Studies in Man on the Relationship of Adrenergic Correlates to Pressor Responsivity

By Joseph D. Sapira, M.D., and Alvin P. Shapiro, M.D.

With the technical assistance of Thelma Klaniecki, Jean E. Yevick, and Jean L. Small

The present study was undertaken as part of a continuing investigation of the mechanisms of cardiovascular responsivenes to noxious stimuli. Its purpose was to determine (1) whether stimuli which raise blood pressure in man would produce concomitant changes in biochemical measures of activation of the autonomic nervous system, (2) whether such changes would vary with different stimuli, and (3) whether they would continue with repetition of the same stimuli.

The inquiry was motivated by several questions concerning blood pressure, homeostasis, and the mechanisms of hypertension. It is often implied that vasoconstriction and pressor responses to noxious stimuli are mediated primarily by discharge of the sympathetic nervous system and that a direct relationship exists between sympathetic arousal and rise in blood pressure. Even if vasoconstriction per se was a consequence solely of neurogenic stimulation, this might not result in proportional blood pressure change, since clinical blood pressure is the final expression of a complex hemodynamic process including inotropic and chronotropic cardiac effects, as well as the net change in peripheral resistance. The latter in turn represents a summation of the changes in constriction and dilatation of various regions of the arterial bed. Furthermore, a blood pressure response of considerable magnitude to various stimuli can persist clinically in the presence of diminished sympathetic activity. This has been demonstrated in normotensive and hypertensive diabetics and in hypertensive patients receiving sympatholytic drugs. When the stimulus in these "denervated" situations is the injection of a humoral material such as the polypeptide angiotensin, the pressor response actually may be enhanced. The cold pressor response, on the other hand, which is said to be mediated primarily by the sympathetic nervous system, is depressed in such subjects, while responses to emotional stimuli may follow either pattern.

Emphasis on the adrenergic nervous system derived also from two additional considerations. First, there is disagreement, recently summarized by Brunjes, concerning whether adrenergic mechanisms, as judged by catecholamine metabolism, are overactive, underactive, or normal in essential hypertension. Physiologically, hyperactivity of the sympathetic nervous system has not been demonstrated in hypertensive subjects, while in fact certain data suggest it is hypoactive. Simultaneous recording of physiological pressor responses and chemical changes in normotensive subjects represents a step in clarifying these questions.

Secondly, phenomena such as the rate at which blood pressure returns to previous levels after its depression by methacholine and the ability to withstand centrifugal effects on blood pressure, have been utilized as measures of sympathetic reactivity. In the centrifugation studies, increased elaboration of norepinephrine in response to aggressive impulses...
seemed to correlate with increased resistance to this vasodepressor stimulus. Other studies have also linked an "anger-out" pattern of personality reaction to sympathotonia, pressor reactions, and norepinephrine elaboration.\textsuperscript{10,11} These considerations are based on the probability that a one-to-one relationship exists between sympathetic arousal and blood pressure change. Although little or no data are available to confirm this direct relationship, and in fact hemodynamic and biochemical measurements after infusion of norepinephrine indicate a lack of correlation,\textsuperscript{12,13} it has become the practice in much psychophysiological research to consider blood pressure and pulse changes as evidence of sympathetic arousal and to include them as part of various "autonomic indices."

To examine these problems, our basic experimental approach consisted of inducing acute pressor episodes in healthy normotensive subjects. Simultaneously, certain chemical constituents of blood and urine that are primarily measures of adrenergic activity were determined.

**Methods**

The subjects were healthy white male volunteers less than 30 years of age. All were undergraduate college students or medical students. No subject had a history of cardiovascular or renal disease or of a neurological disorder. Several had family histories of vascular disease. Volunteers were paid $15 per experimental session. Prior to study, they were told only the manner in which the tests were to be done (that is, the equipment, and the venipunctures), but the stimuli were not described nor were they aware of the specific chemical determinations which were performed.

All experiments were carried out in a quiet, softly lit room equipped with a comfortable bed and a one-way vision window. Subjects refrained from eating and smoking for 8 hours prior to testing, which was performed between 9 a.m. and noon. The morning of the test the subjects voided on arising and recorded the time; subsequent urine was collected by us.

The pressor stimuli consisted of ischemic pain and the cold pressor test. These were applied to the left arm, except in experiment II in which an intravenous saline drip was attached to that arm; accordingly in this experiment the stimuli were applied to the legs. Ischemic pain was produced by placing a blood pressure cuff on the extremity and inflating it to a value at least 10 mm Hg in excess of the systolic blood pressure. When the hand was used, the subject then clenched and unclenched his fist at every beat of a metronome set to 40 beats/minute, for a period of 4 minutes. Leg exercise at the same rate consisted of dorsiflexion of the foot against an elastic band attached to the foot of the bed. Either procedure produces an intense aching and cramping sensation in the ischemic extremity. The cold pressor test consisted of immersing the extremity to the wrist or ankle in ice for 1 minute. Other details of procedure were specific to each of the three experiments and for the sake of clarity will be given in the appropriate section under "Results."

