Reversal of Reflex-Induced Myocardial Ischemia by Median Nerve Stimulation
A Feline Model of Electroacupuncture
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Background—Acupuncture is reported to reduce myocardial ischemia, arrhythmias, and hypertension. To investigate the physiological mechanisms underlying these observations, a model of reflex-induced, reversible myocardial ischemia was developed to test the effects of median nerve stimulation as a surrogate for electroacupuncture.

Methods and Results—Chloralose-anesthetized cats were instrumented to measure arterial blood pressure, left ventricular pressure, left ventricular dP/dt, heart rate, left anterior descending (LAD) coronary blood velocity, and regional wall motion. The LAD artery either was partially occluded or a small diagonal branch was ligated. Subsequently, transient reflex activation of the cardiovascular system was evoked by application of bradykinin (typically 1 μg/mL) to the gallbladder, which significantly increased myocardial oxygen demand (double product), left ventricular dP/dt, and coronary blood velocity and caused ischemia-induced regional dysfunction, evidenced by significant (*P < .05) reduction in normalized wall thickening (10.7 ± 4.2% versus 23.6 ± 2.9%; control versus ischemia; n = 7). However, when median nerves were stimulated with low frequency (5 Hz) to mimic electroacupuncture, bradykinin-induced change in normalized wall thickening was significantly improved (−23.6 ± 2.9% versus 9.8 ± 4.9%; ischemia versus median nerve stimulation, *P < .05) and remained augmented ≥ 1 hour. Results were similar in partial and complete occlusion groups. Significant improvement in wall thickening was associated with unchanged increment of coronary blood velocity and significantly diminished increments of double product and diastolic blood pressure.

Conclusions—These results suggest that stimulation of the median nerve to mimic electroacupuncture diminishes regional myocardial ischemia triggered by a sympathetically mediated increase in cardiac oxygen demand. The mechanism of this effect is related to reduction in cardiac oxygen demand, secondary to a diminished pressor response. These data provide the first documentation of the physiological mechanisms underlying the possible beneficial effect of electroacupuncture in the context of restricted coronary blood flow and augmented myocardial oxygen demand. (Circulation. 1998;97:1186-1194.)

Key Words: bradykinin ■ ischemia ■ coronary heart disease ■ angina ■ ultrasonics

Medical treatment of many clinical conditions with techniques used in traditional Chinese medicine, such as acupuncture and electroacupuncture, has been met with substantial scientific skepticism in Western countries. However, treatment with alternative methodologies has garnered more acceptance in the last several years. It is important, therefore, to fully evaluate the utility and explain the mechanisms underlying these effects of acupuncture and electroacupuncture in terms (ie, physiological principles) that are accepted by Western scientists and physicians. Although in the 1950s acupuncture was touted as a treatment for pain, for instance during surgical analgesia,1 in 1979 the World Health Organization provisionally expanded the list of diseases that could be treated to include acute infections and inflammation, dysfunction of the autonomic nervous system, and peripheral and central neurological diseases.1 Since then, clinical observations have suggested that acupuncture may have therapeutic effects on hypertension, coronary heart disease, certain dysrhythmias, and myocardial infarction.2-7 Richter et al,5 for instance, demonstrated that acupuncture administered three times per week for 30 minutes reduces the number of anginal attacks compared with placebo and increases the threshold for angina during exercise. Also, Ballegaard et al6 observed that electroacupuncture, administered 20 minutes per day for 3 weeks, increased the maximal rate-pressure product for patients with severe, stable angina during exercise. These data suggested clinical efficacy of acupuncture in patients with coronary heart disease, but they did not provide an explanation of the underlying mechanism(s).
Experimental studies in animal models have attempted to provide a physiological framework to explain the action of acupuncture in patient populations. First, it was suggested that acupoints in humans that could affect the cardiovascular system, including Neiguan and Renzhong, were positioned over peripheral nerves that could be located in animals. Second, stimulation of these sites could be shown to lower blood pressure in hypertensive models (eg, spontaneously hypertensive rats and norepinephrine-induced hypertensive rats), raise blood pressure in shock models, or reduce myocardial ischemia. However, these latter studies used hypothalamic stimulation or electric shock and isoproterenol infusion to induce ischemia, which was measured by ECG ST-segment deviation or myocardial necrosis. Each of these models has limited applicability to clinical ischemia, which generally is the result of imbalances between myocardial oxygen supply and demand. Also, measurement of myocardial ischemia was inexact, relying primarily on ECG changes.

