenal and renal sympathetic nerves responds in a nonuniform manner in response to hemorrhagic hypotension. These differential responses result from different integrative control in the central nervous system by cardiopulmonary, sinoaortic, and vagal afferent stimuli. Thus, the response of renal sympathetic outflow is the end result of the integral sum of many inputs to the central nervous system, and this integrative response is highly dependent upon the nature of the afferent inputs.

Other evidence has documented that changes in efferent sympathetic outflow from the central nervous system in response to various afferent stimuli is not uniform (7, 8). Results from the present study also indicate that nonuniform changes in efferent sympathetic outflow from the central nervous system may occur in response to changes in postural position. Thus, the use of renal sympathetic nerve activity as an index of overall sympathetic outflow may or may not reflect changes in nerve traffic which contribute to alterations in arterial pressure. It is our view that renal sympathetic nerve activity is best utilized when taken into consideration with renal function. Taken together, this information will best correlate the relationships between altered renal sympathetic outflow and the regulation of renal vascular and tubular function.

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