Progressive Hypertension in Dogs by Avoidance Conditioning and Saline Infusion

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SUMMARY A group of dogs was trained on a free-operant avoidance-conditioning task that evoked acute increases in arterial pressure and heart rate during each of three daily 30-minute sessions. After 15 days of exposure to this procedure under conditions of normal sodium intake, 24-hour mean levels of arterial pressure remained unchanged. Another group of dogs received continuous intrarterial infusion of isotonic saline at a constant rate of 185 mEq/24 hrs for 15 days, but no avoidance sessions. Again, 24-hour mean levels of arterial pressure did not change significantly. However, 24-hour mean levels of systolic (19.5 ± 6.2 mm Hg) and diastolic (13.7 ± 2.9 mm Hg) pressure rose progressively over a 15-day period in a third group of dogs exposed concurrently to the avoidance schedule and saline infusion procedure. The progressive hypertension was accompanied by no consistent changes in heart rate. These experiments indicate that behavioral stress can potentiate sodium hypertension and provide a new method for the study of physiological and behavioral factors in long-term blood pressure control. (Hypertension 5:286-291, 1983)

KEY WORDS • arterial pressure • avoidance conditioning • dogs • heart rate • hypertension • sodium

DEVELOPMENT of a model of chronic experimental hypertension in large animals with intact renal functions has remained a formidable experimental challenge. Attempts to produce a "neurogenic" hypertension in primates by exposure to avoidance conditioning over periods of months have been successful on occasion,1, 2 but other studies3-4 have been unable to replicate these results and have concluded that long-term elevations in blood pressure do not result from intermittent evocation of cardiovascular "defense" reflexes alone. Similarly, long-term increases in salt intake can produce sustained hypertension in rodents,5 but the preponderance of evidence indicates that hypertension is very difficult to produce in larger animals by salt loading procedures.6

Over the past several years, studies in our laboratory have led to the hypothesis that a combination of avoid-

ance conditioning and saline infusion could generate a reliable model of chronic experimental hypertension. These studies and others7-11 have shown that free-operant avoidance conditioning procedures are associated with characteristic cardiovascular changes in chronically-instrumented dogs during intervals of hours immediately preceding, as well as during avoidance-perform-

ance sessions. The pre-avoidance response consists of a progressive rise in arterial pressure mediated solely by increases in total peripheral resistance, while heart rate and cardiac output remain stable or decrease. This response appears to be a peripheral concomitant of attention to the environment, since it occurs only in well-trained dogs who have learned to quietly await the onset of the avoidance session, and then initiate avoidance behavior promptly following the onset of the stimulus. By contrast, the avoidance performance session is associated with a sustained elevation in arterial pressure mediated by increased heart rate and cardiac output, with decreased total peripheral resistance.

The ability of the kidneys to excrete sodium and water is decreased during avoidance performance ses-

sions.12, 13 Renal excretory functions have not been studied systematically during pre-avoidance periods, but other behavioral situations, which also differentially increase total peripheral resistance but not cardiac

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Supported by Grant 28462 from the National Heart, Lung and Blood Institute.

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Received July 20, 1982; revision accepted November 11, 1982.
output, such as immobile confrontation with a threatening animal, submerging the head in water, and moderate cold stress.

The present study examines acute and long-term cardiovascular effects of moderately increased levels of saline infusion on intact, chronically-instrumented dogs who were maintained on free-operant avoidance conditioning schedules. This study also assessed the effects of avoidance conditioning without increased saline infusion, and of increased saline infusion with no avoidance requirements.

Methods

Experiments were carried out on 10 healthy adult mongrel dogs (mean weight = 16 kg; range = 12–20 kg). Each dog was maintained in a tether system in a 20 sq ft kennel. After habituation to the kennel and training on an avoidance task, each dog was anesthetized with sodium pentobarbital (30 mg/kg), and a Silastic-coated polyvinyl chloride catheter was implanted in the aorta via a carotid artery. After the dog had recovered from surgery, the catheter, which exited from the body in the midscapular region, was attached to a pressure minitransducer (Statham P50) located in the dog's custom-fitted leather vest. The transducer cable ascended vertically through a hollow, flexible tether line attached at the lower end to the leather vest and at the upper end to a fluid and electrical swivel. The swivel was connected to an overhead, counterbalanced boom assembly, attached to a wall plate 7 feet above floor level. The boom assembly moved in concert with the dog, maintaining a constant, minimal tension on the tether. This system enabled continuous monitoring of the dog. Food (Respond 2000, Charles River Company, or Wayne Dogfood, Continental Pet Food Company) and tap water were available continuously. Daily interaction of each dog with experimenters was generally limited to a period of an hour after the 8 a.m. session, during which feeding, sanitation, recalibration, and other procedures were carried out. Core temperature, food intake, and general activity were monitored regularly to ensure maintenance of the dog's physical condition. Antibiotics were administered postoperatively and at other times as necessary.