In view of the limited information on the mechanisms that underlie the potentially beneficial effects of acupuncture or electroacupuncture, we developed a feline model of provokable myocardial ischemia in which myocardial oxygen supply and demand could be evaluated independently. Reflex-induced sympathetic stimulation was used as a physiological trigger of myocardial ischemia. Accordingly, we applied BK to the cat's gallbladder because this maneuver simulates the clinical conditions of inflammation and ischemia or cholecystitis and reflexly activates the sympathetic nervous system to increase arterial blood pressure, myocardial contractility, and, to a lesser extent, heart rate, all of which serve to augment myocardial oxygen demand. Less commonly, chemical activation of abdominal visceral organs causes sympathetically mediated coronary arterial vasoconstriction. As in other studies, we simulated acupuncture by isolating and electrically stimulating the median nerves (corresponding to the Neiguan acupoint). We hypothesized that low-intensity electrical stimulation of predominantly finely myelinated fibers in somatic nerves would lessen the extent of ischemia induced by reflex activation of the cardiovascular system. Furthermore, we hypothesized that the mechanism underlying reduced myocardial ischemia would be related to reduced myocardial oxygen demand rather than increased blood flow, based on the ability of acupuncture to lower blood pressure in hypertension models. Both partial and complete coronary artery ligation protocols were used to model the clinical settings of myocardial ischemia associated with restricted coronary artery blood flow and coronary occlusion, respectively.

**Methods**

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of the University of California, Davis. The studies conformed to American Physiological Society guidelines and principles for research involving animals. Adult cats of either sex (3 to 7.5 kg) were anesthetized by intramuscular or subcutaneous injection of ketamine (40 mg/kg) followed by bolus intravenous injection of α-chloralose (50 to 75 mg/kg). Additional injections of α-chloralose (5 to 10 mg/kg IV) were given to maintain an adequate depth of anesthesia as judged by respiratory rate, jaw tone, and withdrawal response to toe pinch. The trachea was intubated and respiration was maintained artificially (model 661, Harvard Apparatus). Arterial blood gases and pH were measured periodically in all animals with a blood gas analyzer (model ABL-3, Radiometer). Arterial PaO2 and PaCO2 were kept within normal limits (PaCO2 30 to 35 mm Hg and PaO2 >100 mm Hg) by enriching the inspired O2 supply and adjusting the ventilatory rate or volume. Arterial pH was kept between 7.35 and 7.45 and corrected, as necessary, by infusion of 8% sodium bicarbonate. Body temperature was monitored with a rectal probe (model 44TD, Yellow Springs Instrument Co) and maintained at a range of 36° to 38°C by a water heating pad and a heating lamp.

The right femoral vein was cannulated for administration of drugs and fluids. Systemic arterial blood pressure was monitored by a pressure transducer (model 1290, Hewlett-Packard) attached to a cannula inserted into the right femoral artery. A polyethylene tube (PE-90) was inserted into the left ventricle through the left carotid artery to measure LV pressure and to provide an index of dP/dt. 

**Coronary Flow Reduction**

The left chest wall was opened near the midline, two ribs were removed, and the pericardium was incised to expose the heart and coronary artery. Care was taken to prevent drying of the exposed anterior surface of the heart by covering it with a saline moistened gauze. To produce a controlled reduction in regional coronary blood flow, the proximal LAD was isolated and an occluder device was positioned around the artery (n=10). The occluder consisted of a suture snare connected to a manual, screw-controlled device to partially occlude the vessel. A pulsed Doppler flow probe consisted of a small piezoelectric crystal (1 mm² at an angle of 45 degrees). It was positioned over the LAD distal to the occluder to provide continuous measurement of coronary blood velocity (n=6) (model 100, Pulsed Doppler Flowmeter, Triton Technology). In other animals (n=8), a small high diagonal branch of the LAD on the anterior LV wall was tied with 4-0 silk suture to induce regional ischemia.

