Positive and Negative Feedback Mechanisms in the Neural Regulation of Cardiovascular Function in Healthy and Spinal Cord–Injured Humans

J.M. Legramante, MD; G. Raimondi, MD; M. Massaro, MD; F. Iellamo, MD

Background—We tested the hypothesis that in humans, hypertension/tachycardia and hypotension/bradycardia nonbaroreflex sequences that occur within spontaneous arterial pressure (AP) and R-R interval fluctuations are an expression of positive feedback mechanisms regulating the cardiovascular system.

Methods and Results—We studied 15 spinal cord–injured (SCI) subjects (8 tetraplegics and 7 paraplegics) and 8 healthy subjects. The occurrence of nonbaroreflex (NBseq) and baroreflex (Bseq) sequences, ie, hypertension-bradycardia and hypotension-tachycardia sequences, was assessed during rest and head-up tilt (HUT). The ratio between Bseq and NBseq (B/NB ratio) was also calculated. In resting conditions, the occurrence of NBseq was significantly lower ($P<0.05$) in tetraplegics (7.9±1.5) than in paraplegics (16.2±3.2) and normal subjects (19.0±3.5), whereas the occurrence of Bseq was not significantly different between the 3 groups (38.6±11.9 versus 45.4±6.0 versus 47.0±11.9). In tetraplegics, the B/NB ratio showed a marked, significant decrease (from 8.4±4.2 to 1.9±0.8, $P<0.05$) in response to HUT, whereas in normal subjects, it showed a significant increase (from 3.5±0.7 to 9.4±2.7, $P<0.05$). In paraplegics, the B/NB ratio did not change significantly in response to HUT (from 4.5±1.6 to 4.8±1.1).

Conclusions—Our data suggest that nonbaroreflex sequences occur in humans and might represent the expression of an integrated, neurally mediated, feed-forward type of short-term cardiovascular regulation that is able to interact dynamically with feedback mechanisms of baroreflex origin. (Circulation. 2001;103:1250-1255.)

Key Words: reflex ■ baroreceptors ■ nervous system, autonomic

The heart is regulated by feedback systems that include the sinus node, the baroreceptors and chemoreceptors, different cardiovascular sensory endings, and afferent and efferent neural pathways, which gives rise to very complicated dynamics. Relatively simple cardiovascular reflexes can be identified in experimental conditions, but it is unlikely that they act as such when, in closed-loop conditions, a natural hemodynamic event is sensed by different reflexogenic areas. Cardiovascular neural regulation involves both central controllers and peripheral reflex mechanisms operating with negative feedback properties (eg, arterial baroreflexes) that interact in modulating the dynamic heart rate (HR) and arterial pressure (AP) fluctuations.

However, studies performed both in anesthetized and conscious animals have indicated that reflex responses operating with positive feedback mechanisms may contribute to the neural regulation of the cardiovascular system. In addition, it has been reported that stimulation of receptors distributed in the heart and great vessels can cause both an inhibitory cardiovascular reflex, including bradycardia and hypotension mediated by vagal afferents, and an excitatory reflex characterized by hypertension and tachycardia mediated by sympathetic afferents. In this context, we found recently that the continuous relationship between spontaneous AP and heart period fluctuations is neurally modulated not only through negative feedback coupling mechanisms but also through positive feedback (or feed forward) mechanisms. This finding indicates that the analysis of spontaneous fluctuations of AP and HR is a valuable tool to investigate the complexity of neural mechanisms of cardiovascular regulation. By analyzing time series of AP and pulse intervals recorded from anesthetized rabbits using the sequences technique, we demonstrated that spontaneously occurring sequences of consecutive heartbeats characterized by opposite, linearly related changes in systolic AP (SAP) and pulse interval, termed nonbaroreflex to distinguish them from the baroreflex sequences, reflect the operational characteristics of positive feedback mechanisms modulated by the autonomic nervous system. Nevertheless, the occurrence of nonbaroreflex sequences has not been addressed in humans. Accord-
ingly, the aim of this study was to investigate the interrelation between the occurrence of nonbaroreflex and baroreflex sequences, as expressions of integrated positive and negative feedback circuits, considered as part of the complex mechanisms neurally regulating the cardiovascular system.5

In particular, the possible role played by the interaction between these mechanisms to achieve the most adequate regulation of the circulation was addressed in our study. To this aim, we analyzed the occurrence of baroreflex and nonbaroreflex sequences within spontaneous AP and R-R interval fluctuations, in healthy subjects and spinal cord-injured (SCI) patients (tetraplegics and paraplegics) at rest and during orthostatic stress.