Control and experimental samples of blood and urine from each subject were analyzed by the following techniques:

Free fatty acids (FFA) were initially determined in duplicate by the method of Dole.\textsuperscript{14} About one third of the way through experiment I the Trout modification of the Dole method which involves washing with sulfuric acid to remove lactic acid\textsuperscript{15} was instituted and all plasmas in experiment I subsequently were run in duplicate by both methods. Although the values by the Trout modification averaged 240 \(\mu\)Eq/L less than those by the method of Dole, the differences between the pre-test and post-test samples were the same with both techniques. Consequently, since data with the Trout modification were available for only the last two thirds of that study, FFA data in experiment I are reported as the values obtained with the original Dole technique. In experiments II and III determinations were made exclusively by the Trout modification. Endogenous creatinine clearances were determined in all subjects except the first six of experiment I. Creatinine was measured by the Jaffe reaction.\textsuperscript{16} Vanilmandelic acid (VMA) was determined by the method of Sunderman and associates;\textsuperscript{17} when values were too low to read in dilute urine specimens, small amounts of VMA of known concentration were added.

In experiment II urinary free norepinephrine (NE) and epinephrine (E) were measured after being separated on columns of Amberlite CG-50 resin at pH 6.0. After elution, the trihydroxyindoles were formed with potassium ferricyanide and alkaline ascorbate, and simultaneously estimated by differential fluorometry in a Turner 110 fluorometer.\textsuperscript{18} Duplicates were done on all but a few of the samples, the latter usually control urines. The percentage of error of this method was 2.1% for NE and 2.6% for E. In experiment III, the catecholamines were separated from duplicate aliquots of urine on British Drug House...
alumina at pH 8.4; after acid elution they were developed in the same manner as in experiment II. Recovery of added catecholamine by the method used in experiment II was 50 to 75%; by the method used in experiment III, 80 to 90%.

Blood pressures were taken from the right arm by a Colson Model 110 automatic indirect recorder as described elsewhere.\(^3\!,\!^{19}\) Pulse rates were determined by telemetry of precordial electrical activity or monitoring of the ear capillary pulse by a photoelectric cell; each beat-to-beat interval was expressed as pulse rate/minute by a digital cardiotachometer (Gilford Model 120) and recorded linearly on a Texas Instrument Company Recorder. The recorder for the cardiotachometer was synchronized to run whenever blood pressure was determined. Since the latter cycle takes 20 to 30 seconds, a pulse rate record of this duration was made available with each blood pressure cycle, and the average of the highest and lowest points on this record, in beats per minute, was calculated. The base line for the blood pressure and the pulse rate response was calculated by averaging the last four determinations before each administration of the stimulus. The pressor and pulse rate responses were expressed as the difference between the base line and the maximum change with the stimulus. Blood pressure data were converted to a computer mean (diastolic + pulse pressure/3) in order to simplify subsequent calculations.

After each experimental period the subject was interviewed to determine his attitudes and feelings about the study situation in general and the stimuli in particular. In experiment I the subject also completed the Clyde Mood Scale,\(^20\) a self-administered adjective rating test, before and after each stimulus.

The data were expressed in the usual units. The urinary VMA in experiment I was expressed in \(\mu\)g/min but in experiments II and III both the VMA and the free catecholamines were corrected to weight per milligram of urinary creatinine. Techniques for statistical computation were primarily two part analyses of variance\(^21\) or paired difference comparisons, as indicated in more detail in the "Results."

Results

Experiment I

When the subject arrived at the laboratory, a timed ambulatory control urine was collected for analysis of VMA. The blood sample for determination of plasma free fatty acid was drawn, and the apparatus for blood pressure and pulse rate measurements was attached to the subject. The investigator then left the room and the subject rested for 10 to 15 minutes. During this time the subject and the blood pressure and pulse rate monitors were viewed through the one-way window. When cardiovascular base lines had been achieved, the investigator re-entered the room and applied the ischemic pain stimulus.

Blood pressure and pulse rate were determined after 1, 2.5, and 4 minutes, and the stimulus then was discontinued; the same stimulus was repeated three times with a 10 to 15-minute rest between trials. Five minutes following the fourth trial the blood pressure and pulse apparatus were detached, blood was drawn for FFA, and a final urine was collected. Subjects were then told to return 1 or 2 weeks later "to perform the same tests." At that time the identical procedure was repeated.

The results are shown in table 1 and figure 1. The blood pressure response was subjected to an analysis of variance for trials and for days; that is, the responses for the four trials of ischemic pain on each day were compared to each other and the responses on day 1 were compared with those on day 2. There were no significant differences between the trials on each day. Pulse rate increases with ischemic pain were analyzed in the same fashion and also showed no differences for either trials or days. The stimulus thus produced a constant cardiovascular response and even after the eighth stimulus, adaptation had not occurred.

The differences in the response of FFA on day 1 and day 2, on the other hand, were striking. The average pre-test FFA on day 1 was 661 \(\mu\)Eq/L; post-test it was 906 \(\mu\)Eq/L, a mean rise of 245 \(\mu\)Eq/L. Ten of the 11 subjects showed this rise; the eleventh had essentially no change. On day 2, the pre-test level was 737 while post-test it was 658 \(\mu\)Eq/L, an average decline of 72 \(\mu\)Eq/L. The difference in these responses was highly significant \((P = 0.001)\); moreover, since a decline occurred on day 2 in eight of the 11 subjects, while in the remaining three the rise was considerably less than on day 1, all 11 subjects actually had a lessened FFA response on day 2.
Figure 1

Comparisons of cardiovascular responses and biochemical changes following repeated trials with the ischemic pain stimulus (Experiment 1). MBP, mean blood pressure; PR in beats per minute, pulse rate.

