The effect of a 3-h Sidman avoidance operant conditioning schedule (lever pressing to avoid an electric shock) on plasma renin activity and renin substrate concentration was examined in baboons (Papio cynocephalus). Plasma samples were drawn over a 24-h period on both the control and test days, and the avoidance session was presented on the morning of the test day. Plasma renin activity was significantly higher on the test day than at the corresponding hours of the control day at 1, 2, and 3 h after onset of the avoidance test and 30 min after its termination ($P = .032$). The magnitude of the increase in plasma renin activity was not correlated with either the rate of lever pressing or the number of shocks received. Renin substrate concentration was not changed during or after the avoidance session. These data demonstrate that plasma renin activity can be increased by a psychological stimulus.

METHODS

Animal preparation. Six adolescent male baboons (Papio cynocephalus), weighing 9.7–12.6 kg, were adapted to primate restraining chairs for at least 3 wk. A stainless steel electrode was then placed under each animal's abdominal skin by aseptic surgery. After a 4-day period of adaptation to the sound-shielded booth in which all experiments and training were carried out, training on a 20-s Sidman avoidance schedule was begun. During the Sidman avoidance situation, the baboon was required to press a lever in order to avoid an electrical shock to the tail. Each lever press (or shock delivered) postponed that next scheduled shock for 20 s. The discriminative stimulus for the avoidance situation was a 4 x 6 inch red light, which was mounted in front of the animal's tail, and each shock consisted of a 250-ms, 60-Hz train of 4.0–7.5 mA intensity.

Each animal was trained by standard behavior-shaping techniques, under observation by a closed-circuit television system. Onset of the red warning light was first paired with shock delivery; on two occasions during the initial training session, six shocks were presented at a rate of once every 20 s while the red light was on. The next step in training was to allow the animal access to a
leaver which it could press in order to turn off the red warning light and avoid receiving the shock. Gradually
the animal was required to continue pressing the leaver for as long as the red light remained on. Training was carried
out 1-2 h daily for 1-3 wk and was considered complete when the baboon received no more than 6
shocks/h during a 3-h test session on a 20-s avoidance schedule. Because of the potential contaminating effects
of heavy exercise on renin secretion, all animals were also trained to sit quietly during the avoidance situation.
This training was integrated into the shaping procedure after the baboon had passed the initial stage of learning to use the lever; extraneous physical activity was discouraged by delivering a shock when the baboon exhibited vigorous body movements not related to lever pressing while the red light was on. Physical activity was monitored with an accelerometer mounted on the back of the restraint chair and by television observation. Behavioral data (lever-pressing rate, shocks received, and accelerometer signal) were recorded on an oscillograph.

Each baboon was individually housed in a sound-shielded booth throughout the experimental period and for at least 6 days prior to each experiment. A rigid schedule of care and feeding was observed. Booth lights were automatically turned on at 0700 and off again at 2100; feeding, cleaning, and other animal maintenance tasks took place between 1600 and 1700 h. In order to control dietary factors, especially sodium intake, each animal was fed a daily ration of either 150 or 200 g Purina Monkey Chow 25 plus one apple (about 23 or 31 meq sodium, respectively). The amount that each animal was fed was slightly less than that animal's previously determined ad libitum food intake; this assured that all food was consumed within several hours. Water was available at all times. Laboratory sounds were masked by continuous input of 65-70 db white noise. The baboons were maintained in double-tiered primate restraint chairs, which were adjusted to provide the minimum physical restraint necessary to insure protection of the femoral venous cannula.

At the completion of training a chronically indwelling Silastic cannula (0.040 inch ID x 0.085 inch OD) was surgically implanted in one femoral vein. All surgery was carried out under aseptic conditions, using halothane anesthesia after pretreatment with 10 mg/kg ketamine plus 0.2 mg atropine. The animal was permitted a recovery period of 1 wk or more in the animal colony room before the experiment was begun. The cannula was kept patent by constant infusion of sterile isotonic dextrose in water, containing 10 U heparin per milliliter. The infusion totaled 40 ml/day and was infused by means of a Holter model 903 roller pump. An isotonic dextrose, rather than saline, infusion was used because of the renin-lowering effect of sodium. During the experiment, blood samples were drawn through the cannula from outside the booth without opening the booth door or otherwise disturbing the animal. The volume withdrawn (1.5-2.0 ml) was immediately replaced with an equal volume of sterile isotonic dextrose in water.

