Effect of Acute Sympathectomy on Responses to Angiotensin and Norepinephrine

By B. G. Zimmerman, Ph.D.

The vasoconstrictor action of angiotensin has been demonstrated in the perfused vascular bed in dogs and in a variety of other preparations. No autonomic drugs have been shown to block this constrictor action or to prevent the rise in systemic blood pressure induced by intravenous administration of the octapeptide. It is known that angiotensin acts directly on the isolated aortic strip and on perfused denervated vascular beds. Angiotensin, therefore, seems to exert its vasoconstrictor effect through a direct action on vascular smooth muscle.

Since angiotensin has been made more readily available as the synthetic product, several less obvious pharmacological effects have been uncovered. For example, evidence has been presented which indicates that actions elicited indirectly by angiotensin contribute to its direct effect upon smooth muscle. The reports of Khairallah and Page, Ross et al., and Robertson and Rubin suggest that angiotensin is capable of eliciting contractions in the guinea pig and rabbit ileum partly through activation of a parasympathetic mechanism. According to Bickerton and Buckley, angiotensin is capable of raising blood pressure in dogs by acting centrally and stimulating sympathetic pathways.

During preliminary experiments on the hindquarters of the dog, vasoconstrictor responses to angiotensin appeared to be reduced after acute sympathectomy. The present investigation was undertaken to examine this observation.

Methods

The general procedure for studying the vasoconstrictor effect of synthetic angiotensin on the hindquarters vascular bed is described below. Factors which may have influenced the angiotensin response were studied by several modifications of this technique. The method of perfusion was reported in detail by Beck.

Thirty healthy dogs, 8.5 to 17.5 Kg., were used. They were anesthetized with pentobarbital sodium, 30 to 35 mg./Kg., injected intravenously. A longitudinal incision was made in the left flank area, and the abdominal aorta and both lumbar sympathetic trunks were exposed using a retroperitoneal approach. Two pairs of lumbar arteries (L4 and L5) and the inferior mesenteric artery were ligated to reduce collateral blood flow. Blood was led from a cannula in the proximal aorta through a heated rubber coil (38 C.) and a Sigma-motor pump (model T6) to a cannula in the aorta distal to the fifth lumbar artery; the hindquarters were perfused at a constant blood flow through the distal cannula. When a single hindlimb was perfused, blood was pumped from the carotid to the femoral artery. The perfusion tubing and coil increased the animal's circulating blood volume by 51 ml. Heparin, 5 mg./Kg., was used to prevent blood clotting. Systemic arterial pressure and perfusion pressure were measured with mercury manometers and recorded on a kymograph. Any change in perfusion pressure represented a corresponding change in vascular resistance since blood flow through the bed was constant. The Sigma-motor pump was calibrated and found to maintain a constant outflow while the inflow pressure was varied from 10 to 150 mm. Hg.

Experiments were initiated by adjusting the outflow of the pump until the perfusion pressure in the hindquarters was approximately equal to the systemic pressure. The flow required to produce such a pressure varied from animal to animal but was maintained constant in a given experiment. Several minutes were allowed to elapse for the preparation to become stable and for perfusion pressure to become constant. No additional anesthetic was administered after the stabilization period. Intra-arterial injections of pressor drugs were made into the rubber tubing attached to the cannula in the distalorta or femoral artery. All drugs were dissolved in isotonic saline and injected in small volumes, 0.05 to 0.50 ml. In early experiments, graded doses of angiotensin and norepinephrine were injected in the control period. Sometimes the doses were given in replicated fash-
ion. Then, neurogenic vascular tone to the hindquarters was interrupted by sympathectomy at L5, or total sympathetic blockade was produced by intravenous injection of hexamethonium. About 10 minutes were allowed for vascular resistance to become stable after interruption of sympathetic tone before injections of norepinephrine and angiotensin were repeated. It was found that short control periods with single injections of angiotensin and norepinephrine before and after sympathectomy would best contrast the responses of these two agents. A group of eight such experiments were designed, four involving sympathectomy and four others in which a sham sympathectomy was performed. Single doses of norepinephrine and angiotensin were administered in a different sequence in each of four experiments.

