Effect of Massive Sympathetic Nervous System Activation on Coronary Blood Flow and Myocardial Energy Pool

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Our previous work indicates that myocardial ischemia could be the mechanism responsible for the left ventricular (LV) dysfunction that frequently develops after massive sympathetic nervous system (SNS) activation. In this study, coronary blood flow (CBF) and myocardial ATP, creatine phosphate, and lactate concentrations were measured after massively activating the SNS of anesthetized rabbits with an intracisternal injection of veratrine. CBF was measured at time 0 (baseline), and at 2, 10, and 20 min after SNS activation in one group, and at 0, 45, 90, and 150 min in a second group. Myocardial ATP, creatine phosphate, and lactate were measured at 0, 2, 10, 20, 90, and 150 min in separate groups of rabbits. SNS activation caused LV dysfunction in ~60% of the rabbits. SNS-related increases in CBF kept pace with the increases in myocardial energy demand as determined from the systolic pressure-heart rate product. The subendocardial-to-subepicardial blood flow ratio did not change significantly. Myocardial creatine phosphate concentration was depressed 2 min after SNS activation and remained depressed for at least 20 min. ATP fell continuously and was significantly lower than baseline by 20 min. Tissue lactate concentration was elevated at this time. By 90 min, the concentrations of all three metabolites had recovered. These results indicate that myocardial high-energy phosphate compounds fall after massive SNS activation, but ischemia does not appear to be the underlying mechanism.

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Massive activation of the sympathetic nervous system (SNS) as may occur after head trauma, strokes, or epileptic seizures or conditions that mimic SNS activation (e.g. pheochromocytoma or exogenous catecholamine administration) often results in depressed cardiac function. This type of dysfunction is due to excessive concentrations of catecholamines (1), but the underlying mechanism has not been identified. Fleckenstein *et al.* (2) observed a marked increase in cellular Ca^{2+} concentration, decreased myocyte energy content, and myocytolysis in rats administered high concentrations of isoproterenol. They concluded that the myocardial injury was due to Ca^{2+} overload. In contrast, Rona and co-workers (3, 4) reported evidence that myocardial ischemia is the cause. Our previous work indicated that ischemia might be the mechanism responsible for SNS-induced left ventricular (LV) dysfunction because interventions that reduced myocardial energy demand during the intense SNS activity were found to be cardioprotective (1, 5). However, if ischemia is the mechanism, one would expect to find diminished myocardial energy reserves, but this has yet to be observed. For example, Chen and Downing (6) found that myocardial ATP and creatine phosphate concentrations were normal after infusing high doses of norepinephrine into rabbits for 90 min. Likewise, Bittner *et al.* (7) observed normal myocardial high-energy phosphate (HEP) concentrations in dogs with depressed ventricular function 6 hr after massive SNS activation had occurred during the induction of brain death. Consequently, both groups doubted that myocardial ischemia had caused the cardiac dysfunction. However, it should be noted that in both of these studies, the HEP compounds were measured several hours after the initial insult. The onset of massive SNS activation, and to a lesser extent the initial exposure to high doses of norepinephrine, result in a transient period of elevated arterial pressure and tachycardia (hyperdynamic phase), which persists for 15–30 min (1, 5, 7–12). If myocardial ischemia develops, it should occur during this time of intense myocardial energy demand. Thus, in the above mentioned studies, a transient fall in ATP and creatine phosphate concentrations of sufficient

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magnitude and duration to cause injury or dysfunction might have been missed due to the timing of the measurements. Therefore, the aim of the present study was to determine whether myocardial ischemia develops during the period of hyperdynamic activity, and whether HEP concentrations fall during this time.

Materials and Methods

All procedures used in this investigation were approved by the Institutional Animal Care and Use Committee of the Northeastern Ohio Universities College of Medicine and conform to the standards specified in the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.

Animal Preparation. New Zealand white rabbits (3–4 kg) of either sex were anesthetized with pentobarbital sodium (50 mg/kg, i.v.), tracheostomies were performed, and the rabbits were ventilated with 40% O_2/60% N_2. Tidal volume was set at 30 ml and arterial pH and PCO_2 were kept in the normal range by varying the respiratory rate from 10–20 breaths/min. These settings resulted in arterial PO_2s that averaged 130 ± 3 torr. The right femoral artery was cannulated with polyethylene tubing (Intramedic PE 90) to measure arterial pressure and to withdraw samples of blood for blood gas determinations. Arterial pressure was recorded at 200 Hz on a MacLab data acquisition system (AD Instruments, Mountain View, CA), and the heart rate was calculated from the arterial pressure tracing. The right femoral vein was cannulated with a 22-gauge angiocath to administer saline, drugs, and supplemental doses of anesthetic. In experiments in which coronary blood flow (CBF) was measured, a polyethylene catheter (PE 90) was placed in the left ventricle via the right carotid artery and was used to inject fluorescent microspheres. The left femoral artery and vein, respectively, were cannulated in these experiments to collect the reference blood flow and to infuse dextran during the time of blood withdrawal.