**Regional Wall Motion**

Measurements of LV wall thickness were performed with a modified 20-MHz single-transducer sonomicrometer system. The operating principles of the single-transducer ultrasonic instrument are as follows: a 0.5-µs duration burst of 20-MHz pulses is transmitted from the piezoelectric transducer. Ultrasonic waves travel through the myocardial wall muscle and ventricular cavities and reflected echoes are received back by the piezoelectric transducer. The Doppler-shifted echoes are sampled at two successive time intervals, generating two sample volumes, a reference and an interface sampled volume. The Doppler signals from the two sample volumes are amplified, filtered, then applied into RMS-to-DC converter circuits. Steady state is achieved when the reference sample volume remains...
fully within the myocardium, while half of the interface volume remains inside the myocardium and the other half is located within the ventricular cavity. When the endocardium changes position, an amplitude-lock-loop circuit repositions the two sample volumes until steady state is reached again. This closed-loop process results in continuous tracking of the endocardial muscle/blood interface throughout the cardiac cycle. The transducer assembly used in the modified version of the single-transducer sonomicrometer system consisted of a two-dimensional, five-element array arranged in an “X” pattern with each element or group of elements in this array configured as either a transmitter and/or a receiver. The surface area of each crystal was 2 mm²; the sample volume measured by the transducer was ~0.9 mm³. This transducer arrangement has been found to be more versatile and require less time to position on the epicardium and obtain a satisfactory signal from the endocardium than the previously described two-element array. The reliability and accuracy of this ultrasonic single-transducer sonomicrometer system has been published previously.16 The transducer was secured on the serosal surface of the gallbladder with a 1-cm² pledget soaked with BK solution. For each animal, the concentration that provided a control pressor response of 10 mmHg was used throughout the experiment. In most animals, 1 or 10 μg/mL BK was satisfactory, but 4 animals required a different dose (0.1 μg/mL, [n = 2]; 50 μg/mL, [n = 1]; 100 μg/mL BK, [n = 1]). It was determined gravimetrically that each pledget contained 26 μL of solution. Thus, at a concentration of 10 μg/mL, for example, a dose of 0.26 μg BK was applied to the surface of the gallbladder. After the maximum pressor reflex was attained (typically 25 to 60 seconds), the filter paper was removed and the gallbladder was washed twice with normal saline from cotton-tipped applicators to remove BK. To prevent tachyphylaxis, recovery periods of at least 15 minutes were provided between applications.

Assessment of Ischemic Risk and Infarct Areas

The myocardial risk area was determined for hearts subjected to partial occlusion of the LAD (n = 7) and complete occlusion of the LAD diagonal branch (n = 5). For the partial occlusion experiments, the LAD was occluded at the end of the experiment at the site of partial occlusion. The procedure for measurement of risk and infarct areas has been described previously.17 Briefly, patent blue violet dye (0.5%) was injected into the left atrium and the heart was fibrillated by application of direct current to the epicardium. The heart was excised, rinsed in cold saline and sliced in breadloaf fashion into four to six rings from apex to base. The right ventricular free wall was removed and each slice was traced onto clear acetate. The risk area was identified as the nonblue region. The slices were weighed and each slice was traced onto clear acetate. The risk area and infarct area for each slice. The percent areas were converted to grams of tissue weight for calculation of risk and infarct areas as percentages of total LV weight for each heart.

Experimental Protocols

Protocol 1: Effect of Median Nerve Stimulation on Myocardial Ischemia

Animals were allowed to stabilize for 15 minutes before the first application of BK to the gallbladder. When the cardiovascular response to BK was constant (generally after two to four applications), the LAD was occluded partially to reduce flow by ~50% or a proximal diagonal branch of the LAD was ligated and completely occluded. Partial or complete occlusion was maintained throughout the remainder of the experiment. BK was applied twice during the next 30 to 40 minutes. MNS was initiated (0.5-ms pulse duration, 5 Hz, at a current intensity sufficient to produce moderate paw twitches, 0.43 ± 0.07 mA) for 30 minutes, during which time BK was applied twice. This stimulation frequency was similar to that used in clinical electroacupuncture,18 whereas the current was less than that used clinically because we stimulated the nerve directly. After stimulation of the median nerve was terminated, BK was applied every 15 minutes for the next hour. Thus BK was applied during control (2 repeatable responses), coronary arterial occlusion (2 responses), MNS (2 responses), and recovery after MNS (4 responses) for a total of 10 data points. In 4 animals, a 5-ms pulse duration was inadvertently used for MNS. Three of these responded similarly to those animals stimulated by 0.5-ms pulses and were included in the study. As a time control in 5 of the animals, the median nerves also were exposed, the electrode was attached, but the nerves were not stimulated; BK was applied to the gallbladder at 15-minute intervals for a total of 10 applications corresponding to the 10 applications during the nerve stimulation protocol.