We investigated SCI patients because they feature disturbances of cardiovascular neural regulation. Tetraplegic patients are deprived of supraspinal sympathetic control but have intact vagal afferent and efferent pathways,9,10 whereas paraplegics have largely intact cardiovascular neural pathways, as do healthy subjects, but share with tetraplegics the same status of physical (in)activity, possibly resulting in paraplegics having intact cardiovascular neural regulation. Tetraplegic patients are deprived of supraspinal sympathetic control but share with tetraplegics the same status of physical (in)activity, possibly resulting in paraplegics having intact cardiovascular neural regulation. Tetraplegic patients are deprived of supraspinal sympathetic control but share with tetraplegics the same status of physical (in)activity, possibly resulting in paraplegics having intact cardiovascular neural regulation.

We studied 8 healthy volunteers (age 27.6±6.2 years) and 16 SCI patients (8 tetraplegics [age 27.9±6.8 years] with complete traumatic cervical spinal cord injuries between the C4 and C7 vertebrae and 8 paraplegics [age 26.1±7.9 years] with lesions at the thoracic and lumbar levels). The interval elapsed from time of injury in SCI patients ranged from 1 to 19 years. The patients were not bedridden and tended to spend most of their time in a wheelchair. In those patients taking baclofen or diazepam to prevent muscle spasms, the drugs were discontinued at least 2 days before the experiments to avoid any interference with the autonomic and cardiovascular systems. All control subjects were nonsmokers and not involved in regular physical activity. All participants gave their informed consent to the study, and the protocol was approved by the Ethics Committee of the Department of Internal Medicine of University of Rome “For Vergata” and of CTO Hospital.

Statistical Analysis
Each variable was checked for normality of distribution by the Kolmogorov-Smirnov test. Within-group comparisons were performed by paired t test for normally distributed data and by Wilcoxon signed rank test for nonnormally distributed data. Between-group comparisons were performed by 1-way ANOVA for normally distributed variables and the Kruskal-Wallis ANOVA on ranks for nonnormally distributed variables. Pairwise multiple comparison procedures were performed by the Student-Newman-Keuls test. Values are presented as mean±SEM. Differences were considered statistically significant at P<0.05.

Results
Hemodynamic Data
Under supine resting conditions, SAP and diastolic AP were significantly lower and R-R interval was significantly higher in tetraplegics than in paraplegics and normal subjects (see Table). AP responses to orthostatic stress differed among the 3 groups. Whereas tetraplegics showed an AP decrease, paraplegic and normal subjects did not show significant AP changes. R-R intervals significantly decreased in response to HUT in the 3 groups (see Table). None of the subjects complained of symptoms of presyncope, with the exception of 1 paraplegic patient who therefore was excluded from overall analyses.

Experimental Protocol
The experiments were performed in the morning in a laboratory room at ambient temperature. Subjects refrained from food or caffeine ingestion for at least 2 hours before the experiment. The experiments consisted of 10 minutes of supine rest followed by 10 minutes of passive head-up tilt (HUT) at 70°. The table was equipped with straps and footboard to secure patients and prevent movements. Even though the experimental protocol was set up to stop recordings if muscle or bladder spasms were detected, these events were not observed in our SCI subjects.

Spontaneous AP and HR Fluctuation Analysis
Details of this technique have been described previously.5,14,15 Briefly, the beat-by-beat time series of SAP and R-R intervals were scanned by a computer to identify sequences of ≥3 consecutive beats in which SAP and R-R intervals of the following beat changed in the same direction. These sequences are identified as baroreflex sequences.8 In addition, the beat-by-beat time series of SAP and R-R intervals were analyzed to identify spontaneously occurring sequences of ≥3 consecutive beats in which SAP and R-R intervals of the following beat changed in the opposite direction (ie, hypertension/tachycardia and hypotension/bradycardia sequences). These sequences are identified as nonbaroreflex sequences.14,15 A linear regression was applied to each individual sequence, and only regressions with linear r² values >0.85 were accepted. The mean individual slope of the baroreflex and nonbaroreflex sequences, obtained by averaging all slopes computed within a given experimental period, was calculated and taken as a measure of the integrated spontaneous baroreflex sensitivity (BRS) and of the gain of nonbaroreflex mechanisms, respectively, for that period. The spontaneous baroreflex technique has been proven to provide reproducible results during many laboratory tests, including orthostatic stress.15 We also calculated the ratio between the number of baroreflex and nonbaroreflex sequences (B/NB ratio). This index would provide an estimate of the balance between, respectively, negative and positive feedback mechanisms neurally regulating the cardiovascular system.

