Nonlinear Summation of Depressor Effects of Carotid Sinus Pressure Changes and Aortic Nerve Stimulation in the Rabbit

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SUMMARY. We studied the interaction between the carotid sinus and aortic arch baroreflexes' control of arterial pressure in 13 rabbits anesthetized with urethane. The carotid sinus baroreceptor region was isolated to control carotid sinus pressure. The aortic nerve was transected cervically and stimulated with 4-V electrical pulses at 5 Hz and 0.5-msec duration, which caused a 20-30 mm Hg decrease in arterial pressure. Several protocols were used to analyze the interaction. In the first, we compared the combined effect of the stimulation of a unilateral aortic nerve and a simultaneous increase of bilateral carotid sinus pressures from 40 to 70-80 mm Hg to the sum of these effects measured separately. The combined effect was always larger than the sum of the separate effects. In the second protocol, we varied carotid sinus pressures between 40 and 140 mm Hg and, at each carotid sinus pressure, measured the decrease in arterial pressure caused by unilateral aortic nerve stimulation. The fall in arterial pressure was always greater in the middle range of carotid sinus pressure (70-90 mm Hg) than in the lower or higher range. In the third protocol, we increased the intensity of aortic nerve stimulation from 2 to 4 V while raising carotid sinus pressure from 90 to 110 mm Hg. At this increased tonic input level, the combined effect was always smaller than the sum of the separate effects. Thus, we observed a facilitatory summation when the input variables changed from subthreshold to physiological level, but an inhibitory summation when the input stimulus intensity increased from a physiological to a supraphysiological level. We conclude that interaction between the two reflexes depends in part upon the initial level of the input intensity to the receptors. (Circ Res 52: 401-410, 1983)

THE combined effect of two reflex inputs given simultaneously can be equal to, greater than, or less than the sum of their effects given separately. The first case is defined as additive summation (i.e., no interaction), the second facilitatory (synergistic) summation, and the third inhibitory (occlusive) summation. In the recent literature concerning sino-aortic baroreflex summation, all of the three types of summation have been reported. Angell-James and Daly (1970) compared the combined and separate effects of changing pressures in the isolated carotid sinus and aortic arch on reflex control of systemic arterial pressure in dogs on heart-lung bypass. They found that the reflex effect of combined stimulation was smaller than the sum of the effects of separate stimulations. Thus, they concluded that there is inhibitory summation between the two reflexes. In contrast, Donald and Edis (1971) found the sum of the two reflex effects on perfused hindlimb pressure to be approximately equal to their combined effect. Therefore, these authors concluded that the summation is additive. In a similarly pump-perfused hindlimb vascular bed of the rabbit, Guo et al. (1982) found that the two reflex effects on vascular resistance were almost completely redundant, i.e., a strongly inhibitory addition exists between the two reflex controls. In still another study, Kendrick et al. (1979) electrically stimulated the carotid sinus nerve and ipsilateral aortic nerve in the dog and found the effect of combined stimulation on central arterial pressure to be greater than the sum of the separate effects, evidence for a facilitatory summation. The same authors, however, obtained evidence suggestive of inhibitory summation between the reflexes' control of hindlimb vascular resistance.

In the present study, we used the rabbit because its aortic nerve is anatomically separate from the vagus nerve in the neck, and is known to contain only baroreceptor afferent fibers (Neil et al., 1949). We elicited the aortic baroreflex by electrical stimulation of the central end of the transected aortic nerve. To force the carotid sinus baroreflex without eliciting any carotid chemoreflex effect, we isolated the carotid sinuses and increased or decreased their static pressure in multiple steps. Stinnett et al. (1979) recently performed such a study and found that there was inhibitory summation. However, these authors stimulated the aortic nerve with a combination of high voltage and high frequency which would cause a maximum depressor effect. We suspect such intense stimulation may be inappropriate for the analysis of summation between tonically active reflexes such as the carotid sinus and aortic baroreflexes which respond to small physiological pressure fluctuations.
Therefore, we used stimulus parameters which would cause a mild fall of 20-30 mm Hg in mean systemic arterial pressure.

Methods

Animals and Anesthesia

Results were obtained from a total of 19 rabbits of either sex, weighing 4.5 ± 0.17 (st) kg. The rabbits were anesthetized by intravenous injection of urethane (1.0 g/kg), and immobilized with succinylcholine (initial dose, 3 mg/kg). Animals were positive pressure ventilated with room air supplemented with a mixture of 95% O₂ + 5% CO₂. The vagus nerves were cut midcervically to block the afferent fibers from the aortic chemoreceptors and the cardiopulmonary mechano- and chemoreceptors. Mean systemic arterial pressure was measured via a catheter inserted into the femoral artery. Heart rate was monitored by a tachometer triggered by the systemic arterial pressure pulse. Throughout the experiment, heparin and succinylcholine were continuously infused at 200 U/kg per hr and 2.4 mg/kg per hr, respectively. These substances were dissolved in a modified Tyrode’s solution, the pH of which was adjusted to 7.6 by the addition of sodium bicarbonate. This alkaline solution helped to maintain the arterial blood pH at 7.4 ± 0.05.