Experimental Procedures. When blood pressure and heart rate were stable, the SNS was massively activated by inserting a 21-gauge hypodermic needle into the cisterna magna and immediately injecting 280 μg of veratrine (in 0.35 m of saline). SNS activation produces 20–30 min of hypertension and tachycardia (hyperdynamic phase) that peaks in 2–3 min in most rabbits. CBF was measured (15 μm fluorescent microspheres; Molecular Probes, Eugene, OR) under baseline conditions (time 0), and at 2, 10, and 20 min after SNS activation in one group of rabbits (n = 11), and at baseline, 45, 90, and 150 min in a second group (n = 15). CBF was measured at 0, 45, 90, and 150 min in control rabbits (n = 12). These rabbits did not have their SNS activated, but instead were given the same dose of veratrine intravenously. Yellow, crimson, orange, and blue fluorescent microspheres, respectively, were administered sequentially to all three groups. We elected to use no more than four colors so as not to overload the heart with microspheres, and to avoid the problem of crossover fluorescence that arises when microspheres with overlapping excitation and emission spectra are used. The microspheres were sonicated for at least 10 min and were then vortexed vigorously immediately before each administration. One hundred thousand to 500,000 microspheres were administered for each CBF determination. The number depended on the intensity of the fluorescence produced by a particular color and the sensitivity of the photomultiplier tube to light of that wavelength. To help ensure appropriate mixing within the left ventricle, the microspheres were infused at a rate of 1.1 ml/min over 6–27 sec. Blood was withdrawn from the left femoral artery into a heparinized (2000 U) glass syringe at a rate of 2.44 ml/min and was used as the reference for calculating CBF. Withdrawal began a few seconds before the onset of microsphere infusion and continued for ~1.75 min. Dextran was infused simultaneously into the left femoral vein at a rate equivalent to the blood withdrawal rate to minimize any hemodynamic changes. The experiments were terminated 150 min after SNS activation, and the hearts were removed to evaluate LV function.

Isolated heart preparation. One hundred-fifty minutes after SNS activation or at a comparable time in the control rabbits, heparin (1000 U/kg i.v.) was administered, and a midline thoracotomy was performed. The heart was rapidly removed from the animal, immediately arrested in ice-cold (0°C) physiological saline, and then perfused in Langendorf fashion at constant pressure (80 mmHg) and temperature (~32°C). The coronary venous effluent was collected and recirculated. The perfusion solution was aerated with 95% O_2/5% CO_2 and had the following composition (mM): NaCl, 89.0; KCl, 5.0; $CaCl_2$, 2.0; $MgSO_4$, 1.0; $NaHCO_3$, 24.0; Na_2HPO_4, 1.0; CH_3COONa, 20.0; and dextrose, 10.0. The pH and PO_2, respectively, of this solution was 7.4 and approximately 650 mmHg.

Evaluation of LV function. LV pressure was measured by inserting a saline-filled latex balloon connected to a pressure transducer into the left ventricle through a small incision in the left atrium. The balloon was secured by placing a ligature around the atrioventricular groove to constrict the mitral annulus. Before evaluating LV function, the atrioventricular node was crushed with hemostats, and the ventricles were paced with a Grass stimulator at 90 beats/min. After the heart had equilibrated, LV volume was set at 0.1 ml, and LV pressure was recorded. Balloon volume was then increased in 0.3-ml increments until LV volume was 1.6 ml. This procedure was performed in duplicate, and the peak systolic and end-diastolic pressures for the two trials were averaged for each LV volume (preload). The average developed pressure (peak systolic minus end-diastolic) was then plotted as a function of LV end-diastolic volume to generate a curve that was used to evaluate LV performance. The pressure-volume relationship of the balloon was evaluated before each experiment and was judged to be acceptable if no detectable pressures were generated by the balloon over the range of volumes used to evaluate LV function. Since preload, afterload, and heart rate were controlled

in this preparation, a shift in the LV function curve indicated a change in LV contractility. Hearts were perfused at ~32°C and with a heart rate of 90 beats/min to ensure that they were adequately oxygenated during evaluation of LV function. Although this evaluation was made under hypothermic conditions and at heart rates that were lower than normal, the insult producing the LV dysfunction took place in the animal at physiologic temperatures and heart rates. Thus, our method for assessing LV function was a type of assay that allowed us to determine the degree of LV dysfunction that occurred in the animal due to the massive SNS activity.