Because the absolute number of the sequences could depend, in some circumstances, on the current HR, we attempted to introduce an additional index that focused mainly on the fractional occurrence of the sequences, which is independent of recording time and HR. This index, referred to as engagement time, was obtained by dividing the sum of the R-R interval organized in sequences, according to the criteria reported above, by the total recording duration and multiplying it by 100.
Spontaneous AP and HR Fluctuation Analysis
Under resting conditions, the occurrence of nonbaroreflex sequences was significantly lower in tetraplegic than in either paraplegic or normal subjects. In contrast, the occurrence of baroreflex sequences was not significantly different among the 3 groups (Figure 1). Similar results were obtained regarding the occurrence of the 2 types of sequences in terms of engagement time (Figure 2). Under baseline conditions, the B/NB ratio was smaller in paraplegic and normal subjects than in tetraplegics, even though these differences did not attain statistical significance (Figure 1). The resting mean slopes of both baroreflex and nonbaroreflex sequences were not significantly different among the 3 groups (Figure 3).

Orthostatic stress had different effects on the occurrence of baroreflex and nonbaroreflex sequences in the different groups. In paraplegic and normal subjects, nonbaroreflex sequences did not show significant changes, whereas in tetraplegics, these sequences significantly increased in response to HUT (Figure 1). Similar results were obtained regarding the occurrence of nonbaroreflex sequences in terms of engagement time (Figure 2). Conversely, baroreflex sequences showed a marked increase in response to HUT in normal subjects but did not change significantly in SCI subjects; this was true for both the absolute number (Figure 1) and the engagement time (Figure 2).

Consequently, these results are well synthesized by the changes of the B/NB ratio. In tetraplegics, this index showed a marked and significant decrease, whereas in normal sub-

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**Table 1: Cardiovascular Values in Resting Condition and in Response to HUT**

<table>
<thead>
<tr>
<th></th>
<th>SAP, mm Hg</th>
<th>DAP, mm Hg</th>
<th>R-R Interval, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>117.8 ± 2.3</td>
<td>79.4 ± 2.6</td>
<td>924.0 ± 51.1</td>
</tr>
<tr>
<td>HUT</td>
<td>122.8 ± 3.3</td>
<td>82.9 ± 5.6</td>
<td>746.9 ± 18.8*</td>
</tr>
<tr>
<td><strong>Tetraplegics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>100.1 ± 4.3</td>
<td>62.0 ± 2.6</td>
<td>1094.0 ± 68.1†</td>
</tr>
<tr>
<td>HUT</td>
<td>89.7 ± 6.4</td>
<td>50.9 ± 3.3</td>
<td>786.4 ± 50.0*</td>
</tr>
<tr>
<td><strong>Paraplegics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>131.2 ± 6.0</td>
<td>75.7 ± 7.0</td>
<td>743.2 ± 26.9‡</td>
</tr>
<tr>
<td>HUT</td>
<td>132.3 ± 5.0</td>
<td>71.2 ± 4.0</td>
<td>612.8 ± 44.0*</td>
</tr>
</tbody>
</table>

Mean values ± SEM. DAP indicates diastolic AP.
*P < 0.05 vs resting condition; †P < 0.05 vs resting values of normal subjects and paraplegics; ‡P < 0.05 vs resting values of normal subjects and tetraplegics.
jects, it showed a significant increase in response to orthostatic stress (Figure 1). In paraplegics, the B/NB ratio did not show significant changes in response to HUT (Figure 1).

The mean slope of both baroreflex and nonbaroreflex sequences showed a significant decrease in response to HUT in the 3 groups (Figure 3).

Discussion

The major findings of this study are 2-fold. First, sequences of spontaneously occurring consecutive heartbeats characterized by opposite, linearly related changes in SAP and R-R interval, ie, nonbaroreflex sequences (which in the anesthetized animal have been suggested to represent the expression of integrated, neurally mediated mechanisms regulating the cardiovascular system with positive feedback characteristics7), also occur in human beings during unperturbed physiological conditions. Second, disturbances of cardiovascular neural regulation alter the relation that exists between integrated positive and negative feedback circuits neurally regulating the cardiovascular system.