Isolation Of Carotid Sinus

The right and left carotid sinuses were vascularly isolated by ligating the internal carotid arteries, occipital arteries, and all other small branches. As illustrated in Figure 1, the external carotid arteries remained perfused throughout the experiment. Loops of thread were put around the lingual external carotid arteries. These were tightened to occlude the lingual arteries between the experimental runs. To avoid cerebral ischemia blood was conducted via a short tubing (S in Fig. 1) to a plastic chamber, common carotid artery, and isolate the carotid sinuses during the experimental runs. They were released to allow perfusion of the carotid sinuses between the experimental runs. To avoid cerebral ischemia during the experimental runs, a catheter was inserted proximally into each common carotid artery and the arterial blood was conducted via a short tubing (S in Fig. 1) to a catheter inserted into each external carotid artery. Tubes also conducted blood (via C1 in Fig. 1) to a plastic chamber from which catheters conducted blood into the distal common carotid arteries. The plastic chamber was connected with a pressure transducer (P.T.) and a hydrostatic pressure source (H.P.S.). To control carotid sinus pressure, the clamp C1 was closed while another clamp C2 was opened.

Aortic Nerve Stimulation

The left and right aortic nerves were separated from the surrounding tissues and sectioned at the mid-cervical level to prepare for electrical stimulation. Both nerves were stimulated with identical stimulus parameters, and the depressor responses were compared to decide which side caused a greater fall in blood pressure. Left aortic nerve stimulation had a greater depressor effect in the majority of rabbits. That side which caused a greater fall was used for subsequent stimulation. As described in the protocol section, we stimulated a unilateral aortic nerve in most protocols. The stimulus parameters were usually 4 V, 5 Hz, and 0.5 mese in duration.

This choice of stimulus parameters was based on the following findings in the early phase of the study. As stated in the introduction, we wanted to activate the aortic reflex in this study to a mild degree which would cause a fall of about 20 mm Hg in mean arterial pressure when the isolated carotid sinus pressure was set at a physiological level (e.g., 80 mm Hg). We found the particular combination of parameters to cause the desired magnitude of arterial pressure fall in almost all the rabbits. A similar degree of hypotension was attained in some of the rabbits with a lower intensity (0.4 V) and a higher frequency (15 Hz). However, a more uniform degree of hypotension was obtainable among rabbits with the combinations of 4 V and 5 Hz. Therefore, we used this parameter set in most of the stimulations.

Experimental Protocol and Data Analysis

Four protocols were used to investigate the sino-aortic reflex interaction. In the first protocol with nine rabbits, we increased the inputs to both reflexes from subthreshold to a mid-intensity level, separately and then together, to compare the sum of the separate reflex effects against the combined reflex effect. An example of this experimental protocol is shown in Figure 2. In the left panel, the aortic nerve stimulus was step increased from 0 to 4 V (5 Hz and 0.5 mese in duration) while bilateral carotid sinus pressures...
were maintained at 40 mm Hg. This pressure (40 mm Hg) shows its significance in the threshold of the carotid sinus reflex in most of the rabbits. The aortic nerve stimulation was continued for 2 minutes and the steady state fell in mean arterial pressure, \( \Delta MAP_{AN} \), was determined. Next, aortic nerve stimulation was stopped and, as shown in the middle panel of Figure 2, carotid sinus pressure was step increased to 80 mm Hg and maintained at this level for 2 minutes. The steady state fell in mean arterial pressure, \( \Delta MAP_{CS} \), was determined. Finally, carotid sinus pressure was reset to 40 mm Hg and then step increased to 80 mm Hg at the same time that aortic nerve stimulation was begun (right panel of Fig. 2). The steady state combined reflex effect, \( \Delta MAP_{AN+CS} \), was compared with the sum of the separate effects, \( \Delta MAP_{CS} + \Delta MAP_{AN} \).

In the second protocol, we determined the steady state relationship between carotid sinus pressure (CSP) and mean systemic arterial pressure (MAP) over the CSP range between 40 and 140 mm Hg with and without aortic nerve stimulation. Specifically, carotid sinus pressure was set initially at 40 or 140 mm Hg for 1 to 2 minutes without aortic nerve stimulation, and the steady state mean arterial pressure was measured. Aortic nerve stimulation was then started and continued until a steady state fell of mean arterial pressure was recorded. Carotid sinus pressure then was increased or decreased by 10 mm Hg, the steady state mean arterial pressure response was recorded, and aortic nerve stimulation was started again and continued until a steady state fell of mean arterial pressure at the new carotid sinus pressure was determined. The step changes in carotid sinus pressure were continued until it reached the other extreme (i.e., 140 or 40 mm Hg). The direction then was reversed until carotid sinus pressure returned to the original level. The initial level of carotid sinus pressure (40 or 140 mm Hg) was chosen at random for a given rabbit. The same nine rabbits as in protocol 1 were used.