Extraction of microspheres and calculation of CBF. After evaluating LV performance, the atria and right ventricular free wall were removed from the heart. Regional flow was determined by opening the ventricle from base to apex along the septal-LV free wall border. The LV free wall and attached septum were then placed in a flattened position and partially frozen to facilitate the final tissue cuts. The septum was separated from the LV free wall, and the free wall divided into two approximately equal portions by cutting along the midwall. Each of the three pieces (subepicardium to midwall, midwall to subendocardium, and septum) was weighed and then digested with KOH. The reference blood sample was transferred from the withdrawal syringe to a 50-ml polypropylene tube. The syringe and accessory tubing were washed three times with 2% Tween-80, and the diluted blood was digested with KOH. Digestion of both blood and tissue samples was performed at 60°C for 24 h. The digest was vacuum filtered through a 10-μm pore membrane (Poretics, Livermore, Ca), and the filter apparatus washed with Tween-80 and phosphate buffer. The membrane containing the recovered microspheres was transferred to a 30-ml polyethylene tube and the microspheres were dissolved with 2 ml of 2-ethoxyethyl acetate (Cellosolve). Fluorescence was measured using a PerkinElmer (Norwalk, CT) fluorescence spectrophotometer (model 512) at the following excitation (Ex) and emission (Em) wavelengths (nm): yellow, Ex 495-Em 506; blue, Ex 427-Em 470; orange, Ex 532-Em 549; and crimson, Ex 611-Em 633. CBF was calculated by dividing the fluorescence of the tissue sample by the fluorescence of the reference blood and multiplying by reference blood flow (2.44 ml/min). Blood flow of each sample was expressed per gram of tissue by dividing by the sample weight. The coronary vascular resistance of each sample was calculated by dividing mean arterial pressure (MAP) by sample blood flow.

Myocardial HEP and lactate determinations. Thirty six rabbits were anesthetized and instrumented as described above to complete this aspect of the study. Myocardial ATP, creatine phosphate, and lactate concentrations were measured under baseline conditions (n = 11), and at 2 (n = 5), 10 (n = 5), 20 (n = 5), 90 (n = 6), and 150 min (n = 4) after massively activating the SNS with an intracisternal injection of veratrine. At one of the above times, the chest was opened and the heart was rapidly frozen *in situ* using clamps prechilled in liquid N_2. After removing the atria, a small portion of the ventricular tissue was dried to a constant weight at 80°C to determine tissue water content. The remainder of the ventricular tissue was pulverized to a fine powder in liquid N_2. The powdered tissue (~1 g) was deproteinated with perchloric acid (11.6 M) in a 4:1 acid-to-tissue ratio, and the mixture was centrifuged for 20 min at 2°C. The pH of the supernatant was increased to 7.6 with K_2CO_3 (5.0 M), and the neutralized solution was stored on ice for 10 min. Aliquots of the clear supernatant were used immediately to determine ATP and creatine phosphate concentrations using standard enzymatic assays (13, 14). The lactate concentration of the supernatant was also determined enzymatically with a kit obtained from Sigma-Aldrich (St. Louis, MO). The concentrations of the three metabolites were expressed in micromoles per gram of dry weight.

Statistical Analysis. Repeated measures analysis of variance (ANOVA) was used to make multiple group comparisons of LV function, arterial pressure, heart rate, arterial pressure-heart rate (PR) product, CBF, and coronary vascular resistance over time. Statistically significant effects ($P < 0.05$) were followed by Fisher's Protected LSD *post hoc* comparisons to determine which groups were statistically different. Statistically significant time-related changes within individual groups were determined using a 1 within-0 between model ANOVA. Statistically significant changes were followed by contrast comparisons to determine which time points were statistically significant. One-factor ANOVAs were used to determine whether changes in myocardial ATP, creatine phosphate, or lactate concentrations after SNS activation were significantly different from their baseline values. Statistically significant effects were followed by Fisher's Protected LSD *post hoc* comparisons to determine which time points were statistically different.

Results

Massive SNS activation resulted in depressed LV contractility in 15 of the 26 rabbits in which LV function was evaluated. Because contractility was normal in several of the rabbits, the data were analyzed after subgrouping the animals based on their cardiac performance. Left ventricles that failed to generate at least 95 mmHg (at an LV volume of 1.6 ml) were considered to have depressed cardiac function. This number was selected after reviewing records of control hearts studied under similar conditions over the past several years. The validity of this subgrouping is illustrated in Fig. 1. The LV function curve of rabbits considered to have depressed LV contractility was markedly displaced downward ($P < 0.001$), whereas the curve from the subgroup considered to have normal LV function was virtually identical to the curve representing control rabbits.

SNS activation caused a transient increase in MAP, heart rate, and PR product, which peaked in 1–3 min. The SNS-related hemodynamic changes were somewhat different between rabbits that had depressed LV function and those that did not. Although peak MAP and peak systolic

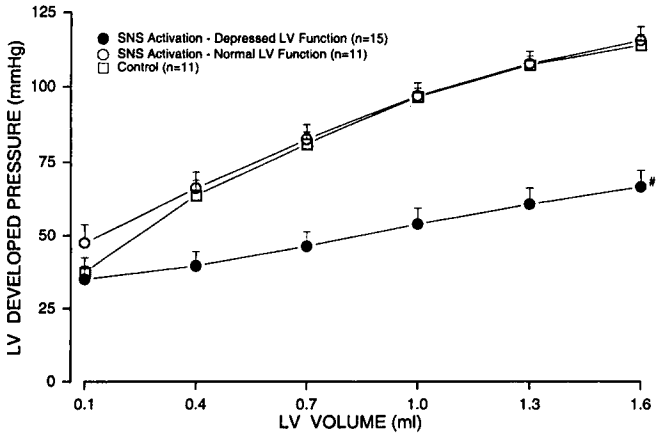

Figure 1. LV function after massive SNS activation. SNS activation resulted in a severe depression of LV contractility in many (58%) but not all rabbits. Control rabbits were administered veratrine intravenously. #, LV function curve significantly different from control rabbits ($P < 0.01$).