Phasic arterial pressure and heart rate of each dog were displayed on a polygraph (Grass model 7C). A DEC PDP 8E digital minicomputer determined systolic, diastolic, and integrated mean arterial pressure at each heart beat and provided on-line printouts of arterial blood pressure and heart rate measures over successive 10- and 60-minute intervals. The transducer to polygraph to computer systems were calibrated three times per week, against a mercury manometer. Calibration error averaged 0.1 ± 0.6 mm Hg at 125 mm Hg (center baseline) and 0.6 ± 0.6 mm Hg at 50 mm Hg (sensitivity or gain).

Patency of the indwelling catheter was maintained by slow but continuous infusion of lightly-heparinized saline (7 USP units/ml) at a rate of 5 ml/hour via a peristaltic pump (Harvard model 607). The rate of saline infusion could be adjusted by changing the speed of the peristaltic pump. During saline-loading experiments, 1.2 liters of isotonic saline were infused into the arterial circulation every 24 hours, resulting in an intake of 185 mEq sodium chloride. This level of salt intake is comparable to the upper end of the human dietary range. During these experiments, dietary sodium intake averaged 35–45 mEq/24 hrs, and dietary potassium intake averaged 25–35 mEq/24 hrs.

The free-operant shock-avoidance task required the dog to press a response panel on the wall of the kennel during each of three, daily 30-minute sessions. During each session, a stimulus light behind the response panel was illuminated, and a 20-second recycling timer was onset. If the timer completed its 20-second cycle, the dog received an electric shock (2–10 mAmp for 0.3 sec). Each time the dog pressed the response key, the timer was immediately reset and the shock postponed another 20 seconds. By maintaining a response rate in excess of one per 20 seconds, the dog could avoid all shocks during each 30-minute session. This contingency generated stable panel-pressing behavior in each dog, resulting in occurrence of less than one shock per hour. After initial training, three sessions were scheduled each day: one at 12 a.m., one at 8 a.m., and one at 4 p.m. During the 7 ½ hour intervals between sessions, the chamber was illuminated but the response panel was not.

Four dogs were run on the avoidance conditioning schedule for 15 days under conditions of normal sodium intake. Four dogs received increased saline infusion (185 mEq/24 hrs) for 15 days but were not exposed to avoidance sessions. Eight dogs received both avoidance sessions and increased saline infusion for 15 days. Most of the subjects participated in two groups. Possible sequence effects were minimized by counterbalancing of the order in which the experiments were programmed, and by spacing of the experiments to permit recovery of basal hemodynamic functions before each.

The results of these procedures were analyzed in terms of acute cardiovascular adaptations to the avoidance schedules, and the rate of change of arterial pressure and heart rate across successive days of exposure to each condition.

Results

Schedule-Induced Cardiovascular Cycles

A pattern of cyclic cardiovascular activity developed in each dog as a result of repeated exposure to the schedule of regularly-occurring avoidance sessions.
Figure 1 presents the mean levels of each cardiovascular measure for each hour of the 24-hour day, averaged for a total of 80 days for the group of 8 dogs on the avoidance-saline procedure. Arrows indicate avoidance sessions.

Figure 1 also shows that systolic pressure increased during the hours between avoidance sessions. The increase in systolic pressure between 1 a.m. and 8 a.m. averaged 6.2 ± 2.2 mm Hg (r = 0.95; p < 0.01). The increase in systolic pressure between 10 a.m. and 4 p.m. averaged 3.2 ± 1.8 mm Hg (r = 0.87; p < 0.01). The increase of 1.5 mm Hg between 5 p.m. and 12 a.m. was not statistically significant. Diastolic pressure levels did not change significantly between sessions.

By contrast, heart rate levels decreased progressively as the onset of the next avoidance session approached. Linear regression analyses showed that heart rate levels decreased by a mean of 12.3 ± 1.9 bpm between 1 a.m. and 8 a.m. (r = 0.85; p < 0.05); 9.0 ± 3.0 bpm between 10 a.m. and 4 p.m. (r = 0.88; p < 0.01); and 7.7 ± 2.7 bpm between 5 p.m. and 12 a.m. (r = 0.94; p < 0.01). These trends were observed in each dog independently of the individual absolute levels of arterial pressure and heart rate. The between-session cardiovascular changes observed in dogs living in the experimental environment partially replicate the divergent changes in arterial pressure and heart rate observed during pre-avoidance periods in previous studies with dogs who remained in a home (nonexperimental) kennel during part of each day. Because of the high positive correlations between heart rate and cardiac output typically observed under these conditions, the data suggest a pattern of divergent changes in cardiac output (decreasing) and total peripheral resistance (increasing) in the maintenance of arterial pressure between sessions.

