Sympathetic-parasympathetic interaction and accentuated antagonism in conscious dogs

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STRAMBA-BADIALE, MARCO, EMILIO VANOLI, GAETANO M. DE FERRARI, DONATELLA CERATI, ROBERT D. FOREMAN, AND PETER J. SCHWARTZ. *Sympathetic-parasympathetic interaction and accentuated antagonism in conscious dogs.* Am. J. Physiol. 260 (Heart Circ. Physiol. 29): H335–H340, 1991.—The heart rate response to vagal stimulation and the interaction with sympathetic activity was evaluated in conscious dogs at rest and during exercise; the latter was used as a tool to physiologically elevate sympathetic activity. In 20 dogs with a healed myocardial infarction and in 7 healthy dogs a bipolar electrode was chronically implanted around the right cervical vagus. Vagal stimulation (3 ms; 2.1 ± 0.7 mA; 2, 4, 6, 8, 10, 12 Hz) was performed while dogs stood on the treadmill (heart rate 120 ± 25 beats/min) and while they exercised (201 ± 17 beats/min). Gradual increases of the frequency of vagal stimulation gradually enhanced the inhibitory effect on heart rate both before and during exercise. During exercise, heart rate reduction was significantly greater than that produced at rest at any frequency of stimulation ($P < 0.001$). This difference widened as the frequency of stimulation increased and the interaction with or without the presence of exercise was significant ($P < 0.02$). Vagal stimulation produced similar effects in the seven dogs without myocardial infarction. These data demonstrate that the vagal-sympathetic "accentuated antagonism" described in anesthetized animals is also present in conscious dogs.

vagal stimulation; heart rate

VAGAL ACTIVITY contributes to cardiac electrical stability (1, 10, 25), whereas a reduction in vagal tone and vagal reflexes is associated with an augmented risk for sudden cardiac death (9, 12, 26). Furthermore, as the vagally mediated antifibrillatory effect seems to be largely secondary to an antiadrenergic mechanism (2, 10), the physiological question of sympathetic-parasympathetic interaction (13) has acquired a new and clinically relevant dimension.

A critical aspect of this interaction is represented by the phenomenon of "accentuated antagonism" described in the anesthetized dog by Levy and associates (14–17), who showed that the heart rate response to vagal stimulation is enhanced in the presence of elevated sympathetic activity. Our group has developed a chronically implantable electrode that allows vagal stimulation in conscious free-moving animals (27). Thus it is now possible to experimentally approach the question of the relevance to the conscious state of the sympathetic-parasympathetic interaction as observed during anesthesia (17).

The effect of autonomic interaction on heart rate was examined specifically because of the significant relationship existing between the reflex control of heart rate and survival during acute myocardial ischemia (31). To enhance the potential clinical relevance of the findings to ischemic heart disease, the present study was performed in dogs with a normal heart and in dogs with a healed myocardial infarction. Also, sympathetic activity was physiologically elevated by exercise at the time of the vagal stimulation.

METHODS

The study was performed in 27 dogs: 20 of them had a healed anterior myocardial infarction, whereas 7 had a normal heart.

Dogs With Myocardial Infarction

Surgical procedure. Anesthesia was induced with 25 mg/kg iv thiopental sodium (Pentothal; Abbott) and maintained by the inhalation of a halotane, nitrous oxide, and oxygen mixture. Through a left thoracotomy a modified Harris-two-stage occlusion was performed on the left anterior descending coronary artery below the first diagonal branch to produce a myocardial infarction. Pentazocine lactate (Talwin, Winthrop, 30 mg im) was given approximately every 8 h for the first 24 h after surgery to control postoperative pain. The guidelines of the American Heart Association on the care and treatment of experimental animals were adhered to throughout the study.

Vagal implant. One month after production of myocardial infarction all the animals were anesthetized with thiopental sodium 50 mg/kg iv. A small neck incision was made and the right cervical vagus was dissected from the carotid artery; a bipolar platinum cuff electrode (Medtronic model SP 5539) was implanted around the nerve, and the lead wires connected to the electrode were tunnelled under the skin to exit from the dorsal surface of the neck.