SYMPATHETIC STIMULATION

In seven experiments, a Harvard electrode was placed on both sympathetic trunks at about L5, and the nerves were sectioned proximal to the electrode. The sympathetic trunks were then stimulated electrically at the appropriate parameters to raise the perfusion pressure to the level existing before sympathectomy. When the pressure became constant, angiotensin and norepinephrine were injected again, and the responses were recorded. The stimulation was terminated, and on the return of the perfusion pressure to the lower level, the vasopressor responses to the two agents were again observed.

SPINAL SECTION

The first cervical vertebra was exposed, and a laminectomy was performed. A segment of spinal cord about 1.5 cm. in length was uncovered by incising the overlying dura mater. Artificial respiration was started, and perfusion of the hindquarters was instituted. After the responses to norepinephrine and angiotensin were recorded, the spinal cord was transected. The injections were repeated after the perfusion pressure had returned to a basal level. In an alternative experiment, xylocaine in a 2 per cent solution was applied locally at the spinomedullary junction to produce a blockade of nervous transmission at this point.

OTHER PREPARATIONS

To limit the action of angiotensin to the hindlimb only, the agent was administered into the innervated extremity, cross-circulated at constant flow with blood from a donor animal. In another experiment, a coil of rubber tubing was placed between the sectioned ends of the inferior vena cava to delay the arrival of angiotensin to the animal's systemic circulation. If a systemic effect of the angiotensin injected into the perfused limb was responsible for the vasoconstriction in that bed, the systemically induced response would follow the local response because of the delayed passage of the drug through the coil.

The drugs used in this study were synthetic isoleucine-5-angiotensin II amide, valine-5-angiotensin II amide, norepinephrine hydrochloride, hexamethonium bitartrate, tyramine hydrochloride, xylocaine hydrochloride, and heparin sodium. The dose of hexamethonium was calculated in terms of the base. The other agents were given in doses expressed as their respective salts. An analysis of variance, split plot design, and linear regression analysis (Snedecor, 1956) were used in the statistical treatment.

Results

Figure 1 illustrates an experiment in which angiotensin (2 µg.) and norepinephrine (4 µg.) were administered intra-arterially before and after sympathectomy of the perfused hindquarters.

*Valine-5-angiotensin II amide (Hypertonsin, Ciba) was supplied by Dr. A. J. Plummer of Ciba Pharmaceuticals Inc.
TABLE 1
Vasopressor Responses Elicited in the Hindquarters by Intra-Arterial Administration of 2 μg. of Angiotensin and 4 μg. of Norepinephrine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Angiotensin</th>
<th>Norepinephrine</th>
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</table>

*Responses elicited 5 to 22 minutes after sympathectomy or sham operation.

*Absolute mm. Hg change in the perfusion pressure.

hindquarters. After sectioning the sympathetic trunks, the perfusion pressure of the vascular bed fell and stabilized shortly afterwards at a lower level. The vasopressor response induced by angiotensin injected 18 minutes after cutting the nerves was greatly reduced, whereas the response to norepinephrine was unchanged. Eight experiments, which were specifically designed so that the results could be statistically treated utilizing an analysis of variance, are shown in table 1. The vasopressor responses to angiotensin (2 μg.) and norepinephrine (4 μg.) are tabulated as absolute values for experiments in four animals before and after sympathectomy and in four animals before and after sham operation. In the sympathectomized animals, the vasoconstrictor effect to angiotensin was reduced (P < 0.001), whereas the norepinephrine vasopressor response was not significantly changed (P > 0.1). Responses to both angiotensin and norepinephrine were not significantly different after sham operation (P > 0.1).

In three experiments, graded doses of angiotensin and norepinephrine were administered in the control period and after sympathectomy. One such experiment is illustrated in figure 2. Pressor responses to angiotensin were diminished after sympathectomy; the dose-response regression line was shifted toward the abscissa, and the slope was decreased. The norepinephrine dose-response regression was essentially unchanged following sympathectomy. It was discovered in these experiments that the dose-response relationships were variable during the progress of an experiment. To avoid the variation due to multiple doses or the time-trend effect resulting from such a procedure, single doses of norepinephrine and angiotensin were given, as previously described.