Under supine resting conditions, the occurrence of nonbaroreflex sequences was significantly lower in tetraplegic patients than in paraplegic and normal subjects, whereas the occurrence of baroreflex sequences was not significantly different in the 3 groups. These results seem to confirm those previously reported in animal experiments7 and to extend to humans the concept that nonbaroreflex sequences are mainly modulated by the autonomic nervous system. Whereas the baroreflex sequences, which are mostly vagally modulated, were similarly represented in the 3 groups, the nonbaroreflex sequences, which are modulated by both autonomic outflows (as indicated by their drastic reduction after selective parasympathetic and sympathetic pharmacological blockades7), occurred less frequently in tetraplegics. These SCI patients do not have supraspinal control of the sympathetic outflow to the heart, whereas paraplegic and normal subjects do have supraspinal control.

This is the first experimental evidence in humans indicating that positive feedback mechanisms16 are operating along with negative feedback mechanisms. The existence of positive feedback mechanisms neurally regulating the cardiovascular system has been postulated on the basis of results obtained in animal models in which the activation of cardiovascular sympathetic afferents evoked reflex responses characterized by consensual changes in AP and HR.1,2 Cardiac sympathetic and parasympathetic afferents have a spontaneous impulse activity, and experimental evidence suggests that physiological hemodynamic events may represent adequate stimuli for this activity.2,5 Stimulation of cardiac sympathetic afferents reflexly increases sympathetic efferent activity and concomitantly inhibits the activity of efferent vagal cardiac fibers, ultimately resulting in an increase in SAP and HR.5 Similarly, stimulation of cardiac vagal afferents may induce a simultaneous reflex increase in vagal efferent and inhibition in sympathetic efferent activity, ultimately resulting in a depressor reflex consisting of decreases in AP and HR.5 In this context, even though sympathetic afferents have been regarded as the main source for positive feedback mechanisms, the complex pattern of cardiac reflexes largely described as mediated by the sympathetic and the parasympathetic system (ie, sympatho-sympathetic, sympatho-vagal, vago-vagal, and vago-sympathetic reflexes16), through their dynamic interactive effects, might be considered a source of nonbaroreflex sequences. These considerations would support our suggestion that in humans, the nonbaroreflex sequences are an expression of positive feedback mechanisms regulating the cardiovascular system primarily via a neural pathway.

Interaction Between Negative and Positive Neural Feedback Mechanisms

The pattern of interaction between negative and positive feedback mechanisms during orthostatic stress was significantly different in tetraplegics versus normal subjects and was expressed by the opposite changes observed in the B/NB ratio. In normal subjects, there was a marked predominance of negative feedback mechanisms in response to HUT, as demonstrated by the striking increase of baroreflex sequences with no changes in the nonbaroreflex sequences, whereas in tetraplegics, the negative feedback mechanisms did not seem to be engaged to a similar extent and positive feedback mechanisms seemed more active, as suggested by the non-significant changes in the occurrence of baroreflex sequences and by the slight increase in the occurrence of nonbaroreflex
sequences. Paraplegic patients who suffer from cardiovascular deconditioning, like tetraplegics, but do have intact neural pathways, like healthy subjects, show an intermediate pattern characterized by impaired baroreflex and unaltered nonbaroreflex engagement, as demonstrated by an unchanged B/NB ratio.

The mechanistic issues raised by the above findings are quite complex, and we shall attempt to analyze them in a more general perspective.

As expected, in healthy subjects, HUT resulted in a marked activation of baroreflex mechanisms, as indicated by the increase in the occurrence of baroreflex sequences. Failure of baroreflex sequences to increase in tetraplegics in response to HUT might be due to an altered peripheral engagement of the arterial baroreceptors as a consequence of the reduced exposure to orthostatic stimuli in these patients. The absence of repetitive gravitational stimuli may therefore result in a reduced ability of baroreceptors to be engaged by spontaneous AP fluctuations during an orthostatic challenge. However, the baroreceptors are able to respond in the same manner as in the healthy subjects once their receptor potential is produced. In fact, baroreceptor afferent and vagal efferent pathways are intact in these patients, and presumably, the central integration of baroreceptor input is preserved, as indicated by the essentially similar BRS behavior in tetraplegics and healthy subjects (Figure 3). In line with this concept, paraplegic patients, who share with tetraplegics a reduced exposure to orthostatic stress, showed a similar lack of increase in the occurrence of baroreflex sequences in response to HUT and similar BRS patterns. A divergent pattern between the extent of baroreflex engagement, as estimated by the sequences technique, and baroreflex gain has been reported during baroreceptor unloading and other conditions and would suggest that the number of baroreflex sequences and spontaneous BRS represent different, although related, aspects of baroreflex functioning, which could even be dissociated.