The CSP-MAP relationship exhibited a fairly large hysteresis, both with and without aortic nerve stimulation, as has previously been reported in the dog (Kumada et al., 1975). The statistical difference in the CSP-MAP relationship between the ascending and descending sequences of carotid sinus pressure change are presented in Table 1. Because the hysteresis was small compared with the overall reflex change in mean arterial pressure, we simplified the analysis by averaging the data during ascending and descending sequences of carotid sinus pressure change presented in Table 1. Because the hysteresis was small compared with the overall reflex change in mean arterial pressure, we simplified the analysis by averaging the data during ascending and descending sequences of carotid sinus pressure change. The parameters of the distribution curves fitted to the CSP-systolic arterial pressure, \( G_{\text{max}} \), maximum slope of the CSP-MAP relation curve fitted to the data, \( CS_{\text{op}} \), optimum CSP at which \( G_{\text{max}} \) was obtained, \( \sigma \), the standard deviation of CSP from CSP, in the gain curve distributed as a function of CSP. *Significantly different at 5% level from the corresponding value during the ascending sequence without aortic nerve stimulation. † and ‡ Significantly different at 5% (†) and 1% (‡) level for the corresponding values without aortic nerve stimulation.

The CSP-MAP relationship was determined by changing CSP from CSP to CSP, was considered to represent the gain over this range of CSP and correlated with CSP, = (CSP + CSP,)/2 as shown in the bottom panel of Figure 3. Paired sets of CSP, − GM relation data were collected from each of the nine rabbits, one set with aortic nerve stimulation and the other set without. We averaged the gain values pooled from all the rabbits at each of the nine CSPs, with or without aortic nerve stimulation, and then fitted a standard normal distribution curve to the data. The parameters of the distribution curves were determined include the maximum gain \( G_{\text{max}} \), the optimum carotid sinus pressure CSP, for the maximum gain, and the standard deviation \( \sigma \) of the CSP values from CSP, (Snedecor and Cochran, 1967). The equations used for the calculation are:

\[
\text{CSP}_n = \frac{10}{m} \sum (\text{CSP}_m \times GM) / \sum GM
\]

\[
\sigma = \sqrt{\frac{\sum (\text{CSP}_m - \text{CSP}_n)^2 GM}{10 \sum GM}}
\]

The sigmoidal CSP-MAP relation curves were obtained by integrating the fitted CSP-gain relationship curves with respect to carotid sinus pressure. The difference between the two parameter sets for the two cumulative distribution curves, one with aortic nerve stimulation and the other without, indicated the effect of aortic nerve stimulation on the CSP-MAP relationship.

In the third protocol, we used four additional rabbits to test the hypothesis that if the inputs to the sino-aortic reflexes are initially at a physiological level and increase to

<table>
<thead>
<tr>
<th>Sequence of CSP change</th>
<th>CSP, (mm Hg)</th>
<th>( \sigma ) (mm Hg)</th>
<th>( G_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending</td>
<td>83.8 ± 3.8</td>
<td>16.4 ± 1.3</td>
<td>2.54 ± 0.22</td>
</tr>
<tr>
<td>Descending</td>
<td>89.2 ± 3.6*</td>
<td>12.4 ± 1.5*</td>
<td>3.61 ± 0.47*</td>
</tr>
<tr>
<td>Averaged</td>
<td>87.4 ± 3.0</td>
<td>14.0 ± 1.2</td>
<td>2.90 ± 0.25</td>
</tr>
</tbody>
</table>

\( \Delta MAP_{AN+CS} \) determined by changing CSP from CSP, to CSP, was considered to represent the gain over this range of CSP and correlated with CSP, = (CSP, + CSP,)/2 as shown in the bottom panel of Figure 3. Paired sets of CSP, − GM relation data were collected from each of the nine rabbits, one set with aortic nerve stimulation and the other set without. We averaged the gain values pooled from all the rabbits at each of the nine CSPs, with or without aortic nerve stimulation, and then fitted a standard normal distribution curve to the data. The parameters of the distribution curves were determined include the maximum gain \( G_{\text{max}} \), the optimum carotid sinus pressure CSP, for the maximum gain, and the standard deviation \( \sigma \) of the CSP values from CSP, (Snedecor and Cochran, 1967). The equations used for the calculation are:

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The sigmoidal CSP-MAP relation curves were obtained by integrating the fitted CSP-gain relationship curves with respect to carotid sinus pressure. The difference between the two parameter sets for the two cumulative distribution curves, one with aortic nerve stimulation and the other without, indicated the effect of aortic nerve stimulation on the CSP-MAP relationship.