The pre-test level of VMA excretion on day 1 was 2.10 μg/min; post-test it was 6.03 μg/min. On day 2, the levels were 2.34 and 5.23 μg/min, respectively. The average increase of 3.94 on day 1 was not significantly different than the lesser rise of 2.09 on day 2. However, it is noteworthy that eight of the 11 subjects had a decreased VMA response on day 2, with only three showing an increased response. Of further pertinence, the three subjects who did not show a decreased response had cardiovascular and FFA responses which were not out of line with those of the rest of the group.

Graphic plots of the pressor response and the pulse response against changes in VMA and FFA were prepared and examined for possible correlations between these parameters, but none were found (that is, they were
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemic pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Day 1)</td>
<td>89</td>
<td>122</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>140</td>
<td>139</td>
</tr>
<tr>
<td>Ischemic pain</td>
<td></td>
<td>169</td>
</tr>
<tr>
<td>(Day 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold pressor</td>
<td>132</td>
<td>126</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>122</td>
<td>134</td>
</tr>
<tr>
<td>Ischemic pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Day 2)</td>
<td>115</td>
<td>135</td>
</tr>
</tbody>
</table>

\*n = 4 only.  
P > 0.05 for all differences.

scattergrams with nonsignificant correlation coefficients). The average creatinine clearances in the four patients in whom these were determined are shown in table 2. There were no significant differences in the clearances for the control and experimental periods on either day.

The Clyde Mood Scale scores are depicted in figure 2. There seemed to be a slightly lower score for each item on the pre-test rating on day 2, but none of these differences were significant statistically. Similarly, the post-test scores on day 1 and day 2 were not significantly different. The poststimulus interviews on the other hand were revealing. Subjects seemed noticeably less tense or concerned about the procedure on day 2. Most voiced this directly, stating, "I was less anxious"; "I knew what to expect"; "I anticipated what would happen next," and so on. In a few, lessened anxiety was implied from comments and mannerisms. It was of interest that several recognized their lessened anxiety on day 2, only in relationship to their retrospective recollection of their feeling on day 1. As one subject put it, "I wasn’t anxious the first time, but today I’m really not anxious."

In summary, the striking finding in this first study was that a constant stimulus produced constant cardiovascular effects in the face of a decrement in at least one biochemical parameter presumably related to the sympathetic nervous system. Thus it appeared that a direct correlation between pressor responsivity and autonomic activity did not exist for this stimulus.

**Experiment II**

The next approach was a comparison of two different stimuli, namely ischemic pain and the cold pressor test. The latter stimulus is said to be mediated predominantly by the sympathetic nervous system in that it is generally diminished or abolished by sympatholytic drugs.22

Accordingly nine new volunteers were studied in the same manner as the first group except for the following additions or differences: The sequence of the two stimuli for each patient was randomized. As before, an ambulatory control urine was collected at the
time of each volunteer's arrival at the laboratory. After each stimulus the subject voided again, and the catecholamines in these urines were considered to represent excretion during the stimuli. An indwelling needle was inserted in the left antecubital vein and kept open by a slow infusion of normal saline. Plasma for FFA was first drawn at the time of venipuncture for insertion of the needle. Additional samples were drawn through the indwelling needle after the 15-minute control period prior to each stimulus. Samples were also taken immediately after and 5 minutes after the end of each stimulus. It should be noted that each stimulus was applied only once in this study, and that the poststimulus urine included that formed during the pre-stimulus control periods and, in the case of the first stimulus of the morning, the period of preparation. The entire study was repeated in 2 or 3 weeks utilizing the same sequence of stimuli as in the subject's first trial.

The data from all subjects are shown in table 3 and include the results of an analysis of variance for days and stimuli. The mean blood pressure responses to the cold pressor test were 16.1 mm Hg on the first day and 13.9 mm Hg on the second. For the ischemic pain the comparable responses were 27.1 and 25.0 mm Hg. There was no significant difference between days for either stimulus, indicating that in two trials neither stimulus evoked tachyphylaxis. However, for both days and on each day, the ischemic pain stimulus caused a significantly greater pressor response than cold immersion. Base-line blood pressures were not significantly different for either days or stimuli.

The mean pulse rate elevations were 11.7 beats per minute for cold and 18.7 beats per minute for ischemic pain on the first day. The comparable values for the second day were 10.2 and 20.9. Again the differences in response between days were not significant, indicating that each stimulus produced a constant effect. However, for the average of both days the ischemic pain response was greater than that to cold; t-tests for each day indicated that for day 1, \( P > 0.05 \), but on day 2, \( P = 0.05 \). Again, base-line values were not different for either days or stimuli.