PR product were not significantly different between the subgroups, peak heart rate was greater ($P < 0.01$) in the group with depressed LV contractility (Fig. 2) and the hyperdynamic phase was longer. MAP returned to baseline in ~20 min in these rabbits compared with less than 10 min for rabbits that had normal LV function. After 20 min, MAP was ~10 mmHg lower than baseline in both subgroups. It remained steady at this level in the rabbits with depressed LV function, but returned to baseline by the end of the experiment in the animals that maintained normal cardiac performance. Heart rate and systolic PR product also fell below baseline 10–20 min after SNS activation, and remained lower for the duration of the experiment in both subgroups. Arterial pressure, heart rate, and PR product did not change significantly at any time in control rabbits that received veratrine intravenously.

The effect of massive SNS activation on CBF is shown in Fig. 3. CBF increased significantly in both subgroups 2 min after massive SNS activation, but fell rapidly and was not significantly different from baseline by 10 min. CBF of rabbits with normal LV function remained at baseline for the duration of the experiment, but fell below baseline in rabbits with depressed LV contractility after 90 min. As shown in Fig. 4, changes in CBF correlated with the changes in systolic PR product. However, the increase in CBF was greater than the increase in PR product during the period of hyperdynamic activity. There was no difference in the CBF-systolic PR product relationship between rabbits that had depressed LV contractility and those that maintained normal LV function.

Changes in regional flow (Fig. 5) followed the same general pattern as the changes in total CBF. Blood flow to the subepicardium, subendocardium, and septum of both subgroups was increased 2 min after SNS activation, but returned to baseline in all three regions within 10 min. Peak blood flow tended to be higher in all regions in the rabbits with diminished LV contractility, but the differences were statistically significant only in the subepicardium. Subendocardial-to-subepicardial blood flow ratio did not change significantly in either subgroup during the period of hyperdynamic activity. During the later stages of the experiment (90–150 min after SNS activation), blood flow to the sub-

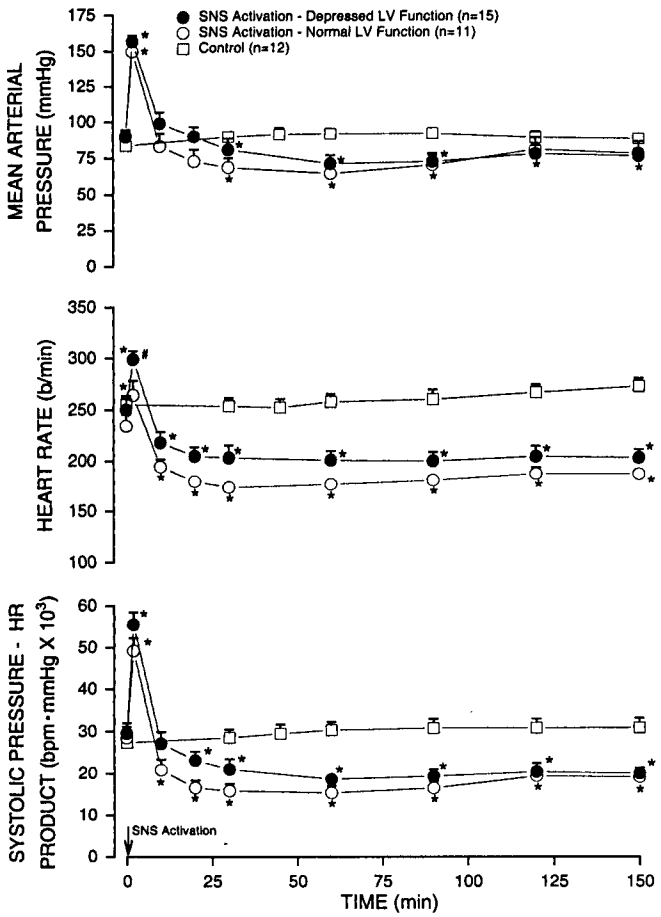

Figure 2. Hemodynamic changes after massive SNS activation. The increase in MAP and systolic PR product at 2 min was similar in both subgroups. #, Significantly greater than subgroup with normal LV function. *, Significantly different from 0 min.