Vagal stimulation protocol. Seventy-two hours after surgery vagal stimulation was performed at rest while

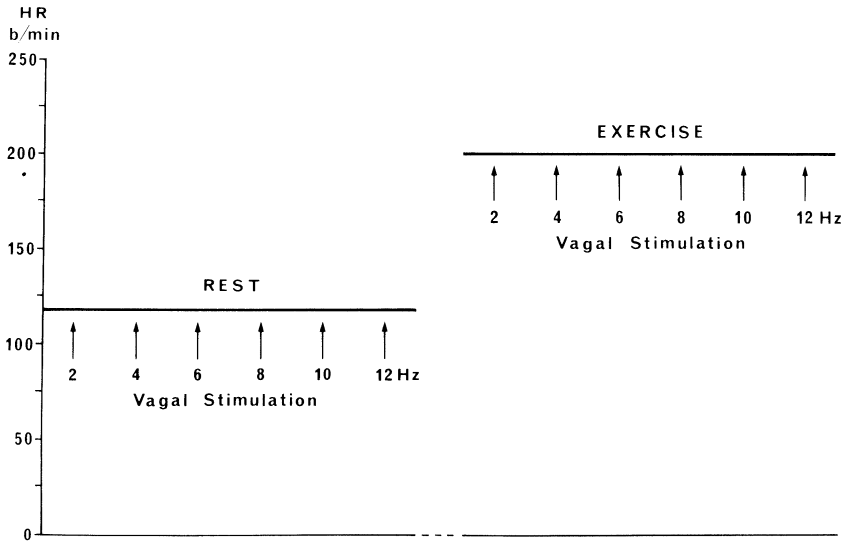

FIG. 1. Protocol of study. Vagal stimulation was performed while dog was standing on treadmill, at 2, 4, 6, 8, 10, 12 Hz and subsequently during exercise when heart rate was ~200 beats/min.

the dog was standing on the treadmill (Fig. 1). The current intensity was 2.1 ± 0.7 mA. Six trains of pulses were delivered at progressively increasing frequencies of stimulation (2, 4, 6, 8, 10, 12 Hz) in 1-min intervals. Each train of pulses lasted 10 s with a pulse duration of 3 ms. The intensity of stimulation was determined by increasing the current at a fixed frequency (2 Hz) until the negative chronotropic response had reached a plateau. These stimulation parameters did not induce any reaction, thus indicating that the animals were not perceiving pain. At higher frequencies (10–12 Hz) a few animals had untoward effects such as retching and/or coughing. These symptoms disappeared immediately with cessation of stimulation or with reduction of the frequency of stimulation.

The same protocol was then repeated during exercise while the dogs were running on a motordriven treadmill (4.8 km/h) at a level that maintained a heart rate of ~200 beats/min (Fig. 1). In three dogs vagal stimulation at 12 Hz was performed at rest after administration of atropine sulfate (75 μg/kg iv). Subsequently propranolol (1 mg/kg) was intravenously injected and vagal stimulation was repeated at the same frequency.

Dogs Without Myocardial Infarction

Seven additional dogs without myocardial infarction were chronically instrumented with right cervical vagus electrodes and subsequently studied with the same protocol utilized in the infarcted animals. Data were analyzed separately and compared with those of the infarcted animals.