In contrast, the levels of FFA when analyzed for days and periods showed no significant differences for periods, indicating that the stimuli had little or no effect. On the other hand, FFA levels were significantly lower on the second test day, although this was limited to the average for all periods on day 1 versus all periods on day 2 (that is, individual t-tests between days for each pair of periods gave values for \( P \) of \( > 0.05 \), although the F value for all periods, between days, was significant).

The FFA data also are shown in figure 3. The major rise on each day occurred prior to the stimuli, although, the highest absolute FFA levels were usually 5 minutes after stimulus. In order to examine the data at the point of maximum change, we chose as an additional index of response, the difference between the high point (the 5-minute poststimulus level) and the low point (the initial level), and compared these by a paired difference analysis for each separate day. By this technique, the change for the cold stimulus on the first day of 164 ± 61 and for the ischemic pain of 145 ± 49 were significant at \( P \) levels of 0.05 and 0.02, respectively. However, on the second day these changes were quite variable and did not present a significant elevation. On neither day, however, were the cold and ischemic pain responses significantly different from each other.

The norepinephrine excretions were variable, and although control and post-cold pressor levels on day 2 appeared higher than on

![Figure 3](Image)

*Curves of responses of plasma free fatty acids in nine healthy subjects in experiment II.*

*Circulation, Volume XXXIV, August 1966*
day 1, this failed to reach a 0.05 level of significance (F = 3.8; for 1/48 degree of freedom, \( P = 0.05 \) when \( F = 4.0 \)). There was no significant difference between controls and stimulus values, or between the two stimuli, on either day. For both epinephrine and VMA, although there was no difference between days, the excretion levels during both stimuli seemed higher than control levels; however, this reached a significant level only for the VMA. When individual t-tests were done, they indicated that the VMA excretion with ischemic pain was higher than the control rate but that during the cold pressor test it was not, although the excretions during each stimulus were not significantly different from each other.

In summary, the only statistically significant change in the catecholamine excretion rates was a greater elaboration of VMA during ischemic pain than during control period. Other excretion rates were not significantly different. Especially noteworthy were (1) the absence of differences between stimuli, although the cardiovascular responses were consistently greater for ischemic pain than for cold pressor, and (2) the lack of difference between days, although, as in experiment I, FFA levels were higher on day 1.*

*The questionable difference in days for NE excretion was in the opposite direction to the FFA decline.
Again in order to minimize the variance and examine the data for its maximum potential for demonstrating change in excretion with the two stimuli, the excretions of both NE and E also were analyzed for each day by the paired differences between the two stimuli. On day 1 the average difference in epinephrine excretion of 0.66 ng/mg creatinine less for ischemic pain was not significant (P > 0.05). On day 2, however, the greater excretion of 1.60 ng/mg creatinine of epinephrine during ischemic pain was significant (P = 0.01). By contrast, on day 1 norepinephrine excretion with ischemic pain was 4.90 ng/mg creatinine greater (P = 0.05), but on day 2 it actually was 4.05 ng/mg creatinine less than that evoked by cold, although this latter difference did not reach a significant level. Thus, the most that the paired difference analysis could demonstrate was that epinephrine excretion was significantly greater during ischemic pain than during cold on the second day but not on the first, whereas ischemic pain evoked higher excretion rates for norepinephrine on the first day but not on the second. Even with this interpretation of the data, the excretion of the sympathetic neurohums had no consistent relationship to pressor responses, which were greater for ischemic pain on both days.

The creatinine clearances were normal for all periods and showed no significant changes (Table 2). The interviews revealed the expected comments concerning lessened anxiety on the second day. The consensus indicated the ischemic pain to be the more unpleasant stimulus.
In several individual subjects cardiovascular responses occurred with minimal norepinephrine excretion. Subject M is of particular interest. Although his pressure and pulse responses to cold were virtually absent and associated with low NE excretion, a marked rise in blood pressure and tachycardia occurred with ischemic pain on both days, with complete absence of NE output. Patient M had a strong family history of diabetes, although not clinically diabetic himself, which is reminiscent of our previous observations of impaired cold pressor responsiveness accompanied by exaggerated humoral reactivity in diabetic subjects without manifest diabetic neuropathy. Although one may question whether this clinically healthy man was a proper “normal subject,” his functional extremism elaborates the point that ischemic pain can evoke a pressor response in the absence of apparent norepinephrine excretion.

Experiment III

Since both stimuli were applied on a single morning in experiments I and II, it can be argued that significant between-stimulus differences in urinary excretion products could be confounded by overlapping. Furthermore, an ambulatory control period may have falsely elevated catecholamines so that stimulus-induced rises in resting catecholamine excretion during recumbency would be judged against an inappropriate and uneven base line. Accordingly, to elaborate differences produced by change and repetition of stimuli, the experiment was repeated in the following manner: Ten new volunteers came to the laboratory, voided, and rested for 1 hour in bed. At the end of this rest period, a timed control urine was collected for catecholamines, VMA, and creatinine, and a blood sample was drawn for creatinine and FFA determinations. The subject was then interviewed and asked to return for a second test “with a different stimulus.” Two or three weeks later the test was repeated with the same protocol, but using the alternate stimulus. Five subjects had the ischemic pain stimulus first and five the cold pressor first.