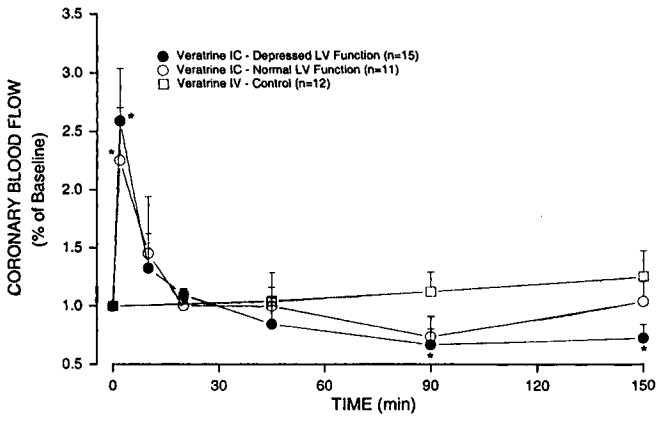

Figure 3. The effect of massive SNS activation on CBF. The changes in CBF were similar in both subgroups during the first 20 min of intense SNS activity. After 90 min, CBF in the subgroup with depressed LV function fell below baseline. *, Significantly different from 0 min.

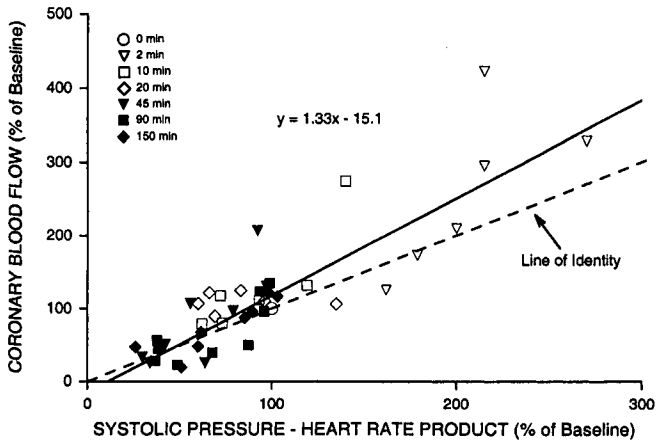

Figure 4. CBF-systolic PR product relationship of rabbits with SNS-induced depression of myocardial contractility. Each data point represents a single measurement taken between 0 and 150 min after SNS activation in 1 of 15 rabbits. The slope of regression line through the data points was significantly greater than unity ($P < 0.01$).

endocardium and septum was significantly diminished in the rabbits with depressed cardiac performance. The lower flow was likely due to the depressed contractile state of these hearts because ATP and creatine phosphate concentrations were normal at this time.

Coronary vascular resistance (CVR) fell 2 min after SNS activation, but the fall was statistically significant only in the subendocardium and septum of rabbits that displayed normal LV function (Fig. 6). A significant fall in CVR was observed in these animals because their baseline CVR was higher than that of the other groups. After SNS activation, there were no differences in vascular resistance between the two subgroups in any of the regions at any time.

The hemodynamic pattern of the rabbits used to determine myocardial HEP concentrations was similar to the rabbits of the CBF experiments that had depressed LV contractility. Although the average peak heart rate was 23 beats/min greater, average peak MAP and peak systolic PR product of 158 ± 4 mmHg and 61,832 ± 2,099 mmHg/beats/min, respectively, were not significantly different. Thus, myocardial energy demand appears to have been about the same in both of these groups.

Creatine phosphate concentration fell from 29.4 ± 1.0 to 23.8 ± 1.8 μmol/g ($P < 0.05$) 2 min after SNS activation and remained significantly depressed for at least 20 min (Fig. 7, top). Baseline myocardial ATP concentration of 22.3 ± 0.5 μmol/g diminished continuously during the hyperdynamic phase and reached a concentration (19.4 ± 1.1 μmol/g) that was significantly lower than baseline ($P < 0.05$) 20 min after SNS activation (Fig. 7, middle). At this