Protocol 2: Effect of Afterload Reduction on Wall Thickening Without Myocardial Ischemia

An additional protocol was used to differentiate between possible effects of afterload reduction (ie, decreased systolic blood pressure) on myocardial mechanical function and myocardial oxygen demand. To eliminate the effect of reduced afterload on myocardial oxygen demand during myocardial ischemia, a group of animals (n = 4) was studied in which an aortic snare occluder was used to produce changes in afterload in the absence of myocardial ischemia. Briefly, animals were anesthetized, intubated, and catherized as described above. A midline sternotomy was performed, a snare occluder placed around the descending aorta distal to the tip of the arterial pressure catheter, and a sonomicrometer wall-thickness crystal applied to the LV wall. The occluder was partially tightened in small increments to produce changes in systolic blood pressure that were similar in magnitude to those produced by BK application during the coronary arterial occlusion and MNS measurement periods in Protocol 1.

Protocol 3: Characterization of Afferent Fibers Activated by MNS

To identify the fiber types activated by MNS in the present study, single-unit afferent recording studies were performed. In three animals, the median nerve was isolated in the upper forelimb near the humerus. The nerve was covered with warm mineral oil, desheathed, and split into fine nerve filaments under a surgical microscope (model OPMI 1-FC, Zeiss). The peripheral end of a filament was draped over one pole of a bipolar recording electrode attached to a high impedance probe (model HIP5, Grass Instruments). The other pole of the electrode was grounded with a saline-saturated cotton thread to the surrounding tissue. The signal of each afferent fiber was isolated and amplified (model P5, Grass Instruments), then processed through an audio amplifier (model AM8B, Grass Instruments) and displayed on a storage oscilloscope (model 2201, Tektronix).

To ensure that the signals measured were from afferent fibers, the nerve action potential was evoked by mechanical manipulation of receptive fields in the paw. Conduction distance was measured with a thread placed from the stimulating to the recording electrode. Conduction time was determined by measuring the latency from the signal of electrical stimulation to recording of the afferent action potential. Conduction velocity of the afferent was calculated by dividing the conduction distance by conduction time. Fibers with conduction velocities <2.5 m/s were classified as C-fibers, whereas those with velocities ≥2.5 m/s were considered as Aδ-fibers.

The median nerve in the foreleg was prepared for electrical stimulation as described above. Each isolated nerve fiber was stimulated at a duration of 0.5 ms and a constant rate of 0.6 to 1.0 Hz. The stimulation current was varied from 0.1 to 3.0 mA to determine the threshold required for activation of each nerve fiber.
Data Analysis

Blood pressure, LV pressure, dP/dt, coronary blood velocity, and regional wall motion were recorded on a polygraph (Brush 260, Gould Inc). Data also were input into a PC-based computer with an A/D converter data interface card and analyzed with data acquisition and analysis software, including a data reduction module (EGAA, R.C. Electronics, Inc). Heart rate was derived from the arterial blood pressure signal and an index of myocardial oxygen demand, the double product, was calculated as systolic blood pressure×heart rate. At each measurement period, data from several heartbeats were measured and averaged. The LV WTh was calculated from the regional wall motion data according to the formula WTh = 100×(ESD − EDD)/EDD, where ESD=end-systolic dimension, calculated from the end of the T wave or 20 ms before peak negative dP/dt; and EDD=end-diastolic dimension, calculated from the peak of the R wave or the onset of positive dP/dt. Normalized WTh was calculated as the following ratio: 100×[maximum WTh response to BK−pre-BK WTh]/pre-BK WTh, as we have described previously. An index of coronary resistance was calculated as diastolic blood pressure/coronary blood velocity, because we measured mean coronary blood velocity rather than coronary blood flow. Changes in mean coronary blood velocity are directly correlated with changes in coronary blood flow.2