Statistical Analysis

The heart rate responses to any frequency of vagal stimulation at rest and during exercise were analyzed

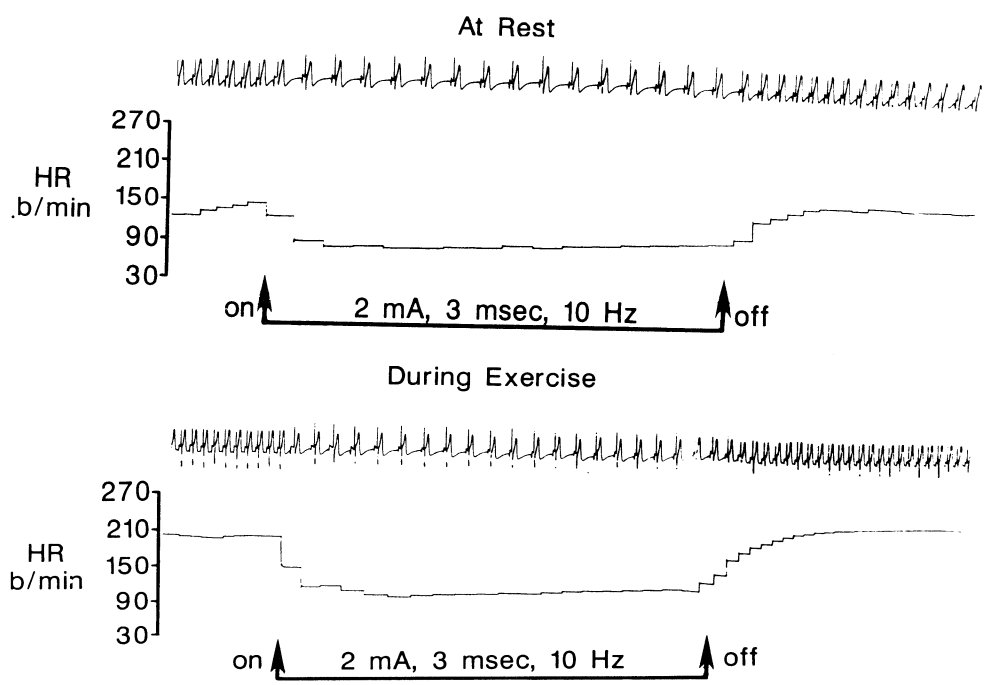

FIG. 2. Electrocardiogram tracings and tachograms illustrating effects on heart rate of vagal stimulation at rest (*top*) and during exercise (*bottom*) in the same dog. Arrows indicate beginning and end of vagal stimulation performed with the specified parameters. Negative chronotropic effect is accentuated during exercise.

both as absolute values and as percent changes. An additional analysis was performed by determining the mean of three R-R intervals before and during vagal stimulation and by calculating the differences both in absolute and in percent values.

Two-way analysis of variance (ANOVA) for repeated measures was used to assess the effect of the frequency of vagal stimulation, the effect of exercise, and the interaction between these two factors. At any frequency of stimulation, the heart rate and R-R interval changes observed at rest and during exercise were analyzed with the Student's t test for paired samples. Mean comparison between infarcted and noninfarcted animals was performed by Student's t test for unpaired samples. The heart rate and the R-R interval changes at any frequency of stimulation were expressed as means ± SD.

RESULTS

The results will be presented according to the actual temporal sequence of the study. Since our main question concerned the autonomic interaction in the postinfarction state, we initially investigated animals with a healed myocardial infarction. After this study was completed we decided to verify if our findings were applicable to animals with an intact heart.

Dogs With Myocardial Infarction

While the dogs ($n = 20$) were standing on the treadmill, their heart rates were measured to be 120 ± 25 beats/min and reached 201 ± 17 beats/min ($P < 0.001$) during exercise at a workload of 4.8 km/h at 0% incline. Vagal stimulation never produced second- or third-degree atrioventricular block both at rest or during exercise. The inhibitory effect of vagal stimulation on heart rate increased progressively as the frequency of stimulation increased from 2 to 12 Hz. This result was true at these frequencies, both at rest (−17 ± 12 and −50 ± 14 beats/min, respectively) and during exercise (−37 ± 17 and −106 ± 25 beats/min, respectively). An example of one typical experiment is shown in Fig. 2. The decrements observed at the various frequencies of stimulation are illustrated in Fig. 3. The main finding of this study is represented by the observation that at any frequency of stimulation, the decrease in heart rate was greater during exercise than at rest ($P < 0.001$). This difference between changes during exercise and rest is also significant when data are expressed as percent changes (Fig. 3); only at 2 Hz the difference did not reach statistical significance.