In the third protocol, we used four additional rabbits to test the hypothesis that if the inputs to the sino-aortic reflexes are initially at a physiological level and increase to
a moderately supraphysiological level, the resultant summation may be quite different from that which is found when the inputs are initially subthreshold and are increased to a physiological level. To test this hypothesis, we initially set carotid sinus pressure at 90 mm Hg and stimulated the aortic nerve with 2 V. We then increased the voltage of aortic nerve stimulation to 4 V with and without a simultaneous increase in carotid sinus pressure to 110 mm Hg. The separate and combined reflex effects were compared as in the first protocol.

In the fourth protocol with another group of four rabbits, we investigated whether bilateral aortic nerve stimulation yields the same results as unilateral stimulation. We also examined how combination of ipsi- vs. contralateral inputs to the sino-aortic reflexes affect the summation. That is, we compared the summation of right side aortic nerve stimulation effect with the right side carotid sinus reflex effect against the summation of the right side aortic nerve stimulation effect with the left side carotid sinus reflex effect.

Statistical Analysis

Statistical significance of the difference in decrease of mean arterial pressure caused by aortic nerve stimulation at different CSPs was first evaluated by a one-way analysis of variance. When these values were found to be statistically significant, the difference between any pair of ΔMAP,ANS at two CSPs was tested with the least significant difference method. The difference between pairs of parameter values (with and without aortic nerve stimulation) derived from the curve fitting was analyzed with the paired t-test.

Results

Protocol 1: Separate and Combined Responses to Carotid Sinus Pressure Change and Aortic Nerve Stimulation from Subthreshold Initial Condition

The response of mean arterial pressure to either aortic nerve stimulation or a step change in carotid sinus pressure reached a steady state in 1 to 1.5 minutes. In the example shown in Figure 2, aortic nerve stimulation alone caused a 5 mm Hg fall in mean arterial pressure (panel A). Raising carotid sinus pressure from 40 to 70 mm Hg caused a 24 mm Hg fall in mean arterial pressure (panel B). These two stimuli together caused a 56 mm Hg fall in mean arterial pressure (panel C). This combined effect is much larger than the sum of the separate reflex responses, 5 + 24 = 29 mm Hg. Figure 4 shows similar data from the nine rabbits studied in this protocol. The diagonal line represents the identity between the combined response and the sum of the separate responses, i.e., an additive summation. Since the points all lie to the left of the identity line, the combined response was always larger than the sum of the separate responses. The difference ranged from 4 to 35 mm Hg, and the mean and standard error were 17.6 ± 3.4 mm Hg. That is, the combined effect was 202% of the sum of the separate effects. This shows facilitatory summation between the two reflex effects. It is important to note that this was the case when the input stimuli were increased from subthreshold to a moderate intensity.

Protocol 2: CSP-MAP Relations with and without Aortic Nerve Stimulation

In the same nine rabbits used in the first protocol, the carotid sinus pressure was changed stepwise between 40 and 140 mm Hg. Average heart rate changed from 240 beats/min at the highest CSP to 270 beats/min at the lowest CSP. The average CSP-MAP relationship data with and without simultaneous aortic nerve stimulation are shown in the top panel of Figure 5. Each dot denotes the mean of nine values of mean arterial pressure and the bars are standard error of the mean. The curves represent the cumulative normal distribution curves fitted to those data. We tested the normality of the distribution of the CSP-gain relation data (Snedecor and Cochran, 1967).
The goodness-of-fit test did not allow rejection of the data as significantly different from a normal distribution curve. The coefficient of skewness of the data was 0.25 and 0.11 for the data with and without aortic nerve stimulation, respectively. These values fell in the range of insignificant skewness. The estimate of kurtosis was -0.19 and -0.40 for the respective curves. These values suggest that the top portion of the relationship curve is only slightly flatter than the normal distribution curve and that the normal distribution curve with the calculated parameters provides an excellent fit to the data. This provided the basis for describing the shift of the CSP-MAP relation curve caused by aortic nerve stimulation in terms of the three parameters of the normal distribution curve, \( G_{\text{max}} \), \( \text{CSP}_0 \), and \( \sigma \).

The shift of the average CSP-gain relationship curve caused by aortic nerve stimulation is shown in Figure 3. The average values of the three parameters of the distribution curves fitted to the data from individual rabbits are listed in Table 1. The changes in \( \text{CSP}_0 \) and \( G_{\text{max}} \) parameters caused by aortic nerve stimulation are statistically significant. Analysis of variance also demonstrated that the response to aortic nerve stimulation was significantly \( (P < 0.05) \) larger during the increasing CSP sequences than during the decreasing sequences.