**Long-Term Cardiovascular Changes**

Effects of 15 days of avoidance schedules and saline infusion upon successive 24-hour mean levels of systolic and diastolic pressure and heart rate for the group of 8 dogs are shown in figure 2. Systolic pressure rose progressively over this period, by an average of 19.5 ± 6.3 mm Hg (linear regression r = 0.96; p < 0.01). Similarly, diastolic pressure levels also increased progressively over this interval, by an average of 13.7 ± 2.9 mm Hg (r = 0.97; p < 0.01). By contrast, heart rate levels showed no consistent changes over the same period.

Table 1 documents the long-term effects of these procedures on individual subjects, presented in terms...
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Mean levels of systolic and diastolic pressure and heart rate for each day of the 15-day exposure to avoidance schedules and saline infusion, averaged for the group of eight dogs.

Four dogs were maintained on avoidance schedules for 15 days under conditions of normal sodium intake. Figure 3 shows that systolic and diastolic pressure levels remained stable across this period under these conditions. Four dogs received increased saline infusion for 15 days during which no avoidance sessions were programmed. Figure 3 shows that there were no significant changes in systolic or diastolic pressure levels for this group of dogs, either.

Table 2 shows the results of these experiments on individual dogs, documenting the consistency of effects between individual subjects. None of the four dogs on the avoidance-only procedure showed a significant elevation in either systolic or diastolic pressure, while the one dog who showed an increase in systolic (but not diastolic) pressure on the saline-only procedure maintained a 24-hour mean heart rate level which was substantially lower than the others. These data confirm the results of previous studies6,21 which indicate that neither avoidance schedules alone nor saline infusion alone produces hypertension over these time periods.

Table 2. Mean Levels of Systolic and Diastolic Pressure and Heart Rate on Day 1 of the Exposure to Avoidance Only or Saline Only and Change in Each Measure Over the 15-Day Period of Each Dog

<table>
<thead>
<tr>
<th>Dog</th>
<th>Systolic pressure (mm Hg)</th>
<th>Diastolic pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 Change at day 15</td>
<td>Day 1 Change at day 15</td>
<td>Day 1 Change at day 15</td>
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<td></td>
<td></td>
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<tr>
<td>Avoidance only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>115</td>
<td>-12.8†</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>107</td>
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<td>73</td>
</tr>
<tr>
<td>5</td>
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<td>75</td>
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<td>3.9</td>
<td>2.2</td>
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<tr>
<td>Saline only</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>111</td>
<td>10.5*</td>
<td>71</td>
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<tr>
<td>5</td>
<td>122</td>
<td>-1.5</td>
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<tr>
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<tr>
<td>SEM</td>
<td>2.6</td>
<td>3.0</td>
<td>2.8</td>
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*p < 0.05.
†p < 0.01.

of systolic and diastolic pressure and heart rate means on Day 1 and the change in each measure over the 15-day period, determined by linear regression analyses. The increase in systolic pressure was statistically significant in seven of the eight dogs, while the increase in diastolic pressure was significant in six. Two dogs showed a significant increase in heart rate while two others showed a significant decrease in heart rate during this period. The magnitude of increase in systolic pressure, which ranged as high as 55 mm Hg over 15 days in an individual case, tended to be an inverse function of the level of systolic pressure on day 1 (r = 0.54; 0.10 > p > 0.05).
Discussion

The results of this study show that blood pressure levels of dogs rose progressively over a period of days during which they received increased amounts of isotonic saline in a context of recurrent avoidance sessions. Heart rate levels did not vary consistently under these conditions. By contrast, blood pressure levels remained relatively stable in subjects who were exposed over comparable periods of time to increased saline but no avoidance sessions, or avoidance schedules with normal sodium intake.

The results appear comparable to those of a previous study, in which blood pressure rose progressively over days in response to saline infusion after renal tissue had been surgically excised. This previous study reported increases in mean arterial pressure averaging 35-40 mm Hg, considerably larger than observed in some (but not all) dogs in the present study, but utilized a saline infusion rate which was almost three times as great. This previous study also reported no significant changes in heart rate over this period, but observed that cardiac output increased during the first few days, due to an increase in stroke volume, followed by a gradual return of cardiac output to pre-experimental levels, and a maintenance of arterial pressure during the later stages of the experiment by a progressively increasing total peripheral resistance.

Presumably, the hypertension induced by avoidance conditioning and saline infusion is associated with alterations in sympathetic nervous system activity that influence renal excretory functions. Previous studies of avoidance conditioning have reported acute decreases in renal blood flow, decreases in renal excretion of sodium and water, and increases in plasma renin activity. In the present experiments, avoidance sessions consumed only 90 minutes of the 24-hour day. It seems likely that normal renal functions between avoidance sessions could compensate for the acute renal changes associated with avoidance performance.