When data were analyzed as absolute R-R interval responses to vagal stimulation, the differences between rest condition and exercise were not evident; however, percent increase in R-R interval induced by vagal stimulation were greater during exercise than at rest (Fig. 4).

The difference between the absolute heart rate responses during exercise and rest tended to widen as the frequency of stimulation increased (Fig. 3); the interaction between frequency of stimulation and presence or absence of exercise is indeed significant ($P < 0.02$). This interaction was not significant when the data were expressed as percent changes.

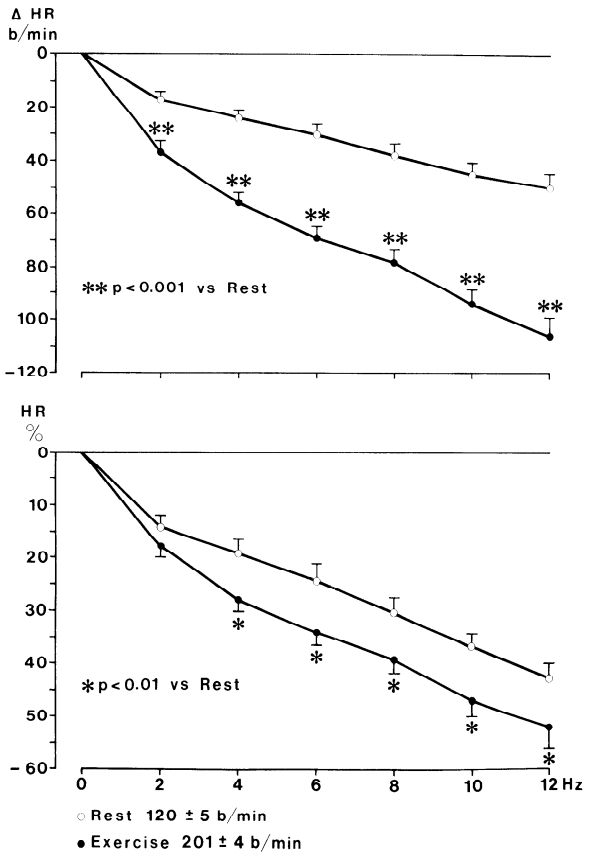

FIG. 3. Heart rate response to vagal stimulation at rest and during exercise in infarcted dogs ($n = 20$). Data (means ± SE) are expressed as absolute (*top*) and percent (*bottom*) changes. Negative chronotropic effect that increases with progressively increasing frequencies of stimulation is greater during exercise than at rest.

In three experiments vagal stimulation was performed after administration of atropine and also after atropine and propranolol. Atropine sulfate (75 μg/kg) increased heart rate by 48 ± 8 beats/min ($P < 0.01$) from 130 ± 14 beats/min. Vagal stimulation (12 Hz) after atropine administration did further increase heart rate in two of three animals (+28 and +37 beats/min), whereas it did not produce changes in one animal (−6 beats/min). Propranolol (1 mg/kg) decreased heart rate by 38 ± 11 beats/min ($P < 0.01$) from 179 ± 15 beats/min. Vagal stimulation (12 Hz) performed after propranolol and atropine no longer changed heart rate (from 138 ± 6 to 134 ± 7 beats/min).

Dogs Without Myocardial Infarction

The effects of vagal stimulation on heart rate in the dogs studied without myocardial infarction were similar to those observed in the infarcted dogs ($n = 7$). While the dogs were standing on the treadmill and during exercise their heart rates (124 ± 10 and 189 ± 21 beats/min, respectively) did not differ from that of the infarcted dogs. Also, the heart rate changes provoked by any frequency of vagal stimulation at rest and during exercise did not differ between the two groups of dogs.