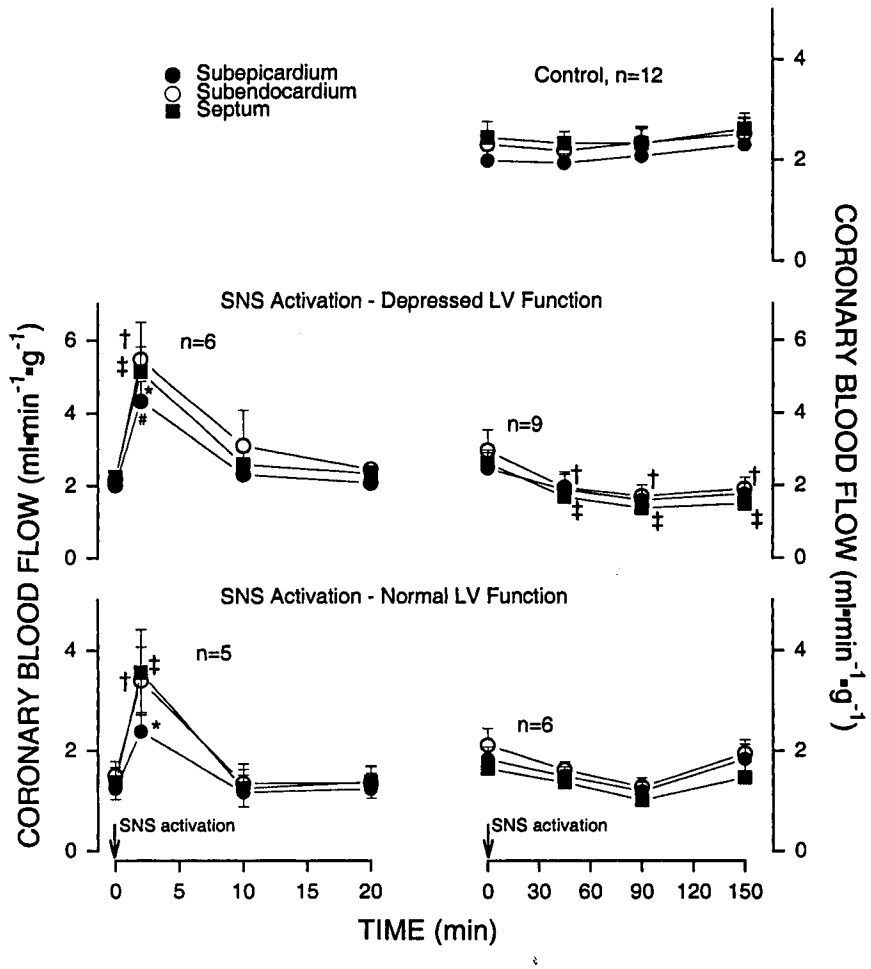

Figure 5. Changes in regional CBF after massive SNS activation. CBF during the first 20 min of SNS activation is shown on the left. A second group was used to obtain measurements from 45–150 min (right). Blood flow to all three regions increased significantly in both subgroups at the peak of the hyperdynamic phase (2 min), but returned to baseline by 10 min (left). After 45 min (panel), subendocardial and septal blood flow was significantly decreased from baseline in the rabbits with depressed LV contractility, but not in rabbits that had normal LV function. Significant ($P < 0.05$) differences from respective values at 0 min: *, subepicardium; †, subendocardium; ‡, septum. #, Significantly greater than rabbits that had normal LV function after massive SNS activation.

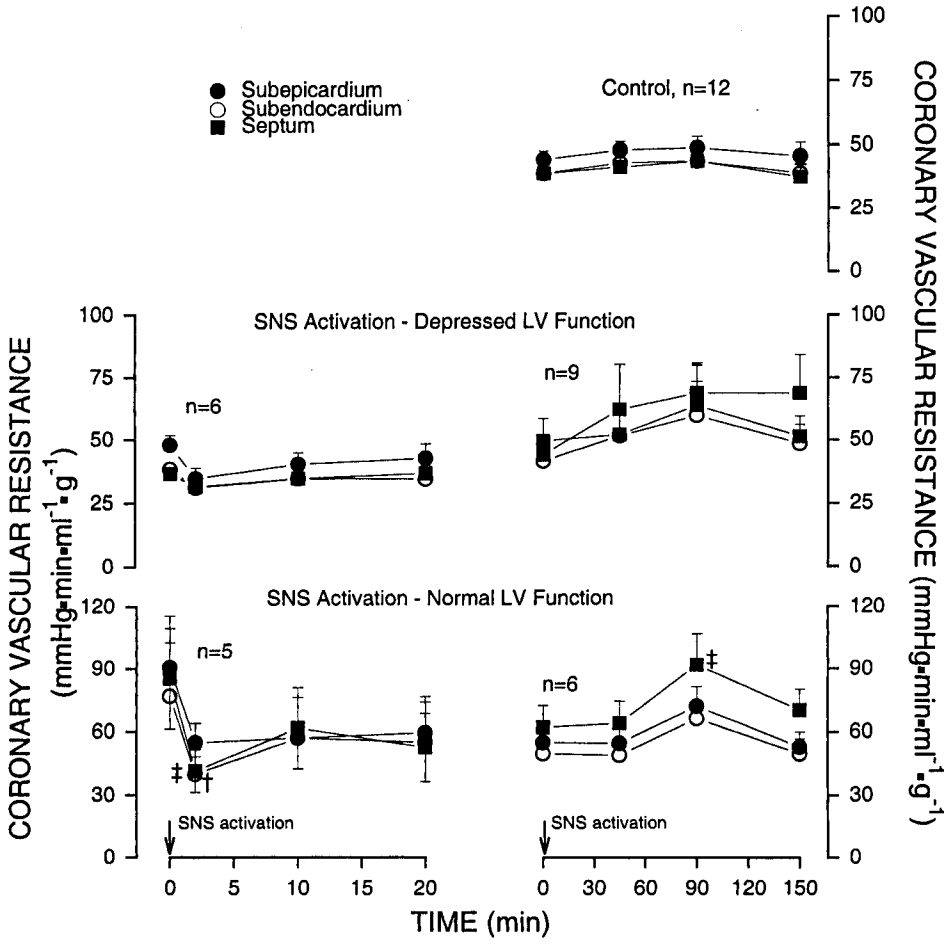

Figure 6. The effect of massive SNS activation on coronary vascular resistance. Although CVR fell significantly in the subendocardium and septum at the peak of the hyperdynamic period in rabbits with normal LV function (lower left), it was not lower at this time than rabbits that developed LV dysfunction (upper left). Significant ($P < 0.05$) differences from respective values at 0 min: †, subendocardium; ‡, septum.

time, myocardial lactate concentration (Fig. 7, bottom) had risen from 13.9 ± 2.9 to 36.8 ± 8.9 μmol/g ($P < 0.05$). By 90 min, the concentrations of all three metabolites had recovered to their respective baseline values.