Data are presented as mean±SEM. The changes in hemodynamic and wall thickening data produced by BK administration in the partial and complete occlusion groups were analyzed by two-way repeated-measures ANOVA. A Bonferroni post hoc test was used to statistically analyze the four preselected between-group comparisons: ischemia versus control, ischemia versus ischemia 1 hour after MNS (sixth time point); and ischemia 1 hour after MNS (10th time point). The two groups did not differ significantly at these time points and therefore were combined for comparison with the control group without MNS. The BK-induced changes in hemodynamic and wall thickening data from these two groups were analyzed by two-way repeated-measures ANOVA. A Bonferroni post hoc test was used to compare the same four time points between groups and to analyze the following preselected within-group comparisons: ischemia versus control, ischemia versus ischemia and ischemia 1 hour after MNS versus ischemia and ischemia MNS. Rest (pre-BK) values for the combined group data were analyzed by one-way repeated-measures ANOVA. Comparisons of rest values with maximal BK responses were analyzed by Student’s t test for paired data or a Wilcoxon signed rank test (nonparametric data). A Bonferroni correction was applied to all multiple comparison procedures. Proportional values were analyzed by the χ2 test. A statistical software package, SigmaStat (Jandel Scientific) was used for these analyses. The level of statistical significance was P<.05.

Results

Animal Model of Reflex-Induced Myocardial Ischemia

The application of BK to the gallbladder resulted in pronounced activation of the cardiovascular system, as evidenced by increased arterial blood pressure, double product, and WTh (Fig 1). In the partial occlusion group, coronary blood velocity without stimulation of the median nerve was reduced by 47% (5.8±0.4 to 3.1±0.3 cm/s; n=6; P<.05) (Fig 2). This degree of flow reduction was not associated with significant changes in resting values of double product, regional function, or diastolic arterial pressure. However, when BK was reapplied to the gallbladder, the normalized WTh was significantly reduced and became negative (10.7±4.2% versus −23.6±2.9%; control versus ischemia; n=7; P<.05), despite increments of coronary blood velocity, diastolic blood pressure, and double product that did not differ from the increments induced by BK before occlusion (Fig 2). The calculated index of coronary resistance was increased from 17.2±2.3 to 32.5±5.7 mm Hg/cm per second by partial coronary occlusion. The change in coronary resistance index during reflex stimulation by BK before occlusion (0.3±0.6 mm Hg/cm per second) was not significantly different from that observed during occlusion (−2.1±2.9 mm Hg/cm per second). In the partial occlusion group, the risk area was 14.3±1.9% (range, 7.1% to 21.4%) of the left ventricle, whereas in the complete occlusion group, the risk area was 2.8±1.5% (range, 0.5% to 8.5%) of the left ventricle (P<.05). There was no evidence of infarction in any animals subjected to partial occlusion. We observed very small infarctions in two of the five animals that received complete occlusion. In one animal, the infarct size was 12.2% of risk area, or 0.4% of the left ventricle, and in the other, 0.9% and 0.005%, respectively.

Effect of MNS on Myocardial Ischemia

Partial Occlusion

In the partial occlusion group, stimulation of the median nerve significantly reduced resting diastolic blood pressure but did not change resting coronary blood velocity, wall thickening, or double product compared with their resting values immediately before stimulation (Fig 2). Importantly, the reduction in regional function during partial coronary occlusion in response to application of BK was improved significantly during stimulation of the median nerve and became a positive value compared with ischemia before stimulation of the median nerve (9.8±4.9% versus −23.6±2.9%, MNS and ischemia versus ischemia). The increased coronary blood velocity resulting from the pressor reflex was not significantly altered by MNS (Fig 2), nor was the coronary resistance index response significantly different.

Figure 1. Computer-captured data from a representative cat showing aortic blood pressure (AoP), LV wall thickness, and the derived parameter, wall thickening (WTh). At each arrow, BK (1 μg/mL) was applied topically to the gallbladder. After control measurements, the LAD coronary artery was occluded partially for the remainder of the experiment. Measurement periods shown are control (a), 30 minutes of occlusion (b), 30 minutes of MNS (0.5 ms-duration, 5 Hz) (c), and after MNS (d). The fall in WTh, associated with the increased sympathetic stimulation reflexly induced by administration of BK during partial occlusion of the LAD, was reversed by stimulation of the median nerve. The bars labeled as ischemia and MNS indicate the periods of myocardial ischemia and MNS, respectively. The actual time course of these interventions is presented in the “Methods” section.
during MNS compared with before MNS (2.3 ± 6.4 and 2.1 ± 6.2 mm Hg/cm per second, respectively). Although the diastolic blood pressure increment was less during MNS, the reduction did not attain statistical significance in this subgroup in which coronary blood velocity was measured.