The first and second blood pressure and pulse responses on any day were not significantly different and were therefore averaged. The complete data are shown in table 4, and the responses alone in figure 4; these indicate

![Figure 4](image-url)

*Comparisons of cardiovascular responses and biochemical changes in 10 healthy subjects following two different stimuli on two different test days (experiment III). Comparison of the two stimuli are represented by the data on the left of the figure; comparison of the order of presentation of stimuli by the data on the right. ( * points out significant differences between the indicated pair of values.)

Circulation, Volume XXXIV, August 1966
### Table 4

#### Experiment III

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean blood pressure (mm Hg)</th>
<th>Pulse rate (beats/min)</th>
<th>Norepinephrine (ng/mg creat)</th>
<th>Epinephrine (ng/mg creat)</th>
<th>VMA (μg/mg creat)</th>
<th>FFA (μEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>R</td>
<td>BL</td>
<td>R</td>
<td>Control</td>
<td>XPTL</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S*</td>
<td>81</td>
<td>28.2</td>
<td>62</td>
<td>33.5</td>
<td>3.82</td>
<td>1.09</td>
</tr>
<tr>
<td>G</td>
<td>84</td>
<td>6.4</td>
<td>52</td>
<td>3.5</td>
<td>12.05</td>
<td>1.49</td>
</tr>
<tr>
<td>H*</td>
<td>82</td>
<td>17.6</td>
<td>65</td>
<td>18.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>He</td>
<td>90</td>
<td>30.8</td>
<td>54</td>
<td>16.0</td>
<td>0.27</td>
<td>0.00</td>
</tr>
<tr>
<td>F</td>
<td>98</td>
<td>36.5</td>
<td>42</td>
<td>8.5</td>
<td>1.95</td>
<td>2.53</td>
</tr>
<tr>
<td>B*</td>
<td>90</td>
<td>36.2</td>
<td>66</td>
<td>11.5</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Bo</td>
<td>97</td>
<td>15.4</td>
<td>64</td>
<td>3.5</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>K*</td>
<td>98</td>
<td>29.8</td>
<td>46</td>
<td>17.0</td>
<td>0.54</td>
<td>0.70</td>
</tr>
<tr>
<td>M*</td>
<td>97</td>
<td>34.0</td>
<td>70</td>
<td>33.5</td>
<td>9.32</td>
<td>14.64</td>
</tr>
<tr>
<td>N</td>
<td>86</td>
<td>11.0</td>
<td>72</td>
<td>4.0</td>
<td>0.00</td>
<td>2.78</td>
</tr>
<tr>
<td>Mean</td>
<td>90.3</td>
<td>24.6</td>
<td>59.3</td>
<td>14.9</td>
<td>2.80</td>
<td>2.32</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>71</td>
<td>14.8</td>
<td>59</td>
<td>8.0</td>
<td>3.91</td>
<td>4.00</td>
</tr>
<tr>
<td>G*</td>
<td>90</td>
<td>42.0</td>
<td>47</td>
<td>23.5</td>
<td>3.03</td>
<td>0.41</td>
</tr>
<tr>
<td>H</td>
<td>83</td>
<td>30.0</td>
<td>66</td>
<td>11.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>He</td>
<td>87</td>
<td>27.3</td>
<td>62</td>
<td>25.2</td>
<td>0.00</td>
<td>1.29</td>
</tr>
<tr>
<td>B*</td>
<td>91</td>
<td>51.0</td>
<td>46</td>
<td>13.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>B</td>
<td>82</td>
<td>23.2</td>
<td>68</td>
<td>4.0</td>
<td>5.26</td>
<td>14.03</td>
</tr>
<tr>
<td>Bo</td>
<td>86</td>
<td>38.7</td>
<td>56</td>
<td>33.5</td>
<td>7.86</td>
<td>11.79</td>
</tr>
<tr>
<td>K</td>
<td>90</td>
<td>15.2</td>
<td>55</td>
<td>2.0</td>
<td>1.85</td>
<td>0.22</td>
</tr>
<tr>
<td>M</td>
<td>95</td>
<td>8.5</td>
<td>65</td>
<td>13.5</td>
<td>7.01</td>
<td>5.61</td>
</tr>
<tr>
<td>N*</td>
<td>88</td>
<td>14.5</td>
<td>71</td>
<td>13.0</td>
<td>2.16</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>86.3</td>
<td>26.5</td>
<td>59.5</td>
<td>14.7</td>
<td>3.11</td>
<td>3.74</td>
</tr>
<tr>
<td>Ischemic pain</td>
<td>89.0</td>
<td>32.0</td>
<td>59.1</td>
<td>22.2</td>
<td>2.67</td>
<td>2.99</td>
</tr>
<tr>
<td>F values of response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By test day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1.0</td>
<td></td>
<td>&lt; 1.0</td>
<td></td>
<td>&lt; 1.0</td>
<td></td>
<td>14.3‡</td>
</tr>
<tr>
<td>By stimulus</td>
<td>7.4‡</td>
<td>25.2‡</td>
<td>&lt; 1.0</td>
<td></td>
<td>&lt; 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BL = base line; R = response; XPTL = experimental; creat = creatinine.
*Ischemic pain.
†P = 0.05
‡P < 0.01
that elevations in all parameters, with the exception of NE, generally occurred with both stimuli. Since we utilized a “crossover design”29 in this experiment, we were able to analyze for both differences between stimuli and between test days, that is, the effect of order of presentation. The responses per se were used in this analysis.