Experimental design. A paired experimental design was used, in which each animal served as its own con-

control. Control and test days were paired within the same week and were identical in all respects except that the Sidman avoidance session was presented between 0930 and 1230 h on the morning of the test day. Two replications were run on each animal, with at least 6 days elapsed between each replication. Blood samples were drawn at 0630, 0930, 0945, 1030, 1130, 1230, 1300, 1430, 1600, 2030, and 0130 h and at 0630 h the next morning on both control and test days. For statistical purposes, plasma renin activity and renin substrate concentration values were expressed as the percentage of the averaged values of the first two samples (0630 and 0930 h) of the day for that replication. After conversion to percentages, replicated sample time values for each animal were averaged and subsequently treated as one data point. Nonparametric statistical analyses were used, as described by Siegel (21).

Assay method. Plasma renin activity was determined by radioimmunoassay of angiotensin I with minor modifications of the methods of Haber et al. (9) and Goodfriend et al. (7), as described by Zehr and Feigl (25). By this method the prepared plasma samples were incubated at 37°C at a pII of 7.4. Aliquots of the same plasma sample which were incubated and assayed on seven different occasions gave a coefficient of variation of 12% (0.84 ± 0.04 SEM ng angiotensin I per ml plasma per h). The radioimmunoassay was conducted in triplicate for each sample, and all samples collected on a given pair of test and control days were both incubated and assayed together.

Each plasma sample was also assayed for renin substrate concentration by incubating the plasma with excess baboon renin in the presence of converting enzyme and angiotensinase blockers until all substrate had been converted to angiotensin I. To prepare the samples for renin substrate measurement, 50 μl plasma were added to 0.125 ml 0.10 M phosphate buffer (pH 7.4, containing 0.01% neomycin sulfate and 0.001% tetracycline HCl), 2.5 μl 10% EDTA, 2.5 μl 0.340 M 8-OH-quinoline sulfate, 1 μl 0.906 M dimercaprol, and 20 μl renin solution. The renin solution was extracted from baboon kidneys by the method of Ila and Goldblatt (8). The prepared plasma was then maintained at 37°C for 1 h. The radioimmunoassay of angiotensin I was carried out as for the plasma renin activity measurement, and all samples from a given replication were both incubated and assayed together. By this method, aliquots of the same plasma sample which were assayed for renin substrate on eight separate occasions gave a coefficient of variation of 14% (650 + 34SEM ng angiotensin I per ml plasma).

RESULTS

Figure 1 displays the mean and standard errors computed for the plasma renin activity values, expressed as percentages, for each of the 12 sample times on control and test days. Renin activity values were found to be significantly higher at 1, 2, and 3 h after onset of the Sidman avoidance test and 30 min after

*1 The angiotensin I antibody was generously supplied by Drs. T. Goodfriend and D. Ball, University of Wisconsin.
termination of the test than at the corresponding times of the control day ($P = .032$, two-tailed sign test, $n = 6$). The control day values for these times were, respectively, 104% ($\pm 8\%$ SEM), 111% ($\pm 8\%$ SEM), 114% ($\pm 10\%$ SEM), and 121% ($\pm 11\%$ SEM); the corresponding test day values were 191% ($\pm 37\%$ SEM), 205% ($\pm 38\%$ SEM), 206% ($\pm 39\%$ SEM), and 171% ($\pm 22\%$ SEM). There were no statistically significant differences between control and test day plasma renin activity values at any other plasma sampling time.

Control and test day renin substrate values were not significantly different ($P > .20$, two-tailed sign test, $n = 6$) at any of the plasma sampling times. Figure 1 shows the means and standard errors of the renin substrate concentrations, expressed as percentages, for each of the 12 sample times on both control and test days. The means and standard errors of the renin substrate concentrations in nanograms angiotensin I formed per milliliter plasma are displayed in Table 1.

The plasma renin activity levels achieved during the avoidance session were not correlated with either shock delivery or lever-pressing rate. The number of shocks delivered during the avoidance session ranged from 4 to 24 and averaged 8.8 ($\pm 2.3$ SEM). The Spearman rank correlation coefficient was $r_s = +0.09$ for the total number of shocks delivered during each avoidance session versus the average of the plasma renin activity values (expressed as percent of control) at 1, 2, and 3 h after onset of the avoidance situation. The mean rate of lever-pressing during the 3-h avoidance session was 36.8/min ($\pm 10\%$ SEM). The Spearman rank correlation coefficient between total lever presses and the plasma renin activity values (averaged as above) for each avoidance session was $r_s = -0.48$, which is not statistically significant.