Discussion

Despite considerable effort, the mechanism responsible for catecholamine-induced myocardial injury has been difficult to identify. One hypothesis is that the injury results from Ca^{2+} overload secondary to intense adrenoceptor activation. This hypothesis was proposed by Fleckenstein *et al.* (2) after observing that high doses of isoproterenol cause a marked increase in Ca^{2+} uptake in the myocardium. Both the myocardial damage and the Ca^{2+} uptake were blocked by L-type Ca^{2+} channel antagonists. Nevertheless, work from other laboratories indicated that ischemia could be the cause (3, 4, 15). In this regard, we previously found that the heart is protected from SNS-induced LV dysfunction by interventions that reduce myocardial energy demand (1, 5). Although this evidence was circumstantial, these findings led us to hypothesize that ischemia was the cause of SNS-induced LV dysfunction. In the present study, ATP and creatine phosphate concentrations fell during the 20-min period of hyperdynamic activity that immediately followed massive SNS activation. However, three lines of evidence indicate that this fall did not result from ischemia. First, SNS-related increases in CBF were greater than the increases in systolic PR product. The systolic PR product is an excellent indicator of myocardial oxygen demand even when contractility is altered by catecholamines (16). Thus, there was no evidence that myocardial oxygen demand exceeded oxygen supply. Second, the CBF-systolic PR product relationship of rabbits that had depressed LV contractility after SNS activation was the same as the CBF-systolic PR product relationship of rabbits that maintained normal LV function. Last, there were no changes in subendocardial-to-subepicardial blood flow ratio as one would expect if ischemia were present. CVR fell to a greater extent immediately after SNS activation in the rabbits that had normal LV function, but this occurred because these animals had greater baseline CVR. After SNS activation, CVR was similar in all rabbits whether they developed LV dysfunction or not. Thus, although myocardial HEP was found to be significantly reduced, there was no evidence that myocardial ischemia had occurred. The reason for the diminished HEP is not known, but high concentrations of catecholamines have been shown to decrease myocardial phosphorylation potential independent of ischemia (17, 18). Ca^{2+} overload, free radical generation, or excessive mobilization of free fatty acids are a few possible mechanisms that could have

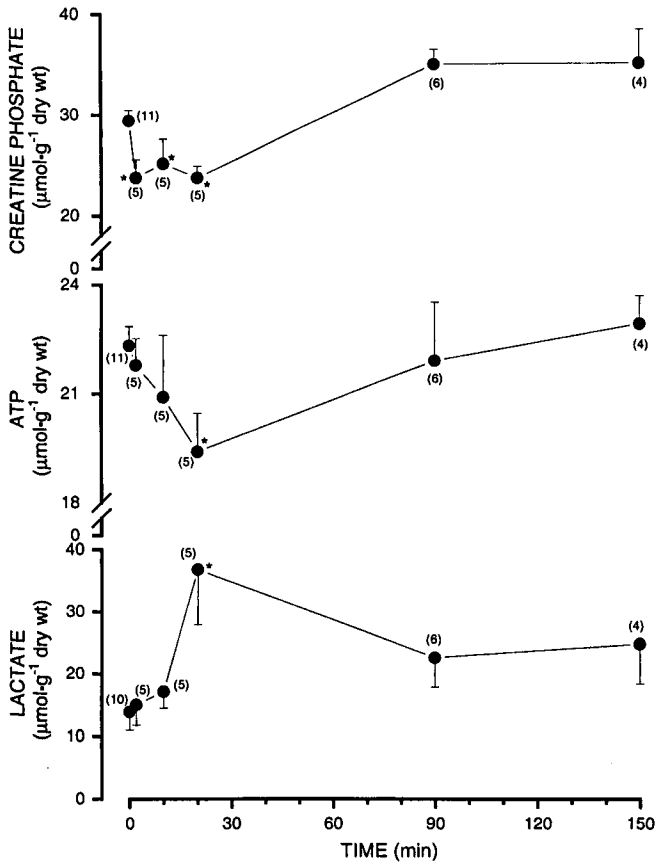

Figure 7. Changes in tissue metabolite concentration after massive SNS activation. Creatine phosphate concentration decreased significantly during the first 2 min of intense SNS activity and remained lower than baseline for at least 20 min (top). ATP concentration (middle) decreased progressively during this time and was significantly lower than baseline by 20 min. Lactate concentration (bottom) was elevated at this time. The concentrations of all three metabolites recovered by 90 min. *, Significantly different from 0 min.

acted alone or in combination to uncouple oxidative phosphorylation. Our previous studies showed that SNS-induced LV dysfunction is ameliorated by interventions that reduce myocardial workload (1, 5). In view of our present findings, it appears that workload reduction is beneficial because of its energy sparing effect, and not because it allows CBF to keep pace with the energy needs of the heart.