Complete Occlusion
In animals with complete ligation of an LAD branch, occlusion provided a similar reduction in the response of wall thickening to application of BK to the gallbladder (Fig 3). Likewise, in this group, stimulation of the median nerve evoked substantial improvement in the response of regional function. Because these responses in the partially occluded and the complete occlusion groups were not significantly different, they were combined into a single group to compare with a time-control group that did not receive MNS.

Combined Partial/Complete Occlusion
Coronary occlusion was associated with a significant decrease in the BK-induced change in regional function in the time-control (n = 4) and MNS (n = 15) groups (Fig 4). Whereas the decrement in regional function evoked by BK was virtually constant for all subsequent repeat applications of BK in controls, stimulation of the median nerve significantly improved wall thickening to a level that differed significantly and remained significantly greater than controls for an hour after cessation of MNS. The improvement in regional function with stimulation of the median nerve was accompanied by a diminished pressor response, as indicated by significantly reduced increments of diastolic blood pressure (n = 18) and double product (n = 18) (Fig 4). The increase in systolic blood pressure during administration of BK was not significantly different during occlusion (38.2 ± 4.5 mm Hg) compared with baseline (42.8 ± 4.6 mm Hg). However, stimulation of the median nerve significantly diminished the increment of systolic blood pressure (23.6 ± 2.8 mm Hg), which did not differ significantly after 1 hour (31.5 ± 4.1 mm Hg) from the increase observed during MNS. The increase in LV dP/dt during BK stimulation at baseline (1111 ± 330 mm Hg/s) was reduced during ischemia (951 ± 311 mm Hg/s) and MNS (591 ± 149 mm Hg/s), but these increments did not differ.
significantly (n=8). The small increases in heart rate produced by BK before occlusion (5±2 bpm) and during occlusion (4±2 bpm), MNS (2±1 bpm), and at 1 hour (2±3 bpm) did not differ significantly. Stimulation of the median nerve did not change resting values of heart rate, arterial blood pressure, LV dP/dt, double product, or wall thickening in the combined partial/complete occlusion group.

Effect of Afterload Reduction on Wall Thickening Without Myocardial Ischemia

Increments in systolic blood pressure produced by partial aortic occlusion were 40±1.9 and 25±1.1 mm Hg, similar to the increments of 38±4.5 and 23±2.8 mm Hg obtained during coronary arterial occlusion and MNS time points, respectively, in Protocol 1. The changes in W'Th produced by these blood pressure increments in Protocol 2 (0.1±1.9% and −0.2±1.4%, respectively) were not significantly different (P>.05).

Characterization of Afferent Fibers Activated by MNS

Both myelinated and unmyelinated nerve fibers were activated by stimulation of the median nerve (Fig 5). Fibers that required a greater threshold current than the highest current used in the ischemia protocol (ie, 1.3 mA) were excluded (n=10), leaving 62 fibers. Of those fibers retained for analysis, 23 (37%) were classified as C-fibers and 39 (63%) as Aδ-fibers (P<.05). The average threshold current for activation of C-fibers (706±78 μA) was higher than that for Aδ-fibers (370±43 μA) (P<.05).

Discussion

The important new findings in the present study were that MNS, mimicking electroacupuncture, significantly improved regional cardiac dysfunction produced reflexly by increased sympathetic stimulation in the context of restricted coronary flow. The mechanism of this salutary effect was related to diminished cardiac oxygen demand rather than improved blood supply. These results provide a physiological basis for the reputed therapeutic efficacy of acupuncture in the management of some forms of angina.

Normal cardiac mechanical function is associated with a high rate of aerobic metabolism, a condition that makes myocardial contractile function particularly vulnerable to reductions in oxygen supply. The balance between coronary blood supply and myocardial oxygen demand is crucial for maintenance of normal cardiac contractile function. In the present study, reduction of coronary blood supply by partial occlusion of the LAD tended to decrease resting regional contractile function, but the decline in function did not attain statistical significance. This finding suggests that the coronary supply/demand ratio at rest was not seriously compromised, perhaps related to (1) the delivery of additional coronary blood flow to the region at risk by collateral blood vessels, which are more prevalent in cats than in rats, rabbits, or pigs but less than in dogs,22 (2) increased extraction of arterial oxygen, and/or (3) reduction of myocardial oxygen requirements. We believe the latter explanation is unlikely...
because there was no change in double product, a parameter that is related closely to myocardial oxygen demand.19 However, because contractility tended to decrease and because the double product does not account for changes in contractile function, a determinant of myocardial oxygen demand,23 we cannot absolutely discount a decrease in myocardial oxygen requirements. It is likely that an increase in oxygen extraction played a minor role because oxygen extraction in the heart is near maximal at rest.24