The decrease in arterial pressure caused by aortic nerve stimulation was found to be greater in the middle range of CSP than in the higher or lower range (lower panel of Fig. 5). When the data from all rabbits were pooled and examined by analysis of variance (Table 2), the depressor effects of aortic nerve stimulation at CSPs of 70 and 80 mm Hg were significantly greater \( (P < 0.05) \) than those at CSPs of 40, 100, 120, and 130 mm Hg. The decrease in arterial pressure at CSP of 90 mm Hg was found to be greater \( (P < 0.05) \) than that measured at CSPs of 110, 120, and 130 mm Hg.

To determine whether this finding was specific to the particular combination of aortic nerve stimulus parameters \( (4 \text{ V and 5 Hz}) \), we stimulated the aortic nerve of two additional rabbits with a combination of lower voltage \( (0.4 \text{ V}) \) and higher frequency \( (15 \text{ Hz}) \). We used these parameters because Douglas et al. (1956) reported that such a combination of relatively low intensity and high frequency stimulus parameters excited predominantly fast-conducting fibers in the rabbit. The results obtained from the two rabbits are shown in Figure 6. No significant difference could be demonstrated between the results obtained using these stimulus parameters and the results with the control stimulus of 4 V and 5 Hz.

The increase in the depressor effect of aortic nerve stimulation with the increase of carotid sinus pressure from the threshold to a physiological level leads us to
the conclusion that there is facilitatory summation between the two depressor reflexes in this range of the reflex input. On the other hand, the decrease in the depressor effect of aortic nerve stimulation as carotid sinus pressure was further increased from the physiological to supraphysiological range suggests the opposite, i.e., that there is inhibitory summation between the two reflexes. This hypothesis was validated by the third experimental protocol.

**Protocol 3: Summation over Mid-to-High Intensity Range of Inputs**

The relationship between the combined reflex depressor effect and the sum of the separate reflex effects, obtained by increasing the stimulus voltage of aortic nerve stimulation and raising carotid sinus pressure from 90 to 110 mm Hg, simultaneously or separately, is shown in Figure 7. The points are all below the identity line; the combined effect was smaller than the sum of the separate effects by 11.0 ± 2.63 mm Hg, or by 29 ± 10% of the latter. This result is in sharp contrast to that shown in Figure 4, in which all points were above the identity line, but is consistent with the trend shown in the high CSP range in the lower panel of Figure 5.

![Figure 6. Relationship between carotid sinus pressure (CSP) and depressor effect of aortic nerve stimulation with 0.4 V, 15 Hz, and 0.5 msec. Data obtained in two rabbits are shown with open and closed circles.](image)

![Figure 7. Relationship between combined effect (on ordinate) and sum of separate effects (on abscissa) of aortic nerve stimulation and carotid sinus reflex. Carotid sinus pressure, initially at 90 mm Hg, was raised to 110 mm Hg, while the intensity of aortic nerve stimulation was increased to 4 V, at constant frequency (15 Hz) and duration (0.5 msec). The solid line indicates the identity which represents additive summation. Compare with Figure 4.](image)

**Protocol 4A: Summation with Bilateral Aortic Nerve Stimulation**

The effect of stimulation of bilateral aortic nerves with the stimulus parameters of 0.1-0.5 V, 20 Hz, and 0.5 msec in duration on the CSP-MAP relationship curve is shown in the top panel of Figure 8. The depressor effect of the aortic nerve stimulation is shown as a function of carotid sinus pressure in the bottom panel. In both graphs, the circles represent the mean values from four rabbits and the bars the standard error of the mean. Comparison of this figure with Figure 5, in which unilateral aortic nerve stimulation was used, indicates a strong similarity. That is, the depressor effect of aortic nerve stimulation is greater over the physiological range of 70 to 90 mm Hg than in the sub- or supraphysiological range of CSP, whether the aortic nerve is stimulated unilaterally or bilaterally.

### Table 2

<table>
<thead>
<tr>
<th>CSP (mm Hg)</th>
<th>MAP ± SD</th>
<th>ΔMAP ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>134.8 ± 7.3</td>
<td>9.9 ± 6.8</td>
</tr>
<tr>
<td>50</td>
<td>133.6 ± 7.4</td>
<td>13.3 ± 6</td>
</tr>
<tr>
<td>60</td>
<td>128.2 ± 7.6</td>
<td>16.7 ± 6.5</td>
</tr>
<tr>
<td>70</td>
<td>123.2 ± 8.2</td>
<td>21.3 ± 7.0</td>
</tr>
<tr>
<td>80</td>
<td>106.5 ± 10.8</td>
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<tr>
<td>90</td>
<td>83.4 ± 12.5</td>
<td>18.7 ± 11.1</td>
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<tr>
<td>100</td>
<td>63.9 ± 11.0</td>
<td>11.1 ± 9.6</td>
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<tr>
<td>110</td>
<td>45.4 ± 8.4</td>
<td>6.8 ± 5.9</td>
</tr>
<tr>
<td>120</td>
<td>40.4 ± 6.4</td>
<td>5.8 ± 5.8</td>
</tr>
<tr>
<td>130</td>
<td>39.0 ± 2.8</td>
<td>7.0 ± 5.8</td>
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</tbody>
</table>

LSD (least significant difference): If the depressor response at a given CSP differed from the depressor response at any other CSP by more than this LSD value (9.14 mm Hg), the difference is statistically significant at 5% level. See text for CSPs at which the significant difference in depressor response occurred.