The progressive 24-hour blood pressure changes occurring under these circumstances apparently involved changes in renal function between avoidance sessions. During the hours between sessions, heart rate decreased progressively, sometimes dramatically, reaching a low point immediately prior to the onset of the avoidance stimulus. That the cardiac changes during these intervals did not reflect merely a "calming down" of the subject is suggested by the fact that systolic pressure levels did not also decrease, but remained stable or increased somewhat under these conditions. Rather, this cardiovascular response appears to reflect a progressive fall in cardiac output and rise in total peripheral resistance, associated with the subject's inhibitory behavioral orientation in anticipation of the next avoidance session. Zanchetti and co-workers observed that a combination of bradycardia and peripheral vasoconstriction in various bradycardia and peripheral vasoconstriction in various bradycardia and peripheral vasoconstriction in various contexts in cats during immobile confrontations with other animals. Decreases in renal blood flow levels were also observed during these intervals, which could be prevented by section of the renal sympathetic nerves. Similar changes in renal functions may occur gradually over periods of hours in dogs awaiting the onset of free-operant avoidance sessions, in which prompt initiation of behavior requires vigilant attention to the environment. Such changes could decrease the ability of the kidneys to excrete sodium at the same time that total peripheral resistance is increasing.

The hypertension induced by avoidance conditioning and saline infusion was reversible by termination of the experimental contingencies. In some cases, blood pressure levels returned to pre-experimental levels within 24 hours, while in other cases, blood pressure levels decreased gradually over periods of 7-14 days following termination of avoidance sessions and saline infusion. Individual differences may have been a function of concurrent changes in environmental conditions accompanying termination of the experimental procedures. For some subjects, overhead room illumination increased when avoidance-saline procedures were terminated (a condition previously associated with monitoring of baseline cardiovascular activity). For some dogs, overhead room illumination was not increased coincident with termination of the experiment procedures, because other dogs were continuing on avoidance schedules. The occurrence of these individual differences suggests that conditioned stimuli continued to exert effects upon cardiovascular activity for days following termination of unconditioned stimulation. The observation that the hypertension was reversible also suggests that the blood pressure elevations were not mediated by brain or kidney damage.

The results of this study suggest that the long-term effects of psychological stress and sodium intake are not additive, but synergistic, in the sense that the occurrence of one potentiates the hypertensive effects of the other. According to this view, high levels of sodium intake will not produce hypertension as long as renal functions remain normal. Conversely, psychological stress conditioning will produce chronic elevations in arterial pressure as a function of excessive intake of sodium and water. Fluid volume levels of experimentally hypertensive dogs may or may not be absolutely increased over levels observed in the same subjects prior to the increases in arterial pressure.

However, just as the sympathetic nervous system can alter renal regulation of salt and water, so also do changes in sodium intake influence aspects of nervous system activity. Within the last few years, studies have been reported that show that the lymphocytes of human subjects on high sodium diets had 50% more beta-adrenergic receptors than did those of subjects on a low sodium diet. These subjects also had increased chronotropic responses to infusions of isoproterenol. Similarly, the platelets of hypertensive subjects were found to have twice as many alpha adrenergic receptors on a high sodium diet. Moreover, the cardiovascular effects of norepinephrine infusion depend upon concurrent levels of sodium ingestion. Intrarenal infusion of norepinephrine was found to result in sustained hypertension in dogs, and it was subsequently showed that...
the magnitude of hypertension was a function of the concurrent level of sodium intake. The hypertensive response was associated with decreases in renal blood flow and glomerular filtration rate, and increases in renal vascular resistance. Similar results have recently been reported in studies of the effects of intravenous norepinephrine infusion on blood pressure of human subjects, which also reported that high salt intake was associated with decreases in basal levels of sympathetic nervous system activity. Moreover, renovascular hypertension has been associated with increases in levels of circulating catecholamines, and can be prevented by administration of 6-OHDA, which selectively destroys noradrenergic neurons. Thus, sympathetic nervous system variables, on the one hand, and renal mechanisms involved in sodium metabolism, on the other, may mutually influence each other in ways which significantly determine long-term blood pressure adaptations.

In summary, this study provides a method for reliable development of hypertension in a large, intact laboratory animal preparation which may facilitate understanding of the role of environmental factors and physiological mechanisms in long-term blood pressure control. The implication of the present finding is that salt and “stress” are synergistic in their effects upon blood pressure, and that understanding of the role of one requires concomitant consideration of the other.

Acknowledgments

The authors are grateful to Drs. Ray A. Olsson and Celso Gomez-Sanchez for their critique of an earlier version of this manuscript.

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Progressive hypertension in dogs by avoidance conditioning and saline infusion.
D E Anderson, W D Kearns and W E Better

Hypertension. 1983;5:286-291
doi: 10.1161/01.HYP.5.3.286

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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