Some Contributions to the Pharmacology of Synthetic Angiotensin

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SINCE THE SUCCESSFUL synthesis of various angiotensins and their amides, these polypeptides have become available in amounts which have permitted a more extensive study of their pharmacology than was possible before with the natural products. It is not surprising, therefore, that a number of new findings have been reported recently, indicating qualities so far unknown and leading to a partial revision of our ideas about various pharmacodynamic properties of angiotensin. We will limit ourselves to reporting on the following investigations of synthetic angiotensin: (1) conditions under which tachyphylaxis can be demonstrated, (2) effects on the venous pressure, and (3) action on arterial and venous pressure in experimental hypotension.

In almost all of our experiments, synthetic valyl-5-angiotensin II-amide (octapeptide) was used. The material had a purity of 98 per cent, the remaining 2 per cent being the corresponding free acid. In a few comparative experiments, the free octapeptide was given instead and showed exactly the same results as the amide. Most of our experiments were done in nonanesthetized trained dogs. Whenever anesthesia was given, it will be specially indicated.

Tachyphylaxis to Angiotensin II and Renin

Tachyphylaxis may be defined as the transient diminution of response to repeated doses of the same substance and has to be distinguished from refractoriness, which can occur spontaneously and is not directly related to previous exposure to the drug in question. Although it was known to occur with renin, tachyphylaxis was not thought to occur with angiotensin. However, with angiotensin, according to Page and Helmer, a state of spontaneous refractoriness may be observed in some animals. Mainly as a result of the work of Goldblatt et al., we are quite well informed on the conditions under which renin produces tachyphylaxis, but similar studies, especially with high doses, had not so far been done on angiotensin II.

In small and medium doses, both angiotensin II and renin show a linear relationship between the log of the dose and arterial blood pressure elevation. The curves for the 2 substances run parallel, but the impure hog renin product available to us had to be administered to either dogs or rats in doses about 10,000 times higher in order to obtain a blood pressure increase as great as that caused by angiotensin II. If, however, angiotensin II is administered in doses higher than 10 μg./Kg. and if these doses are preceded by smaller doses of angiotensin II, the hypertensive reaction becomes even less pronounced than that produced by smaller doses. As a result, the dose-response curve is not asymptotic but, in fact, even declines after reaching a maximum.

While repeated injection of a medium dose of angiotensin II (1 μg./Kg.) always elicits the same increase of blood pressure, a high dose, of the order of 20 to 50 μg./Kg. depresses the reaction to smaller subsequent doses (fig. 1). Within a period of 40 to 60 minutes, responsiveness to low doses may be restored to the level which existed before the high dose. Unlike angiotensin II, the reaction to norepinephrine is not depressed in the same way, nor does a high dose of norepinephrine provoke a similar diminution of succeeding lower doses of either norepinephrine or angiotensin II.

A comparable phenomenon of tachyphylaxis is observed if angiotensin II is given in the form of an infusion. While a dose of 0.1 μg./Kg./min. provokes a constant elevation of
arterial blood pressure during the period of administration, a dose 10 times higher leads to an initial peak of arterial pressure, which is then followed by a partial decline of the pressure, despite the maintenance of a constant rate of infusion. This transient effect on blood pressure is more pronounced if 15 \( \mu g/\text{Kg.} \cdot \text{min.} \) are given. In spite of continuous steady infusion of this dose, blood pressure falls to nearly the initial values within 5 to 10 minutes. On cessation of the drug, the reaction to small or medium doses of angiotensin II remains diminished, while the response to norepinephrine is not changed. Similar results can be observed with the infusion of increasing doses of renin. With low doses, an elevated blood pressure may be maintained, while there is only a transient initial rise with a high dose.

These observations, made in the nonanesthetized dog, suggest that the response to angiotensin II is mediated through the renin-angiotensin system. The transient effect observed with high doses could be attributed to the rapid metabolism of angiotensin II by plasminogen activator, which is known to degrade angiotensin II into its inactive metabolites.

**Figure 1**

Tachyphylaxis induced by a high dose (50 \( \mu g/\text{Kg.} \)) of angiotensin II on subsequently injected small doses (1 \( \mu g/\text{Kg.} \)). Venous pressure (above), heart rate (middle), and arterial pressure (below). Unanesthetized dog.

**Figure 2**

Effect of increasing doses of norepinephrine (NE) and of angiotensin II (H) on venous pressure (above), heart rate (middle), and arterial pressure (below). Unanesthetized dog.
thetized trained dog, are very similar to those we made in the anesthetized nephrectomized rat.\textsuperscript{5}

A large dose of angiotensin II diminishes the pressor reaction, not only to this polypeptide, but also to renin, and a high dose of renin reduces the hypertensive response to a subsequently administered dose of angiotensin II.\textsuperscript{6} This cross-tachyphylaxis is further evidence that the pressor effect of renin is mediated by release of angiotensin in vivo. The difference in the shape of the blood pressure curve after the injection of renin and after angiotensin may depend on the fact that renin liberates angiotensin over a certain period at a slowly decreasing rate, the release being sustained until the enzyme is completely destroyed or inactivated.

The results of these experiments led us to the conclusion that although angiotensin II does not cause tachyphylaxis in small or medium doses, it does so in high doses. In our opinion, this effect of angiotensin is responsible for the well-known tachyphylaxis with high doses of renin.