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**TABLE 2**

Mean Systemic Arterial Pressures (MAP) and Depressor Responses to Aortic Nerve Stimulation (ΔMAP) at Different Levels of Isolated Carotid Sinus Pressure (CSP) Averaged from Nine Rabbits

<table>
<thead>
<tr>
<th>CSP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>ΔMAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>134.8</td>
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<td>50</td>
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<td>120</td>
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The effect of stimulation of bilateral aortic nerves with the stimulus parameters of 0.1-0.5 V, 20 Hz, and 0.5 msec in duration on the CSP-MAP relationship curve is shown in the top panel of Figure 8. The depressor effect of the aortic nerve stimulation is shown as a function of carotid sinus pressure in the bottom panel. In both graphs, the circles represent the mean values from four rabbits and the bars the standard error of the mean. Comparison of this figure with Figure 5, in which unilateral aortic nerve stimulation was used, indicates a strong similarity. That is, the depressor effect of aortic nerve stimulation is greater over the physiological range of 70 to 90 mm Hg than in the sub- or supraphysiological range of CSP, whether the aortic nerve is stimulated unilaterally or bilaterally.

**Protocol 4A: Summation with Bilateral Aortic Nerve Stimulation**

The effect of stimulation of bilateral aortic nerves with the stimulus parameters of 0.1-0.5 V, 20 Hz, and 0.5 msec in duration on the CSP-MAP relationship curve is shown in the top panel of Figure 8. The depressor effect of the aortic nerve stimulation is shown as a function of carotid sinus pressure in the bottom panel. In both graphs, the circles represent the mean values from four rabbits and the bars the standard error of the mean. Comparison of this figure with Figure 5, in which unilateral aortic nerve stimulation was used, indicates a strong similarity. That is, the depressor effect of aortic nerve stimulation is greater over the physiological range of 70 to 90 mm Hg than in the sub- or supraphysiological range of CSP, whether the aortic nerve is stimulated unilaterally or bilaterally.
We also studied the summation between the depressor effects of left and right aortic nerve stimulations. When we compared the effect of bilateral stimulation to the sum of the effects of separate stimulation, the mean difference between the pairs was 1.4 ± 1.4 (±) mm Hg, which was not statistically significant. Therefore, summation between the left and right aortic nerve reflexes appeared to be simply additive, at least when the intensity of individual stimulations is low enough to cause only mild decreases in arterial pressure.

Protocol 4B: Ipsilateral vs. Contralateral Summation

In the four rabbits used in protocol 3, we studied the summations of right aortic nerve stimulation effect with the effects of the left vs. right side carotid sinus baroreceptor reflex. The results are plotted in Figure 9. In agreement with the earlier work by Kendrick et al. (1979), facilitatory summation was observed only with inputs to the ipsilateral carotid and aortic reflexes. Summation between the contralateral sino-aortic reflexes was simply additive, as shown by the data distributed along the identity line.

Discussion

Two reflex systems which share some part of their effector pathway can summate in an inhibitory, additive, or facilitatory manner. Two important determinants of the type of summation are the magnitude of change in the input variable (e.g., ΔCS) and the absolute level of the input variable on which the input signal is superimposed (e.g., CSP). In the present study, we used small increments of carotid sinus pressure and low intensity of aortic nerve stimulation to produce mild decreases in arterial pressure. When the initial level of the receptor input was subthreshold, we observed facilitatory summation between effects of the carotid sinus reflex and aortic nerve stimulation.

In a similar study, Stinnet et al. (1979) used a very intense stimulus to the aortic nerves of 8-V and 80-Hz pulse bursts synchronized with the heart beat. When carotid sinus pressure was set at a subthreshold level, aortic nerve stimulation decreased arterial pressure by 70 mm Hg, lowering arterial pressure to 36 mm Hg. As the initial level of carotid sinus pressure...
was increased above the threshold, aortic nerve stimula-
tion caused smaller decreases in arterial pressure.
The decrease in the effect of aortic nerve stimulation was proportional to the initial fall in arterial pressure caused by the carotid sinus reflex. The result is indicative of a strongly inhibitory summation between the two reflexes over the entire range of the carotid sinus reflex. The possible reason for the difference between this study and our present study is the intensity of the aortic nerve stimulation. To test the possibility, we stimulated the aortic nerves with the same voltage and frequency as Stinnet et al. used in those four rabbits in which the effect of bilateral stimulations was studied. Our results were quite similar to theirs. When we stimulated the aortic nerve first and then increased carotid sinus pressure, the carotid sinus reflex could decrease arterial pressure only 20 mm Hg over the entire receptor pressure range from 40 to 140 mm Hg. The strong inhibition seen in these experiments indicates a large overlap between the central and/or the peripheral effector pathways of these reflexes.