Plasma renin activity was higher during the first Sidman avoidance test (average of percentage values at 1, 2, and 3 h after onset of the avoidance situation) than during the second replication for five of the six animals; this difference is not, however, statistically significant ($P > .20$, two-tailed sign test). There was no consistent pattern of differences between the first and second replications in the number of shocks delivered or in lever-pressing rates.

**DISCUSSION**

When the baboons in this study were presented with a Sidman avoidance situation, plasma renin activity rose to twice the control day values within 1 h and remained at this level for the remainder of the 3-h test session. Plasma renin activity was still significantly elevated 30 min after termination of the avoidance session (Fig. 1). Blood samples were drawn at intervals during the following 18 h, as well as at corresponding times during the control day in order to test the possibility that the avoidance condition might have prolonged effects on the renin-angiotensin system. This has been observed for urinary 17-hydroxycorticosteroids and noradrenaline following 72 h of avoidance behavior in rhesus monkeys (15). However, prolonged effects of the 3-h avoidance session were not observed; plasma renin activity did not differ statistically between control and test days at 2 h after the avoidance session or at any later plasma sampling time (1600, 2030, 0130, or 0630 h) (Fig. 1).

Plasma renin activity is a measure of the rate of in vitro formation of angiotensin from endogenous renin substrate and reflects the plasma concentration of renin substrate as well as of renin. The plasma concentration of human endogenous renin substrate is lower than that required for a maximal rate of angiotensin formation (17); therefore, changes in renin substrate concentration can alter plasma renin activity values even when renin concentration stays the same. The baboon plasma renin substrate concentration values reported in this study (Table 1) are slightly lower than those reported for human plasma (19). The formation of renin substrate is increased by angiotensin (11) and by glucocorticoids (18). Although both circulating glucocorticoid (15) and

**Table 1. Plasma renin activity and renin substrate concentration on control and Sidman avoidance test days**

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma Renin Activity, ng ang I/ml per h</th>
<th>Renin Substrate Concentration, ng ang I/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SEM</td>
<td>Mean SEM</td>
</tr>
<tr>
<td>0630</td>
<td>1.41 $\pm$ 0.36</td>
<td>1.52 $\pm$ 0.36</td>
</tr>
<tr>
<td>0930</td>
<td>1.58 $\pm$ 0.32</td>
<td>1.84 $\pm$ 0.35</td>
</tr>
<tr>
<td>0945</td>
<td>1.48 $\pm$ 0.31</td>
<td>2.37 $\pm$ 0.46</td>
</tr>
<tr>
<td>1030</td>
<td>1.47 $\pm$ 0.35</td>
<td>3.24 $\pm$ 0.83</td>
</tr>
<tr>
<td>1130</td>
<td>1.39 $\pm$ 0.36</td>
<td>3.31 $\pm$ 0.82</td>
</tr>
<tr>
<td>1230</td>
<td>1.60 $\pm$ 0.37</td>
<td>3.46 $\pm$ 0.92</td>
</tr>
<tr>
<td>1300</td>
<td>1.76 $\pm$ 0.50</td>
<td>2.91 $\pm$ 0.67</td>
</tr>
<tr>
<td>1430</td>
<td>2.06 $\pm$ 0.71</td>
<td>2.51 $\pm$ 0.93</td>
</tr>
<tr>
<td>1600</td>
<td>2.06 $\pm$ 0.62</td>
<td>2.72 $\pm$ 0.90</td>
</tr>
<tr>
<td>2030</td>
<td>1.49 $\pm$ 0.41</td>
<td>1.59 $\pm$ 0.43</td>
</tr>
<tr>
<td>0130</td>
<td>1.64 $\pm$ 0.37</td>
<td>1.68 $\pm$ 0.37</td>
</tr>
<tr>
<td>0630</td>
<td>1.45 $\pm$ 0.35</td>
<td>1.72 $\pm$ 0.34</td>
</tr>
</tbody>
</table>

Each mean and SEM is for 6 animals, with 2 replications per animal. The Sidman avoidance session occurred between 0900 and 1200 h on the morning of the test day only. *P = .032, two-tailed sign test.*