The mean blood pressure response to ischemic pain averaged 32.0 mm Hg and to cold pressor, 19.2 mm Hg; these are significantly different. There was no difference in the average pressor response between test days. Similarly the pulse rise was 22.2 beats/minute to pain and only 7.4 beats/minute to cold; these are significantly different. Again there was no difference between days. The free fatty acid rise with ischemic pain was 320 µEq/L and to the cold immersion only 175 µEq/L. Although suggestively higher for the ischemic pain, the difference fails to reach a significant level (P = 0.10). For the between-days effect, however, the greater response on the second test day is probably significant (F = 5.3 which is just at the 0.05 level for 1 and 8 degrees of freedom). Thus, in contrast to the previous two experiments in which the same stimuli were given on both test days, in this experiment, when a “new” stimulus was given on the second day, the FFA response was not dampened, and in fact seemed to increase.

The catecholamine analyses revealed no significant differences for NE excretion for either test days or stimuli. The epinephrine responses were not different between the stimuli; however, they were significantly greater on the first test day than on the second, although the cardiovascular responses were not and the change in FFA response was in the opposite direction. The VMA data were incomplete but the trend seemed to mirror that of the epinephrine response. As in the earlier experiments the creatinine clearances were not significantly different for any period (table 2).

**Discussion**

The striking findings in the three experiments taken as a whole, were the consistency of the cardiovascular responses in relationship to a given stimulus regardless of its order of presentation or its repetition, and the consistent lack of relationship of these cardiovascular responses to the biochemical correlates of adrenergic arousal. Specifically, in experiment I, the cardiovascular responses to ischemic pain were consistent on a given day and between trials, but FFA rose on one day and fell on the second; while VMA excretion showed a less impressive rise with the second exposure. In experiment II, two different stimuli were shown to cause different cardiovascular responses, yet FFA elaboration did not differ, and there were no consistent parallel changes in catecholamine excretion. Again, FFA mobilization was less with repetition of the stimuli on a second trial although the cardiovascular responses were unchanged. In experiment III, the cardiovascular responses to two different stimuli were again different, but still unaccompanied by significant differences in catecholamine excretion. On the other hand, with the alternate stimulus now introduced as a “new stimulus” on the second trial, FFA response did not fall, despite the seemingly discrepant observation that epinephrine excretion declined.

A number of implications of the findings deserve discussion. To consider them, appropriately, however, certain problems concerning methodology in these and similar studies require exposition. First of all, caution should be exercised in judging the accuracy or specificity or both of the various biochemical measures of autonomic activity which were used; it would be more appropriate to call them estimates. Without exception they are modified by nonautonomic factors, such as the higher centers of the nervous system and the circulating levels of the various noncatecholamine hormonal substances. For instance, plasma free fatty acids are a family of blood lipids which are released from adipose tissue in response to adrenergic arousal.24 Since their average half-life is less than 5 minutes,25 they can be used as a convenient moment to moment index of the state of the autonomic nervous system assuming all other factors to be constant. This latter point bears emphasis.
PRESSOR RESPONSIVITY

since many naturally occurring humoral substances have been shown capable of altering plasma levels of FFA\textsuperscript{26}; in fact some or all of these hormones may be responsible for recent observations that the same dose of a catecholamine may produce different degrees of lipolysis in animals exposed to several types of noxious stimuli.\textsuperscript{27, 28}

Secondly, although VMA is an end metabolite of both norepinephrine and epinephrine, it is several metabolic steps away and hence is a somewhat sluggish and indirect measure. Even the urinary catecholamines are somewhat indirect, in the sense that as measured in urine they reflect only a small amount of that which has been elaborated. After release from its storage site a norepinephrine molecule must escape the action of two different enzyme systems concerned with its destruction. It then must avoid hepatic conjugation with either sulfate or glucuronide, in order to reach the kidney where it also probably must escape tubular reabsorption in order to be excreted. Even after chemical detection, we do not know whether such a molecule of free norepinephrine originated in the adrenal medulla, the heart, a vasoconstrictor receptor site, or a sympathetic nerve fiber ending. Only the last of these qualifications does not apply to epinephrine, the other free catecholamine measured in this study, but since epinephrine stimulates both alpha (vasoconstrictor) and beta (vasodilator) receptors, its physiological origin is equally obscure. Superimposed on these considerations is an inherent fault in the experimental design; namely, urine collected after each stimulus contained a mixture of presumably high catecholamine urine from the relatively brief stimulus period and presumably lower catecholamine urine from the prior base-line period. These difficulties, which have been recently summarized by von Euler\textsuperscript{29} as well as methodological problems of the catecholamine measurements per se and the wide variation from patient to patient, may have made it infeasible to distinguish differences in excretion rates between different physiological responses. Thus, differences in blood pressure and pulse responses to the two stimuli may have been more capable of recognition by the physiological measurements employed than were their adrenergic biochemical correlates. Some of these latter problems would be at least partially alleviated by a reproducible technique for determining plasma catecholamines on small samples, a methodological problem which still has not been effectively solved. However, with the urinary techniques used, whereas calculations of averages might mask differences, correlations or trends should have been present and these too were not readily apparent.