The renal responses to high doses of angiotensin II also reflect a similar tachyphylaxis in that, during infusion, glomerular filtration rate and renal plasma flow are reduced markedly at the onset, but the effect partially wears off, despite continued administration at the same rate.\textsuperscript{7-9} Corresponding observations were made in the dog with regard to sodium excretion.\textsuperscript{1}

**Effects of Angiotensin II on Venous Pressure**

In addition to arterial pressure, central venous pressure was recorded in either the superior or inferior vena cava of the nonanesthetized dog. Simultaneously with the increase of arterial blood pressure, produced either by angiotensin II or by renin, a comparable elevation of the venous pressure was observed. The threshold dose for an increase of venous pressure for angiotensin II, as well as for norepinephrine, was nearly 10 times higher than that required for an effect on
arterial pressure (fig. 2). By comparing the action of angiotensin II and norepinephrine on arterial and venous pressure, we found that the potency (on a weight basis) was 10:1 for the arterial and about 5:1 for the venous pressure. That means in doses with equal pressor activity on the arterial side, angiotensin II has only about half the activity of norepinephrine on the venous side (fig. 3). After a high dose of angiotensin II, tachyphylaxis is evident in the venous pressure response in the same way as on the arterial side (fig. 1).

The effect of angiotensin II on central venous pressure is definitely diminished, either by anesthesia with pentothal or by atropinization, while the action on the systolic and especially the diastolic pressure is enhanced (fig. 4). Simultaneously, the reflex bradycardia which accompanies the pressor effect is abolished and is often replaced by an increase of heart rate.

Central venous pressure depends on the venous volume, as well as on the tonus of the venous system which is influenced by humoral and nervous factors. In our experiments, no marked vasoconstrictive effect on the conjunctival veins was observed after instillation of angiotensin II into the rabbit’s eye. In the skin-muscle region of the cat, Folkow et al. found angiotensin II to have a much more pronounced effect on the precapillary resistance vessels than on the venous side. From these findings, it was concluded that the angiotensin receptors are mainly located in the resistance vessels, while receptors sensitive to norepinephrine are present in the resistance as well as in the capacitance vessels. This conclusion is in accord with findings in the human being.11 The more pronounced di-

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**Figure 4**

Effect of pentothal anesthesia on venous and arterial pressure response of angiotensin II (H) and of norepinephrine (NE). From above: respiration, venous pressure, heart rate, arterial pressure.
rect effect of norepinephrine on the venous wall may contribute to its stronger action on the venous pressure. The influences exerted by anesthesia and by atropine suggest that the increase of venous pressure is mainly of reflex origin. The efferent part of this reflex mechanism seems to be mediated through the vagus, while the afferent pathways remain to be identified. We can only assume that the increased tone of the pressor receptors elicited by the hypertensive reaction plays a decisive part in this reflex mechanism.

**Angiotensin II in Experimental Hypotension**

Hypotension was produced either by the injection of bacterial endotoxin (1.5 mg./Kg. isolated from *Serratia marcescens*) in the non-anesthetized dog or by exsanguination in the dog anesthetized with pentothal.

*Serratia* endotoxin provoked a steep fall of arterial pressure, which afterwards partly recovered but did not reach its previous value. As in the cat, the responsiveness to either angiotensin II or norepinephrine gradually declined following the injection of the endotoxin. On the venous side, this effect was more marked, and after 30 to 40 minutes, the response to both substances was abolished (fig. 5).

The gradual disappearance of the venous pressure reaction under the influence of endotoxin suggests that the underlying mechanism differs from that which is involved during anesthesia and atropinization. It may be that subsequent to endotoxin administration, the venous blood reservoirs are depleted, resulting in reduced venous volume so that additional reflex venous constriction does not become effective.

In dogs in which hemorrhagic hypotension was elicited by rapid exsanguination, it was possible to demonstrate the pressor effect of angiotensin II during the state of extremely low systemic blood pressure, while the reaction to norepinephrine was either abolished or markedly diminished. This was the case even when, before blood loss, the pressor response to the test dose of norepinephrine was more marked than that to angiotensin II.
spite of the absence of effect on the blood pressure, norepinephrine may have an undesirable effect on the heart muscle, as can be demonstrated by simultaneous recording of the electrocardiogram (fig. 6). In various experiments, it was possible to obtain a partial restoration of blood pressure after exsanguination by the infusion of angiotensin II (0.2 μg./Kg./min.), which persisted even after the infusion was stopped. From these observations, we may conclude that if, in various forms of hypotension, the administration of a vasoconstrictor agent is envisaged as a therapeutic measure, angiotensin II will be more suitable than norepinephrine, since it may act under conditions in which there is already refractoriness to norepinephrine and also since it has a less adverse effect on myocardial function.

Summary

The examination of synthetic valyl-5-angiotensin II-amide in the conscious dog revealed the following:

1. Both the arterial and the central venous pressure responses exhibit tachyphylaxis when high doses of angiotensin II are administered, although not when medium or low doses are given. Cross-tachyphylaxis can be demonstrated between renin and angiotensin II but not between either of these and nor-
epinephrine. These results suggest that tachyphylaxis to renin is due to tachyphylaxis to angiotensin.

2. Angiotensin II and norepinephrine provoke a dose-dependent increase in central venous pressure. The threshold dose is about 10 times higher than that necessary for the effect on arterial pressure. In doses eliciting the same increase of arterial blood pressure, norepinephrine is about twice as active on the venous pressure as angiotensin II.

3. Anesthesia and atropinization both reduce or abolish the effect of angiotensin II as well as that of norepinephrine on the venous pressure, indicating the reflex nature of the underlying mechanism.

4. In endotoxin hypotension, the pressor response to angiotensin II and to norepinephrine is diminished, and the venous pressure response becomes gradually depressed until it is completely abolished.

5. During hemorrhagic hypotension, the response to angiotensin II may be retained or even enhanced and is less likely to have an adverse effect on the myocardium than norepinephrine.

References
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