DISCUSSION

This study demonstrates the feasibility of vagal stimulation in conscious animals both at rest and during

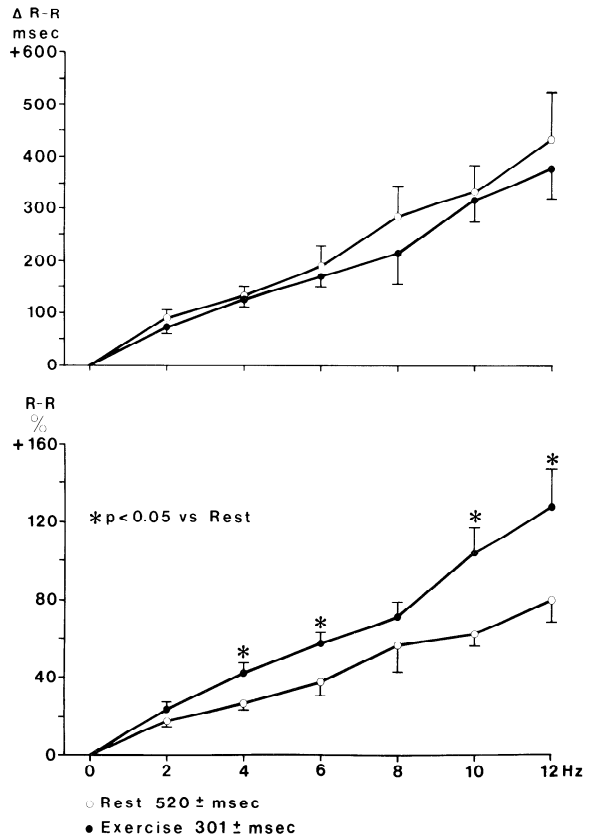

FIG. 4. R-R interval response to vagal stimulation at rest and during exercise in infarcted dogs ($n = 20$). Data (means ± SE) are expressed as absolute (*top*) and percent (*bottom*) changes.

exercise and thus provides novel information on the characteristics of the sympathetic-parasympathetic interactions at the sinus node level in the conscious state. The results allow an analysis of the phenomenon of accentuated antagonism in unanesthetized animals with a normal heart as well as a healed myocardial infarction.

Characteristics of Preparation

To study the effects of vagal activation in the conscious animal the intact right cervical vagus was stimulated; this implies excitation of both efferent and afferent vagal fibers. Therefore, in interpreting the results one should also consider the possibility of a reflex reduction in sympathetic activity secondary to stimulation of the afferent vagal fibers (24). This possibility was tested by stimulating the vagus after atropine administration.

If afferent vagal fibers had played a major role in the negative chronotropic effect, vagal stimulation performed after atropine would have still reduced heart rate through the reflex inhibition of sympathetic efferent activity; actually, it did produce a modest increase in heart rate. This was probably due to the concomitant stimulation of the efferent sympathetic fibers running in the cervical vagosympathetic trunk (18). Blocking the effect of efferent vagal activity with atropine unmasks the effect of stimulating these sympathetic fibers. This interpretation is supported by the fact that β-adrenergic blockade with propranolol prevented this slight increase in heart rate during stimulation of the vagosympathetic trunk. The effect of propranolol also makes unlikely the possible involvement of the intriguing phenomenon termed "excess tachycardia," which apparently involves non-β-adrenergic mechanisms (19).

In the present study, a continuous vagal stimulation with a progressively increasing frequency was used. This may be a limitation of our study because the physiological vagal discharge to the heart occurs in pulse-synchronous bursts (6, 7, 11). As a consequence, the cardiac response to a given vagal stimulus depends on the phase of the cardiac cycle during which it is applied (3, 15, 28). One cannot exclude that a phasic vagal stimulation in conscious animals might result in different and more complex vagal-sympathetic interactions on the heart, as observed in anesthetized animals (29, 32).