Returning then first to experiment I, with the above limitations in mind, it appears that despite a decrease in "autonomic arousal" on day 2 as compared with day 1 by biochemical parameters, cardiovascular responses were unchanged. Whatever adaptation occurred was seen in the biochemical correlates but not in the physiological responses.* There was a dissociation between the magnitude in change in blood pressure, pulse rate, lipolytic activity,

---

\*At the suggestion and with the guidance of Dr. Lincoln Gerende, Assistant Professor of Biostatistics in the Department of Social and Preventive Medicine, an additional and considerably more complex analysis of variance of the blood pressure and pulse data in experiment I was also performed. This analysis takes into account the variability of response between individuals by the fact that the same subject was tested on both days. Accordingly, the residual error term is reduced by removing it sources of variability due to individuals and to interactions between individual and periods and individuals and days. From this analysis, emerges the curious observation that the pressor response is significantly slightly greater on day 2 than on day 1 ($P = 0.05$), while the slightly lower pulse response on day 2 is also statistically significant ($P = 0.01$).

Insofar as this analysis is applicable in the present situation, it would expand the dissociation referred to above by further indicating a dissociation of the cardiovascular response itself. Thus, a lower pulse rate response and a higher pressor response on day 2 would suggest that while the chronotropic response of the heart correlates with the adrenergic predictors, the pressor response operates independently. Perhaps indeed a higher cardiac output on day 2, with no decrease in peripheral resistance causes a greater pressor response. This is an attractive possibility in keeping with other suggestions from our data, but one which requires much further study.
and of the excretion of end-products of catecholamine hormones in response to ischemic pain, as reflected in the average results. Moreover, correlations were lacking between these various measurements among the individual subjects.

Explanation for these phenomena can at best be only speculative, but the most attractive conjecture to us is that the pressor response to ischemic pain is mediated at least in part by a pathway other than the autonomic nervous system. It is known for instance that ischemic pain results in elaboration of vasopressin, a powerful vasoconstrictor. It is possible that other humoral vasopressors, such as angiotensin II are elaborated in the kidney or elsewhere,* or that vasoactive polypeptide materials are produced locally in the area of ischemia. The latter source is unlikely since the ischemic area was cut off from the circulation by the occluding cuff during the period of response, while the lack of change in GFR (that is, creatinine clearance) mediates against the possibility of angiotensin elaboration from the kidney. It is worth noting that Bogdonoff and associates have shown that vasoactive polypeptides, for example, angiotensin and vasopressin, which sharply raise blood pressure, have no effect on levels of FFA, while Mirsky and Goldman have demonstrated actual lowering of plasma FFA with vasopressin and several of its analogues. At any rate, if the cardiovascular responses, and particularly the pressor response, result from stimulation of some other pathway than the autonomic nervous system (ANS), then the changes in FFA and perhaps VMA might be considered as measurements of the ANS response to the anxiety surrounding the tests and the pain experienced during them, rather than of the hemodynamic response. Likewise, the hemodynamic response to ischemic pain would have to be considered as independent of the level of anxiety of the subject. Gunnells and associates have shown the corollary, namely that reduction of cerebral activity by meprobamate does not alter the hemodynamic response to the stimulus of exogenous epinephrine. This hypothesis of a pressor response which is "autonomically independent" is also in keeping with the observations recently reported by Harris and associates who have demonstrated after production of beta-adrenergic blockade that hypnotic suggestion of fear and rage produces pressor responses of similar magnitude to those elicited before blockade, although other parameters of the autonomic reaction changed.

It is also possible that catecholamine products which have vasoconstrictor function but no lipolytic effect are elaborated by ANS arousal but this is unlikely. There is considerable evidence that catecholamines must have certain molecular configurations in order for them to be lipolytic. However the vasoconstrictor amines, such as phenylephrine, metaraminol, and methoxamine, which affect FFA are synthetic and not biological compounds, while degradation products such as normetanephrine and VMA, which fail to affect levels of FFA, are not vasoconstrictors at physiological levels.

A third explanation for the lack of correlation of biochemical and physiological measurements is purely a quantitative one. Adrenergic arousal sufficient to cause a rise in levels of FFA for instance may be in excess of that necessary to cause a hemodynamic response, as suggested in studies in dogs by Froberg and Oro. Hence on the second exposure, a lesser adrenergic arousal occasioned by lessened anxiety concerning the test may have reflected itself in the failure to elevate the level of FFA, but was still sufficient as not to diminish the pressor and pulse rise.