In three experiments, ganglionic blockade by hexamethonium, 10 mg./Kg., affected the response to angiotensin in the same manner as did sympathectomy. The response to norepinephrine was increased by this dose of the blocking agent.

Experiments were done to examine the manner by which sympathectomy results in a decrease in the vasoconstrictor action of angiotensin. In seven preparations, after the response to angiotensin was reduced as a result of sympathectomy, the distal ends of the sectioned sympathetic trunks were stimulated electrically. Such an experiment is illustrated in figure 3. The response to angiotensin (2 μg.) was 85 mm. Hg and to norepinephrine (4 μg.) was 58 mm. Hg. Following sympathectomy, the response to angiotensin was reduced to 40 mm. Hg, whereas the response to norepinephrine was unchanged. Subsequently, sympathetic stimulation was instituted, and the perfusion pressure was in-

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ANGIOTENSIN AND NOREpinephrine

FIGURE 2

Effect of graded doses of angiotensin and nor-epinephrine injected intra-arterially upon the vas-cular resistance of the dog's perfused hindquarters in one experiment. The increase in resistance (pressor response) is equal to the absolute rise in perfusion pressure in mm. Hg. Curves are plotted for one set of angiotensin and norepinephrine responses before and after an acute lumbar sympathectomy.

creased approximately to the control level. The response to angiotensin was increased to 77 mm. Hg during the stimulation period. The response to norepinephrine was reduced. Perfusion pressure fell when the stimulation was terminated, and again the response to angiotensin was diminished. Though in this animal the response to norepinephrine happened to be decreased at this time, the effect of this pressor amine differed from that of angiotensin during sympathetic stimulation. The response to angiotensin was increased during stimulation while the response to norepinephrine was decreased.

The results of the seven experiments in which the sympathetics were stimulated are presented in table 2. The pressor response to angiotensin before and after sympathectomy and the pressor effect obtained during stimulation are listed. In six animals, the angiotensin response was increased during sympathetic stimulation; however, in only two experiments was a response of the control magnitude obtained. The response to norepinephrine was decreased during sympathetic stimulation.

In two additional experiments, norepinephrine was infused intra-arterially at a rate which restored perfusion pressure to the control level. Responses to angiotensin were not increased by the exogenously administered norepinephrine.

Since sympathetic stimulation did not re-establish a response to angiotensin of the control magnitude, an additional mechanism was considered. A vascular reflex evoked by angiotensin seemed a possibility because the vasoconstrictor action of angiotensin depended upon physically intact or unblocked sympathetics. To test whether such a reflex took place at a level below the medulla, the response to angiotensin was elicited before and after the spinal cord was transected at Cl. The pressor response was reduced after cervical spinal transection as it was following lumbar sympathectomy which indicated, therefore, that angiotensin did not elicit reflexes at the spinal level.

In two animals, the administration of tyramine into perfused hindlimbs resulted in equivalent vasoconstriction before and after sympathectomy. The response to angiotensin was reduced in these same experiments after the nerves were sectioned.

In all experiments, relatively small doses of pressor agents were administered intra-arterially to perfused hindquarters in order to produce a local response. In several experi-
ments, the intra-arterial administration of 2 µg. of angiotensin increased the systemic blood pressure (20 to 50 mm. Hg) after some delay. Since in these few cases the systemic circulation of the animal was exposed to effective concentrations of angiotensin, it was possible that the perfused bed could be affected by nervous actions elicited elsewhere by angiotensin. Experiments were done in which drugs could be administered solely to the hindlimb of the experimental animal. In an experiment in which the hindlimb of a dog was cross-circulated with the blood of a donor animal, the angiotensin response was reduced by sympathectomy, and the response to noradrenaline was unchanged, as found in previous experiments. Similar results were obtained in a preparation in which the venous drainage of the perfused hindlimb was delayed in reaching the animal’s systemic circulation. Pressor responses to intra-arterially administered angiotensin, therefore, can be attributed mainly to a direct action on the perfused vascular bed.