The lack of significant changes in the occurrence of nonbaroreflex sequences in healthy subjects was not completely unexpected. Because both sympathetic and parasympathetic activity contribute to their occurrence, the reciprocal changes in sympathetic and parasympathetic outflow evoked by HUT could have abolished their ultimate effect on the occurrence of nonbaroreflex sequences. The mechanisms responsible for the greater activation of positive feedback mechanisms, as expressed by the increased occurrence of nonbaroreflex sequences, in response to orthostatic stress in tetraplegics are unknown.

As discussed above, the complex pattern of cardiovascular reflexes evoked by the activation of sympathetic and vagal afferents might be considered as one of the sources of the nonbaroreflex sequences. Vagal and sympathetic cardiovascular afferents send inputs to the medulla and to the spinal cord in response to normal cardiovascular events, and the likelihood exists that simultaneous but opposite cardiovascular reflex mechanisms are activated by the same event. This information is transmitted, through ascending spinal pathways, to the brain stem and integrated along with afferent inputs from baroreceptors to produce the most adequate cardiovascular responses through changes in sympathetic and parasympathetic efferent activity. However, these reflexes also exist in spinal preparations, thus suggesting that spinal structures, at least under particular conditions, could be sufficient to integrate them. In this context, it has been reported that most spinal cord reflexes appear to be inhibited by supraspinal centers. It is tempting to speculate that when the autonomic nervous system is challenged, as during orthostatic stress, partial loss of this complex regulation, as occurs in tetraplegics, might allow a greater expression of positive feedback mechanisms than could be fully integrated at the spinal level. Removal of supraspinal inhibition during stress would correspond to the observation that nonbaroreflex sequences increased in response to HUT in tetraplegics. This interpretation would fit well with the observation of a central neural occlusive interaction between simultaneous stimulations of sympathetic and parasympathetic cardiac afferents that limits opposing cardiovascular reflex responses. Nevertheless, the occurrence of nonbaroreflex sequences in subjects with intact neuraxis suggests that positive feedback mechanisms may provide a fine-tuning of the cardiovascular system even in the intact state.

Potential Limitations of the Study

Only a small number of beats appear to be organized in sequences characterized by a nonbaroreflex pattern both in humans (~7%) and in animals (~5%). This finding should not be interpreted as indicating that positive feedback mechanisms are not engaged for a prominent fraction of time or that they modulate the cardiovascular system only in a sequencelike fashion. It may only reflect an intrinsic limitation of the sequence method.

Furthermore, our study focused mainly on the interrelation between negative and positive neural feedback mechanisms in dynamically modulating the spontaneous AP and HR fluctuations rather than the absolute contribution of the single mechanisms. In this context, it is not surprising that negative feedback mechanisms, such as the arterial baroreflex, are the main buffering mechanism and are predominant in the physiological regulation of the cardiovascular system.

We cannot exclude the possibility that an enhanced endocrine response to HUT occurs in tetraplegic patients in an attempt to compensate for postural hypotension. This response includes increased levels of vasopressin and angiotensin II, and these might have contributed through a vasomotor action to the interaction of the various mechanisms. In addition, the possible contribution of circulatory mechanisms that mechanically couple HR and blood pressure in determining the occurrence of nonbaroreflex sequences cannot be ruled out and has been recognized previously. However, previous experimental evidence that the occurrence of nonbaroreflex sequences was markedly and significantly decreased after complete pharmacological autonomic blockade clearly shows that the autonomic nervous system is critical in the modulation of the expression of these sequences.

In summary, our study provides 3 novel and interesting findings. First, sequences of consecutive heartbeats within spontaneous AP and HR fluctuations, characterized by con-
sensual, linearly related increases or decreases in SAP and HR, ie, nonbaroreflex sequences, also occur in humans. Second, our data confirm and extend to humans previous results obtained in animals suggesting that spontaneous AP and HR fluctuations are neurally modulated not only through negative feedback (baroreflex sequences) but also through positive feedback (nonbaroreflex sequences) mechanisms. Finally, our data show that an altered interrelation between negative and positive neural feedback mechanisms characterizes the response to orthostatic stress in tetraplegic patients compared with healthy subjects, thus suggesting that a balance between these 2 mechanisms is essential to achieve the most adequate neural regulation of the cardiovascular system.

Additional studies are needed to reveal whether the combined evaluation of negative and positive neural feedback mechanisms could give some pathophysiological insights in those states characterized by disturbances in autonomic cardiovascular regulation, such as chronic orthostatic intolerance and neurally mediated syncope, as well as in several cardiovascular diseases.

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**References**
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