Bradykinin was applied to the gallbladder to reflexly augment sympathetic stimulation of the heart and vasculature, and, thereby, to increase arterial blood pressure, LV pressure, LV dp/dt, and heart rate.14 This reflex generally leads to increased myocardial oxygen demand and increased coronary blood flow and oxygen extraction.15 The elevated sympathetic drive exacerbated the imbalance in coronary supply and demand, as evidenced by a marked reduction in regional function when coronary blood flow was restricted. The use of BK to reflexly induce a transient increase in sympathetic stimulation provides a model of physiological stress similar to that occurring during surgical manipulation of the biliary tract,25 inflammatory conditions involving abdominal organs,26 exercise,27 and mental stress,28 some of which have been shown to provoke angina when coronary blood flow is limited by a stenosis.

In the present study, stimulation of the median nerve for 30 minutes did not change baseline heart rate, blood pressure, LV dp/dt, regional function, double product, or coronary blood velocity. However, the pressor response and maximal LV dp/dt during reflex stimulation were markedly inhibited, the double product was significantly reduced, and coronary blood flow response was unchanged, whereas the response of regional myocardial function was improved. The mechanism underlying the augmented wall motion in the context of restricted coronary blood flow appears to be related to decreased myocardial oxygen requirements, as suggested by the reduced double product. Similarly, in a dog model of myocardial ischemia in which coronary blood flow was reduced and BK was administered into the coronary circulation to simulate angina, stimulation of the Neiguan acupoint reduced whole-heart oxygen consumption, prevented the fall in coronary sinus blood pH, and increased regional contractile force.29 However, this study is complicated by the fact that BK may have reduced ischemia through local endothelium-dependent vasodilation.30 Another study reported that electroacupuncture reduces ischemia through a direct effect of BK on coronary blood flow and hence oxygen supply, but rather reduced myocardial oxygen demand.

The BK-induced increment in systolic arterial blood pressure was reduced significantly during MNS. It is possible that the augmentation of systolic wall thickening by BK was related to a reduction of afterload. To test this possibility we performed Protocol 2, in which the changes in systolic pressure produced by BK administration during coronary occlusion and coronary occlusion + MNS were reproduced in animals without coronary occlusions, namely, in the absence of myocardial ischemia. Thus the confounding effects of reduced afterload and reduced myocardial oxygen demand in the setting of myocardial ischemia, both of which could increase regional myocardial function, were separately evaluated. The insignificant changes in WTh in Protocol 2 suggest that the magnitude of the decrease in systolic pressure (ie, afterload) associated with MNS was not sufficient to significantly alter WTh. Therefore it is likely that the significant improvement of regional wall motion during MNS was related to reduced myocardial oxygen demand associated with lowered blood pressure rather than a mechanical effect resulting from reduced afterload.

Resting diastolic blood pressure was significantly reduced in the partial occlusion group by MNS, but in the combined partial/complete occlusion group the decrease did not attain statistical significance. It is unclear why the resting diastolic blood pressure was more consistently lowered in the partial occlusion group. The greater variability of data in the combined group contributed to the statistical finding of nonsignificance. We believe that the data from the larger number of animals is statistically most correct, but acknowledge a potential for significant lowering of resting diastolic blood pressure by MNS.

Direct electrical stimulation of the median nerve superimposed on our experimental paradigm of reversible ischemia markedly improved the ischemic state as indicated by the significant increase in wall thickening during chemical stimulation of the gallbladder (Fig 4). Furthermore, improved contractile function remained relatively constant during the next hour. These findings have important clinical implications because MNS mimics stimulation of the Neiguan acupuncture point used in traditional Chinese medicine for treatment of angina.7 This acupoint is located on the forearm and overlies the trunk of the median nerve.31 A previous study in cats suggested that electroacupuncture of the Neiguan acupoint can reduce ischemia caused by coronary artery ligation and reperfusion.32 However, in that study ST-segment changes were the only index of ischemia, and measurements were obtained only during reperfusion. One member of our group also has reported that low-frequency, low-current electrical stimulation of Neiguan or the median nerve in rabbits inhibits arrhythmias induced by electrical stimulation of the hypothalamic perifornical area or midbrain periaqueduct gray matter.33 Although this latter study did not examine the mechanism by which acupuncture reduces arrhythmias,
both reports, along with data in the present study, provide compelling evidence for a beneficial response to this form of traditional Chinese medicine.