We did not determine how long the energy pool of the myocardium remained depressed, but diminished HEP concentrations persisted for at least 20 min after the SNS was activated before recovering. In a previous study (19), norepinephrine was infused into rabbits at a dose that simulated the hemodynamic changes that typically occur after massive SNS activation. We found that the norepinephrine-induced depression of LV contractility had reversed within 4 days. Thus, our finding that the energy pool of the myocardium is diminished for at least 20 min, along with our previous observation that catecholamine-induced LV dysfunction is reversible, suggests that the dysfunction could be a type of myocardial stunning.

In previous studies, our veratrine model of SNS activation produced severe depression of LV contractility in 70–80% of rabbits (1, 8, 11). However, in the CBF experiments of this study, the number of rabbits that did not develop LV dysfunction after veratrine administration was higher than we typically observe. For reasons that are unknown to us, the hemodynamic pattern was different in these animals. Normally there is a brief fall in arterial pressure immediately after the veratrine injection. Approximately 15 sec later, tachycardia develops and arterial pressure climbs steadily for 2–3 min to a peak that is ~2 times greater than baseline. Arterial pressure then begins to decline slowly to baseline over the next 20–30 min. The initial hypotension was absent in the rabbits that did not develop LV dysfunction. Arterial pressure rose quickly (often reaching a peak in <1 min) and then rapidly returned to baseline within a few minutes. Moreover, heart rate tended to be lower at all times after SNS activation. The abbreviated period of intense SNS activity along with lower heart rate would have made it easier to maintain normal myocyte energy balance and might explain why these animals did not have impaired LV function. Although it is unclear why so many of the rabbits had this hemodynamic pattern and failed to exhibit LV dysfunction, these animals were nevertheless valuable because they provided an opportunity to compare CBF after SNS activation between rabbits with and without LV dysfunction. This comparison helped to support our conclusion that myocardial ischemia did not develop.

There were four limitations in our experimental design that should be considered. First, CBF, LV performance, and myocardial HEP could not be determined in the same heart. Consequently, we could not confirm that creatine phosphate and ATP concentrations did not fall in the hearts with normal LV function nor was it possible to evaluate the cardiac performance of the hearts in which HEP was measured. However, the hemodynamic pattern in the rabbits that were involved in the HEP determinations was similar to the pattern that occurred in the rabbits of the CBF experiments that had depressed LV function, and were typical of the pattern that we have consistently observed in previous studies. Thus, we believe that we would have observed LV dysfunction in most of these rabbits if it could have been evaluated, and that the energy condition of these hearts was representative of hearts with depressed contractility. Secondly, the microspheres used to measure CBF could not be injected into the left atrium, which is the preferred site. This would have been extremely difficult, if not impossible, in the closed-chest rabbit preparation that we used. To help ensure appropriate mixing in the left ventricle, the microspheres were administered over several seconds using an infusion pump. This approach appeared to work well. In control animals, CBF was consistent from measurement to measurement throughout the 150-min duration of the experiment. Furthermore, our CBF values were within the range of expected values for anesthetized animals. Average baseline CBF (2.15 ml/min/g) was similar to that previously reported for open chest, pentobarbital-anesthetized rabbits (20) in which CBF was measured using radioactive micro-

spheres that were injected into the left atrium. The third limitation was that vehicle (saline) was not administered intracisternally to control animals. This would have been risky because the resulting increase in intracranial pressure could have activated the SNS (Cushing response), and we did not want to take this chance. In this regard, any increase in intracranial pressure that was associated with the veratrine injection may have helped to increase the intensity of SNS activation in the experimental group. The final limitation was that isolated hearts were studied under hypothermic conditions and at lower than normal heart rates. This was done to ensure that the metabolic needs of the heart were met and that oxygen supply-demand balance was maintained. When comparing LV function with this approach, one must be certain that differences are due to the experimental insult and are not the result of an ischemic preparation. LV function of our control hearts has been stable and very consistent from study to study over the past several years. Consequently, we have the utmost confidence that our preparation is yielding reliable results. It is important to note that the consequences of massive SNS activation occurred in the intact animal under normal temperature and heart rate conditions. Thus, our heart preparation functioned as an assay that allowed us to determine the degree of dysfunction that occurred when the heart was in the animal.

In summary, massive SNS activation led to a decrease in myocardial energy reserves that did not appear to result from myocardial ischemia. Although the decline in energy supply is transient, it seems to be of sufficient duration to cause myocardial stunning, which is consistent with our previous observation that this type of LV dysfunction is reversible.

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