The present findings suggest, however, that we cannot necessarily extrapolate this concept of summation to the physiological situation and expect to observe an equally strong inhibitory summation between the sino-aortic reflexes. During certain physiological circumstances, such as postural changes and respiration, arterial pressure changes are limited to 10 to 20 mm Hg. It is reasonable to expect that the input signals to the sino-aortic baroreflexes will be correspondingly small and, consequently, summation between the two reflexes is governed by a rule valid for small input signals rather than a maximum stimulation.

As a gross approximation of the circumstances in which the two reflexes are responding to a mild pressure increase from the normal level, we increased carotid sinus pressure from 90 to 110 mm Hg and the intensity of aortic nerve stimulation from a finite level at 2 V and 15 Hz to a slightly higher level of 4 V and 15 Hz. Under this condition, we observed a mild degree of inhibitory summation (Fig. 7). When the initial inputs to the reflexes were subthreshold and then increased toward a physiological level, we found a moderate degree of facilitatory summation between the two reflexes. Therefore, one of the conclusions from the present study is that the nature of summation between the sino-aortic reflexes, (facilitatory, inhibitory, or additive) depends very critically on (1) the initial level of the stimulus to the reflex receptors, and (2) the magnitude of the signals (i.e., change in the input) used to study the summation. The validity of this conclusion for physiological circumstances is limited, however, by the unphysiological nature of the inputs given to the baroreceptor afferents, particularly to the aortic nerve, in the present study.

It is difficult to understand the reasons for conflicting findings on summation between the sino-aortic reflexes in the literature. Angell-James and Daly (1970) found a moderate degree of inhibitory sum-

mation between the canine sino-aortic reflexes with systemic vascular resistance as the output variable. They used nonpulsatile pressure forcing of both baro-
sensory areas increasing the pressure by 9 to 20 mm Hg, both separately and simultaneously. Unfortu-
nately, the control levels of carotid sinus and aortic arch pressure are not given. It is also not clear whether the results reported were steady state responses or peak values of resistance changes. A similar inade-
quate description of input stimuli makes it difficult to infer why Donald and Edis (1971) found a simply additive summation between sino-aortic reflex con-
trols of canine hindlimb resistance. They might have changed both receptor pressures over a small range above and below the normal arterial pressure. If this were the case, summation could be simply additive, judged from the curve in the bottom panel of Figure 5. This hypothesis needs to be examined in different vascular beds and in different animal species.

More recently, however, Guo et al. (1982) found quite a different type of summation between sino-
aortic reflex controls of hindlimb resistance. Working in the rabbit, these investigators increased and decreased baroreceptor pressure with injections of phenylephrine and nitroglycerine, respectively. They monitored reflex change in arterial pressure of the hindlimb pressure perfused with a constant flow. From a series of well-controlled denervation experiments, they concluded that—with both baroreceptors intact—there is extremely strong inhibitory addition between their reflex effects on hindlimb vascular resistance, but a simple addition between their reflex controls of heart rate. This study differs from ours, in that Guo et al. used a dynamically changing pressure with a maximum duration of 30 seconds as the input, and measured peak transient responses of heart rate and hindlimb resistance as the output. We used step changes in stimulus intensity and measured steady state changes in mean central arterial pressure, which incorporates reflex changes in both total peripheral resistance and cardiac output. Therefore, the two studies are not immediately comparable. However, there is qualitative similarity between them in that both results indicate inhibitory summation between the reflex responses to increasing or decreasing input signals superimposed on a mid-level tonic stimulation to the receptors. From a quantitative viewpoint, the inhibitory summation was extremely strong in the reflex control of hindlimb resistance, whereas it was rather moderate in the reflex control of arterial pres-
sure. Whether this marked quantitative difference can be attributed solely to the above-mentioned differences in the input stimulus and in the nature and time course of measured output variables remains to be investigated.

Alternative and partial explanation for the disparate summation patterns is that different investigators studied the summation of reflex controls in different cardiovascular processes. Angell-James and Daly (1970) analyzed the reflex summation in the control of total peripheral resistance. Donald et al. (1971),
Kendrick et al. (1979), and Guo et al. (1982) studied the reflex summation in the hindlimb vascular resistance. That this might be an important factor is suggested by the findings of Kendrick et al. (1979) and Guo et al. (1982) that the summation was inhibitory in hindlimb resistance but facilitatory or additive in central arterial pressure and heart rate.