The effect of sympathectomy on hind-quarter vascular resistance and the concomitant reduction in the angiotensin response are depicted for 17 experiments in figure 4. Values for the control perfusion pressure and control responses to angiotensin were taken as 100 per cent, and the values obtained 5 to 25 minutes after sympathectomy were taken as a percentage of control. The per cent decrease in the angiotensin response was plotted against the per cent decrease in the control perfusion pressure. In all cases, the response to angiotensin was decreased after lumbar sympathectomy. The slope of the regression line $Y = 0.93X - 21.0$ proved to be significantly different from zero ($P < 0.01$) and indicated that there was a significant relationship between the reduction in the response to angiotensin and the decrease in vascular resistance obtained after sympathectomy. In two of these experiments, valine-5-angiotensin II amide was utilized, and the results did not differ qualitatively from those obtained using isolonic-5-angiotensin II amide.

Discussion

These experiments have demonstrated a difference in the direct vascular actions of synthetic angiotensin and noradrenaline. The vasopressor response to noradrenaline was not significantly changed while the response to angiotensin was reduced after the vascular tone was diminished by acute sympathectomy. The decreased effect could not be attributed to angiotensin tachyphylaxis or to a time trend since responses to angiotensin were unaltered after a comparable period of time following a sham sympathectomy. A direct result of acute sympathectomy is passive vasodilatation of the perfused vasculature due to the loss of sympathetic vasoconstrictor tone. It has been suggested that vasoconstrictor re-

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### TABLE 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Angiotensin</th>
<th>Parameters of Stim.</th>
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</tr>
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</table>

*In volts.
†In cycles/sec.
‡In msec.

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spontaneous activity in general would be reduced by an increase in blood vessel caliber. However, in this study, and as reported by others, the vasoconstrictor response to norepinephrine is not altered following the increase in blood vessel diameter brought about by the abolition of sympathetic tone. The decreased response to angiotensin following sympathectomy cannot, therefore, be explained by decreased vascular reactivity due to vasodilatation. Thus, it appeared that angiotensin was able to contract vascular smooth muscle by another mechanism in addition to its direct effect on the blood vessels. The possibility was considered that locally injected angiotensin could produce indirect vasoconstriction in a manner similar to catecholamine-releasing agents such as tyramine. Since it was found that the vasopressor response to intra-arterially administered tyramine was not affected by acute sympathectomy, it was apparent that angiotensin was not acting like tyramine. The results indicated, furthermore, that a spinal vascular reflex was not involved since a cervical spinal transection reduced the response to angiotensin. The possibility of a vascular reflex taking place through the medulla or higher centers, though unlikely, cannot be completely ruled out. Since spinal transection, ganglion blockade, and lumbar sympathectomy all reduced the response to angiotensin, it appeared that the abolition of centrally mediated sympathetic tone was involved in the mechanism.

Evidence was presented which indicated that the reduction in the response to angiotensin depended upon the degree of neurogenic tone possessed initially by the vascular bed (fig. 4). The initial degree of sympathetic tone was estimated by the reduction in perfusion pressure obtained after sympathectomy. It was shown that in vascular beds in which sympathetic tone made a large contribution to the initial level of vascular resistance, i.e., perfusion pressure, the percent reduction in the response to angiotensin was also large. Vascular sympathetic tone is maintained by the release of norepinephrine from sympathetic nerve endings. Possibly, the release of transmitter may sensitize the vascular smooth muscle to angiotensin. The reverse hypothesis is also tenable, namely, that the octapeptide may facilitate in some manner the vasoconstrictor action of the neurohumor tonically released from the nerves. When norepinephrine was infused intra-arterially following sympathectomy at a rate which restored the control level of perfusion pressure, the response to angiotensin was not changed. The vascular smooth muscle was not sensitized, therefore, by exogenously administered catecholamine. If tonic firing of the sympathetic reflexes is responsible for facilitation of the response to angiotensin, one would presume that electrical stimulation of the sectioned sympathetic nerves would restore the control response to angiotensin. The incomplete recovery of the response which was obtained may have been due to differences between electrically induced and physiologically stimulated sympathetic neurohumoral release. For instance, the frequency of stimulation which was employed to maintain the control perfusion pressure is greater than that considered to be in the physiological range.
addition, stimulation of the lumbar sympathetic nerves results in the release of norepinephrine as well as a cholinergic and possibly a histamine-like substance. These vasodilator agents are not thought to be tonically secreted during the resting sympathetic discharge. It is possible, therefore, that sensitization to the vascular action of angiotensin depends upon a certain background of neurohumor, tonically released by normally functioning sympathetic nerves.