The effects of vagal stimulation on heart rate might have been affected by myocardial infarction (25). To exclude this possibility a group of seven dogs without myocardial infarction was studied with the same protocol used in the infarcted dogs. The observation that no differences were present between the two groups of dogs indicate that 1 mo after myocardial infarction, the capability to reduce heart rate in response to an increase in vagal activity is well preserved.

Chronotropic Effect of Vagal Stimulation

The heart rate response to vagal stimulation was a function of the frequency of stimulation; the inhibitory effect increased as the frequency of stimulation was progressively augmented. This observation in conscious dogs has to be placed in the context of previous findings.

Besides some rather crude attempts described by Hoff (4), vagal stimulation became a physiological tool in 1846 with the Weber brothers (31) and was generally restricted to anesthetized animals. In 1932 Rosenblueth (20) showed in anesthetized cats that the change in heart rate evoked by vagal stimulation was a hyperbolic function of stimulation frequency. Indeed, the negative chronotropic effect increased steeply from 0 to 5 Hz and then more gradually until a plateau was reached at ~15 Hz. In 1969 Levy and Zieske (17) showed in anesthetized dogs that the plateau was reached at 6–7 Hz of frequency of stimulation.

In the present study, the plateau phase described previously (17, 20) was not observed with stimulation frequencies up to 12 Hz. Two possible reasons might explain this seeming discrepancy. One reason relates to the conscious state of the animals. In the conscious animal the plateau phase might occur at higher frequencies of stimulation due to the absence of anesthesia. Frequencies of stimulation above 12 Hz were not employed because they would have probably created discomfort to most dogs. The second reason relates to the fact that we stimulated the right vagus nerves, whereas left or bilateral stimulation was employed in the other two studies. It is known that the right vagus has a more profound influence on the sinus node compared with the left vagus (18). These factors may have acted synergistically to displace the plateau toward higher values of frequency of stimulation.

Sympathetic-Parasympathetic Interactions

The negative chronotropic effect was greater during exercise compared with rest, and this difference widened by increasing progressively the frequency of stimulation.

After the onset of exercise, vagal activity is almost completely withdrawn, whereas there is a major increase in sympathetic activity that progresses in relationship with workload. We have used a constant level of submaximal exercise to obtain a state characterized by minimal vagal activity and by physiologically elevated sympathetic activity. In this setting our findings demonstrate that the vagal-sympathetic accentuated antagonism on heart rate described in anesthetized animals is also present in conscious dogs.

Rosenblueth and Simeone (21) were among the first to demonstrate results consistent with the concept of "accentuated antagonism" as subsequently defined by Levy in 1971 (13). They observed in anesthetized cats that the magnitude of the heart rate reduction produced by vagal stimulation was reduced by bilateral stellate ganglionectomy. One year later, in 1935, these conclusions were confirmed by Samaan (22) who showed that the negative chronotropic effect of vagal stimulation is enhanced by a concomitant electrical stimulation of the stellate ganglia. More recently, Warner and Russel (30) and Levy and Zieske (17) have carefully quantified the extent of the vagal-sympathetic interaction on heart rate stimulating the vagus with and without a simultaneous sympathetic stimulation.

Pathophysiological Implications

When acute myocardial ischemia occurs, the antifibrillatory effect of vagal activation is partly dependent on direct electrophysiological effects and is largely secondary to the decrease in heart rate resulting in reduced oxygen consumption (25). A potential obstacle for the maintenance of an optimal heart rate (5) is represented by the fact that during acute myocardial ischemia an excitatory cardio-cardiac sympathetic reflex is elicited (24); this reflex increases heart rate and contributes to the onset of life-threatening ventricular tachyarrhymthias (23). The protective effect of vagal stimulation depends largely on the ability to lower heart rate; if under conditions of elevated sympathetic activity vagal activation could not be as effective as under condition of resting sympathetic tone, its protective effect might be lost. The present experiments prove that this is not the case because, even during increased sympathetic activity, vagal stimulation can lower heart rate to relatively similar levels.

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