An obvious limitation of the present study is electrical stimulation of the aortic nerve and consequent nonphysiological nature of afferent signals of the aortic baroreflex. Since we neither recorded afferent nerve impulses nor measured the conduction velocity in the excited aortic nerve fibers, we cannot tell how closely the electrical stimulation used in our study simulated the physiological tonic activation of the aortic baroreflex and the small changes in its input. For this reason, our conclusion regarding the reflex summation may not be immediately applicable to all physiological situations. Even with this possible limitation, the study supports two concepts advanced by Kendrick et al. (1979). Using dogs, they activated both carotid sinus and aortic nerves by applying a moderate intensity of electrical stimulation separately and simultaneously. They found a fall in central arterial pressure with simultaneous stimulation of these nerves that was 50% greater than the sum of the falls caused by separate stimulations of these ipsilateral nerves. This is quite similar to our finding presented in Figure 4. When they stimulated the carotid sinus and aortic nerves on the opposite sides, they found simply additive summation. Again our results presented in Figure 9 agree with this finding by Kendrick et al. Therefore, our study demonstrated that their concept is valid, in the rabbit as well as in the dog, that facilitatory summation exists between the ipsilateral sino-aortic reflexes when the input stimulus is increased from subthreshold to a mild to moderate intensity, but simple addition exists between the contralateral reflex responses to the similar stimuli.

In an attempt to analyze summation between the sino-aortic reflex as these reflexes restore arterial pressure after an acute mild hemorrhage in conscious dogs, Hosomi and Sagawa (1979) estimated the open-loop gains of the carotid sinus reflex and the lumped vagally mediated reflex system for arterial pressure control. To estimate the gains, they measured the falls in arterial pressure after repeated hemorrhage perturbation, under intact and denervated conditions. They found that the data were consistent only with an assumption that there is a very strong facilitatory interaction between the two reflex forces which restore blood pressure. This finding is in direct contrast to the concept suggested by the present result that there is inhibitory summation between the sino-aortic reflexes when the input signals are decreases of receptor stimulation from the physiological tonic level. Again, the reason for this discrepancy is not clear. The hemorrhagic stimuli used in that study are more natural but also far more complex, invoking many more reflexes than those in the present study. The gain estimation depends on the linear assumption which is known to be valid only in a very narrow range of receptor pressure. Lumping the multiple cardiopulmonary reflexes and aortic baro- and chemoreceptor reflexes into a single hypothetical reflex for arterial pressure control is another simplification which is difficult to justify. Further experimental studies with well-defined and yet natural inputs specific to the sino-aortic reflexes are warranted.

Apart from these disparate findings in the literature, the dependence of the type of summation on the initial level of stimulation and the magnitude of the input signal can be explained simply by considering the sigmoidal nature of the input-output relationship of the sino-aortic reflexes. Two separate inputs both from subthreshold to a mild intensity level will produce rather small individual outputs because they fall in the flat portion of the sigmoidal curve near the threshold. If these inputs are given simultaneously, adding to each other and increasing the input size to their sum, this combined input will span the steeper portion near the mid-point of the sigmoid curve and will produce a greater reflex output than the sum of the two separate outputs. Thus, the type of summation will be judged to be facilitatory. If the same small input signals fall in the relatively linear middle range of the relation curve, the separate and combined reflex effects will be approximately the same, and simply additive summation will be seen between the two reflexes. If these small positive input signals are superimposed on a supraphysiological level of a tonic stimulus, their combined reflex effect will be smaller than the sum of the individual effects because the sigmoid curve is flat again in this range. Thus, inhibitory summation will be observed. As long as there is a saturation plateau in the reflex output, one can always observe inhibitory summation by feeding two inputs, one of which is large enough to cause saturation of the reflex output. The above explanation, although not unique, is perhaps the simplest mechanism and, as such, deserves consideration.

In conclusion, the present study demonstrated the presence of a nonlinear summation between the carotid sinus and aortic baroreceptor reflex control of arterial pressure. Whether the interaction is facilitatory (synergistic) or inhibitory (occlusive) depends upon the initial conditions and stimulus magnitudes. If the stimulus inputs to both reflexes are initially subthreshold, the depressor effect provoked by simultaneous and mild stimulations of the two reflexes will be greater than the sum of arterial pressure falls caused by the same but separate stimulations. Thus, the summation is facilitatory. By contrast, if the initial condition were a more physiological one, in which moderate frequencies of the baroreceptor afferent impulses were arriving at the reflex center, further and simultaneous increases of the stimuli to the two receptors would produce a depressor effect smaller than the sum of the separate reflex responses to the same increases in the stimuli. The summation, then, is seen as inhibitory. This dependence of the nature of the summation on the baseline level of the input

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stimulus may explain at least some of the contradictory findings in the literature concerning the summation in the sino-aortic baroreceptor reflex control of arterial pressure.

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INDEX TERMS: Carotid sinus baroreflex • Aortic arch baroreflex • Facilitatory interaction • Inhibitory interaction • Initial condition dependence of interaction
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