A fourth explanation for the dissociation may be in the differing proportions of NE and E elaborated and the resultant differences in which areas of the vascular bed are constricted or dilated and to what extent the heart is stimulated. Brod has pointed out that one cannot consider hemodynamic responses in terms of uniform changes in peripheral resistance and cardiac output, but rather that among

---

*It is of interest for instance, that a renin-like material has recently been isolated in high titer from the salivary gland of mice.
Different vascular beds responding simultaneously, some may dilate and others may constrict. Moreover, a cardiac output rise without corresponding fall in peripheral resistance might produce the same pressor result as a peripheral resistance rise with no change in output. The pressor response in effect represents a final expression of these different circulatory adjustments. Thus, the identical pressor changes which we noted with repetition may still reflect a situation in which entirely different hemodynamic adjustments have occurred. With different adjustments taking place, the biochemical parameters of ANS arousal might have changed although the end cardiovascular responses were the same.

In experiment II the dissociation between pressor responses and biochemical parameters was emphasized by comparing the ischemic pain to the cold pressor stimulus. The pressor responses from the two stimuli differed and yet the biochemical changes did not. This study and experiment III further indicated the importance of the experimental setting. For instance, in experiment II the setting and the stimuli were held constant for both trials, yet, as judged by the levels of FFA, the baseline degree of arousal upon which stimulus responses were superimposed was lower on the second trial than on the first. This decrease in arousal with duplicate setting and stimuli we interpret as a further example of the relief of tension in the subject by awareness of what was to follow. The novelty or "newness" effect was no longer present as indicated in the subjects’ comments. In experiment III the setting was held constant but a new stimulus was given on the repeat trial; now the FFA rise was not lost, but in fact was slightly higher, whereas the different stimuli had not themselves produced a significant difference in this response. Taken together the results in the three experiments suggest that the novelty of the stimulus may be a more important determinant of adrenergic activation than the nature of the stimulus per se or the response which it elicits. Similar results of a new experience on FFA levels have been demonstrated in medical students interviewing psychiatric patients. The interrelationships of other hormones with catecholamines in elaboration of FFA offer a tempting suggestion to explain the foregoing data. ACTH or cortical steroids or both for instance appear to be necessary for NE and E to mobilize FFA at optimum rates. One can postulate that in the presence of a new experience when there is a greater degree of anxiety and an increase in elaboration of steroids, FFA is released to a greater extent by the same quantity of norepinephrine and epinephrine (for example, on day 1, in experiment I), even though the hemodynamic response is unaffected.

Although the studies to date seem to raise problems rather than offer solutions, they do make it clear that assumptions about sympathetic activation from the hemodynamic responses of blood pressure and pulse rate alone are apt to be misleading. These physiological responses are "homeostatic end results," and their component parts, including the ANS, are confounded. Those theories and experiments which attempt to interpret acute and chronic psychosomatic disorders only in terms of the meaning of the stimulus, need to take this into account.

As a final observation, it is of interest that the simple stimulus of ischemic pain produces a consistent pressor response which seems to be predictable when repeated in the individual subject. In view of the variability of hemodynamic response to other stimuli which have been used in appraising blood pressure reactivity, this operationally simple, significantly pressor, and long-recognized procedure is probably worthy of further consideration for use in epidemiological studies. In this respect, family history data from the 21 subjects from experiments I and III who received ischemic pain to the arm were available which permitted their division into six who had a history of hypertension in a first degree relative (father, mother, or sibling), and 15 who did not. The average pressor responses and standard errors to their first exposure to ischemic pain were 35.9 ± 3.9 mm Hg in those with a family history of hypertension, and 26.4 ± 1.82 mm Hg
in those without such a history, a difference significant at the \( P = 0.02 \) level.

**Summary**

A study was designed to examine the adrenergic contribution to cardiovascular responsiveness to acute stimuli, with particular attention to the pressor component of this response. Data indicated a dissociation between pressor responses, catecholamine excretion, and FFA elaboration which can be summarized as follows:

1. The same stimulus (ischemic pain) given on different days, produced the same pressor responses but with an apparent decrease in adrenergic activation.

2. Different stimuli on the same day (ischemic pain and cold pressor) gave pressor responses of different magnitude, but adrenergic activation was not consistently greater with the greater pressor response.

3. Different stimuli given on different days maintained a consistent difference in pressor responses. However, adrenergic activation may have correlated with the order of presentation, rather than the nature of the stimulus or the response.

4. Among other implications from this discordancy between cardiovascular reactivity and adrenergic activation are the following: (a) Factors other than the autonomic nervous system (ANS) may be important in the mediation of pressor response, particularly to such stimuli as ischemic pain, and (b) the ANS response itself may more closely correlate with emotional factors concomitant to the stimulus, than to the stimulus itself. Finally, the results point up the inappropriateness of using cardiovascular responsiveness as a precise indicator of activation of the ANS in physiological and psychophysiological studies.

**Acknowledgment**

The assistance of Eileen Tyrrell, predoctoral trainee in Clinical Pharmacology (National Heart Institute Training Grant T1-HE 5467) in the statistical analyses is gratefully acknowledged.

**References**


*Circulation, Volume XXXIV, August 1966*
PRESSOR RESPONSIVITY


Studies in Man on the Relationship of Adrenergic Correlates to Pressor Responsivity

JOSEPH D. SAPIRA, ALVIN P. SHAPIRO, Thelma Klaniecki, Jean E. Yevick and Jean L. Small

Circulation. 1966;34:226-241
doi: 10.1161/01.CIR.34.2.226
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1966 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/34/2/226

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/