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**Fig. 1.** Plasma renin activity and renin substrate concentration on control and Sidman avoidance test days. Solid line denotes control day and broken line denotes avoidance test day. Values are expressed in percentage, with 100% equal to average of first 2 samples of day for each replication. Vertical lines indicate $\pm 1$ SEM. Each mean and SEM is for 6 animals, with 2 replications per animal. Stars indicate a significant difference between control and test days by 2-tailed sign test ($P = .032$).
angiotensin levels would be expected to rise during the Sidman avoidance situation, the 3-h avoidance session in this study was not accompanied by any statistically significant change in plasma renin substrate concentration. The observed increases in plasma renin activity are therefore most likely to be due to an increase in renin concentration rather than renin substrate concentration.

The sympathetic nervous system is believed to be able to increase renin secretion rate by causing renal hemodynamic changes which result in decreased blood pressure in the afferent arterioles, through stimulation by circulating catecholamines, and by direct neural stimulation of the juxtaglomerular cells of the kidney (6). There is evidence suggesting that each of these three mechanisms could bring about increased renin secretion during the avoidance situation. Forsyth (5) reports a statistically significant decrease in renal blood flow during Sidman avoidance testing; Smith et al. (22) have demonstrated decreased renal blood flow in baboons during similar stressful situations. Catecholamine excretion (15), blood pressure, heart rate, and cardiac output (5) increase during Sidman avoidance testing (5); these observations suggest an increase in the activity of the sympathetic nervous system which could include increased neural stimulation of the juxtaglomerular cells, as well as stimulation of renin release by increased circulating catecholamines.

The observed increase in plasma renin activity could also be a consequence of decreased renin clearance rate due to a fall in hepatic blood flow (10). This possibility is unlikely, however, since hepatic artery blood flow has been observed to triple in rhesus monkeys during Sidman avoidance conditioning, in the face of considerably smaller decreases in gastrointestinal, splenic, and pancreatic blood flow (5).

This study was designed to restrict the test stimulus as much as possible to that of the psychological component of free operant avoidance behavior per se. Several previous studies of the physiological effects of the Sidman avoidance procedure utilized avoidance sessions which lasted for 72 continuous hours (5, 15); thus, the animals were subjected to the additional stimulus of severe disruption of their normal sleeping and food intake patterns. The avoidance session in this study was limited to 3 h, presented at a time of day when the animal was normally awake. The paired experimental design controlled for diurnal variation in plasma renin activity as well as possible effects of the blood sampling procedure. Additionally, the data collected during the control day provide information concerning the resting levels and 24-h fluctuations in plasma renin activity and renin substrate concentration in baboons under the feeding, lighting, and housing conditions of this study (Table 1).

Although light exercise does not affect plasma renin activity, renin activity can be increased by heavy exercise (12); each baboon was therefore trained to sit quietly while lever pressing during the avoidance session. There was no statistically significant correlation between the rate of lever pressing during the avoidance test and the magnitude of the rise in plasma renin activity.

Pain associated with shock delivery is also unlikely to be an important stimulus to the increased plasma renin activity observed during the avoidance session. It is known that in rats painful stimuli, such as intramuscular injection of hypertonic saline (2) or repeated electrical shock (14), are accompanied by elevated plasma renin activity. The number of shocks received by each baboon in this study, however, was small (8.8 per session, or 2.9 per h), and the magnitude of the rise in plasma renin activity was not correlated with the number of shocks received. Furthermore, in five of the seven replications in which no shocks were delivered during the first 5 min of the Sidman avoidance session, plasma renin activity levels were greater after 15 min of avoidance behavior (0945 h) than at the onset of the test session (0930 h). The data from one of these replications are shown in Fig. 2.

In conclusion, this study demonstrated that plasma renin activity can be increased by a stressful behavior situation. Disturbances of the renin angiotensin system are believed to contribute to some categories of essential hypertension as well as to renovascular hypertension (3); it is possible that inappropriate elevation of plasma renin activity in response to behavioral stress could be a factor in the development of cardiovascular disease.

We gratefully acknowledge the expert technical assistance of Mr. David J. Taylor and Mr. Fellner Smith.

This study was supported in part by Public Health Service Grants RR00166, GM00666, and HL04741 from the National Institutes of Health.

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Received for publication 9 January 1976.
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