One may speculate that a difference in the blood flow distribution in the hindquarters takes place after sympathectomy in addition to dilatation of the blood vessels. For instance, a loss of sympathetic tone may open arteriovenous shunts and permit the passage of injected vasoconstrictor substances through the perfused bed before the smaller resistance vessels can be affected by these agents. In such a situation, however, the pressor effect of all intra-arterially administered agents would be diminished following arteriovenous shunting. In the present experiments, the response to angiotensin was differentially affected after sympathectomy so that such a shunting process does not appear to be involved. Secondly, if sympathetic tone maintains the perfusion of specific areas of the hindquarters more sensitive to the vasoconstrictor action of angiotensin, and removal of tone shifts the distribution of flow to regions less sensitive to angiotensin, a reduction in the pressor effect could be obtained following such a pattern. However, to explain the results by means of such a mechanism, blood flow must be shifted following sympathectomy from resistance vessels, which are more sensitive to angiotensin than norepinephrine, to vessels less sensitive to angiotensin than norepinephrine. Evidence to support such a view has not been found.

Summary

The vasopressor response to angiotensin administered intra-arterially to the perfused hindquarters of the dog was reduced significantly after acute sympathectomy. Responses to norepinephrine and tyramine remained unchanged. Similar results were obtained following ganglionic blockade with hexamethonium and cervical spinal transection. After the response to angiotensin was reduced following sympathectomy, the cut lumbar sympathetic nerves were stimulated electrically. During the period of stimulation, an increase in the vasoconstrictor effect to angiotensin was obtained, but complete restoration to the control magnitude was not seen consistently. Possible mechanisms for the reduction in the response to angiotensin following sympathectomy are discussed. The present evidence suggests that the tonic discharge of normally functioning sympathetic nerves is capable of facilitating the response to angiotensin and that sympathectomy results in an abolition of this effect.

Acknowledgment

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References


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Book Reviews

This monograph pertains to experiences of the author in examining the brains of patients dying of apoplexy. The illustrations of pathological specimens are excellent. Some of the conclusions pertaining to the pathogenesis of apoplectic attacks are as follows: In permanent arterial hypertension, increase in vasomotor sensitivity represents a paramount pathogenetic factor in the development of cerebral conditions. Atherosclerosis of cerebral arteries is not of decisive importance in the pathogenesis of hypertensive apoplexies, although it may further the development of neurogenic circulatory disturbances. On the other hand, atherosclerosis is a decisive factor in non-hypertensive apoplexies caused by thrombosis or by stenosis. In these cases, increase in vasomotor sensitivity accompanying sclerotic vascular changes is also to be considered an important pathogenetic factor.
It is interesting to note that vasomotor nervous sensitivity of the cerebral vessels in man is less important than sensitivity to metabolites. The situation in abnormal vessels appears to be different.

Many investigators will welcome this index of over 13,000 references which deal with the action of drugs on the cardiovascular system. These articles were derived from 400 medical publications, published from 1951 to 1955, which were examined on a "page-by-page" basis. Any omission of the literature on cardiovascular agents during this five-year period would include articles published in periodicals other than the "selected 400."
The group responsible for this volume should be encouraged to include in the index as soon as possible articles published prior to 1951 and subsequent to 1955. One suggestion for future indices is to include periodicals other than the "selected 400," perhaps not examined on a page-by-page basis, but simply on the basis of the title of the article. If this is done, then the index can be depended upon to cover all publications on cardiovascular agents.
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