It has been recognized for many years that pressor or depressor responses can be evoked by stimulation of somatic nerves.\(^3\)\(^4\) The nature of the blood pressure response is dependent on both the frequency of stimulation and the predominant fiber type that is activated. At low stimulation frequencies (1 to 5 Hz), stimulation of A-fibers alone or A- and C-fibers results in a depressor effect, whereas activation of C-fibers alone produces a pressor response.\(^3\)\(^6\) In the present study, both myelinated (A\(_\delta\)) and unmyelinated (C) fibers in the median nerve were activated by the low-frequency, low-intensity parameters that we used. However, a larger proportion of finely myelinated fibers were stimulated, suggesting that the depressor effect observed was related to the predominance of activation of A\(_\delta\)-fibers.

Stimulation of the median nerve produces a sympathoinhibitory effect, which results in decreased cardiac sympathetic drive, vasodilation, and reduced blood pressure.\(^3\)\(^7\) The centers involved in this inhibition include the nucleus arcuatus in the hypothalamus, ventral periaqueduct gray, and nucleus raphe obscurus, with a projection to the rostral ventrolateral medulla (rVLM).\(^3\)\(^8\) Release of endorphins, serotonin, and \(\gamma\)-aminobutyric acid (GABA) mediates inhibition of cardiac sympathetic neurons in the rVLM.\(^3\)\(^8\) The \(\mu\)- and \(\delta\)-, but not the \(\kappa\)-, opioid receptors of the rVLM, are activated to induce the depressor effect under resting conditions.\(^3\)\(^9\) Thus previous studies of the central neural structures activated by MNS have documented the presence of an important inhibitory effect on sympathetically mediated vasoconstriction. Although these studies did not investigate the response to stimulation of the median nerve during reflex activation of the sympathetic nervous system, it is possible that similar pathways and the endogenous opioid system are involved in the diminished pressor and double product reflex responses during gallbladder stimulation.

Further studies will be necessary to determine (1) if the inhibitory effect of stimulation of the median nerve on the pressor response and the improvement of cardiac contractile function are due to activation of the aforementioned sympathetic inhibitory pathways, (2) if activation of opioid receptors mediates the reflex responses, (3) if stimulation of other somatic nerves can elicit the same response, and (4) if transcutaneous acupuncture or electroacupuncture of the Neiguan acupoint produces the same effects as direct stimulation of the median nerve. In addition, the results of the present study, taken together with the limited clinical studies of acupuncture treatment for angina or acute myocardial infarction patients in China\(^,\)\(^2\)\(^7\) Sweden\(^,\)\(^5\) and Denmark\(^,\)\(^4\) suggest that further clinical investigation of this relatively inexpensive therapeutic intervention is warranted.

In summary, MNS, used to mimic electroacupuncture, substantially improves regional cardiac wall motion during myocardial ischemia induced by restriction of coronary blood flow and superimposed stimulation of the cardiovascular sympathetic nervous system. Improved regional contractile function suggested reduced myocardial oxygen demand. Despite the decreased oxygen demand, coronary blood flow in response to application of BK was unchanged, suggesting that the maintenance of blood flow contributed to the improved supply/demand relationship and hence the reduction in myocardial ischemia. These results provide a physiological basis for the reported efficacy of acupuncture in the treatment of angina pectoris. Activation ofafferent input from somatic nerves may be a potentially important therapeutic element in maintaining cardiovascular health and in the treatment of ischemic heart disease.

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**References**

17. Amsterdam EA, Stahl GL, Pan H-L, Rendig SV, Fletcher MP, Longhurst JC. Limitation of reperfusion injury by a monoclonal antibody to C5a
Median Nerve Stimulation Inhibits Cardiac Ischemia

Reversal of Reflex-Induced Myocardial Ischemia by Median Nerve Stimulation: A Feline Model of Electroacupuncture
Peng Li, Koullis F. Pitsillides, Stephen V. Rendig, Hui-Lin Pan and John C. Longhurst

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