Renin and Angiotensin Tachyphylaxis

By K. D. Bock, M.D., and F. Gross, M.D.

IN THE paper which first described the pressor effect of kidney extracts, Tigerstedt and Bergman reported that, on repeated intravenous injection of the substance which they called renin, the response in the rabbit became less pronounced. This observation was confirmed by various investigators, and the effect was denoted as renin tachyphylaxis. Goldblatt et al., in a careful analysis of this phenomenon, demonstrated that the response to repeated injections of the same dose of renin is diminished only if the intervals between the doses are so short that the blood pressure cannot return to its initial value. Hence, the authors concluded that residual constriction of the arterioles following the first injection of renin is a factor at least partly responsible for the phenomenon of tachyphylaxis. This assumption is in agreement with the observation that the animal with a diminished response to renin showed also a reduced sensitivity to angiotensin (Goldblatt, Page et al.). On the other hand, all authors working with angiotensin stated that, in contrast to renin, this polypeptide does not exhibit tachyphylaxis. However, we have already shown that after the administration of high doses of synthetic angiotensin, the reaction to subsequent smaller doses is diminished, i.e., a degree of tachyphylaxis develops, even when the blood pressure is allowed to return to normal between the single injections. Therefore, we reinvestigated the problem of renin tachyphylaxis: (a) to determine the exact dose-response relationship for pure synthetic angiotensin and to compare its characteristics with that of renin; (b) to define the conditions under which angiotensin tachyphylaxis develops; and (c) to investigate the influence of renin on the response to angiotensin, and vice versa. In all these experiments, we limited ourselves exclusively to the blood pressure as a single parameter, ignoring the other actions of renin and angiotensin.

Methods

Forty-four experiments were performed in 14 male dogs weighing from 12 to 28 Kg. The animals were trained to lie quietly on their backs slightly turned to one side during the whole duration of the experiment without anesthesia. Blood pressure was continuously recorded by the Sanborn manometer after percutaneous puncture of the femoral artery. Clotting was avoided by a continuous drip of a solution of heparin (40 units Liquaemin per ml. 0.9 per cent saline), releasing between 5 and 10 drops per minute. The heart rate was recorded by a special method developed in our laboratories, using either the pulse-pressure wave or the R wave of the electrocardiogram as a signal impulse. In a few experiments, venous pressure in the inferior or superior vena cava was also recorded by the Sanborn manometer.

All injections were given intravenously in the brachial vein. The following substances were administered: (a) renin extracted from hog kidneys* according to the method of Green and Page, 0.3 mg./Kg. of the crude product having an activity of about 1 Goldblatt unit; (b) synthetic val5-angiotensin II-amide (Hypertensin, Ciba), containing about 98 per cent val5-angiotensin II-amide and 2 per cent val5-angiotensin II (dicarboxylic acid); (c) l-norepinephrine (Arterenol).

Results

Dose-Response Relationships

In the conscious dog, when the maximum pressor response to angiotensin is plotted against the log. dose, the curve is linear, provided that high doses are administered without preceding similar doses (fig. 1). If, however, repeated doses in the medium range of 5 to 10 µg./Kg. are injected, subsequent higher doses...
doses elicit a relatively reduced response, indicating a tachyphylaxis (see below). The dose-response curve of renin runs parallel to that of angiotensin, although, using the crude preparation of renin available to us, high doses were necessary. However, in view of the longer duration of the action of renin, the two dose-response curves would have a different slope, if, instead of the maximal increase in systolic blood pressure, the area under the blood-pressure curve were taken as a parameter.

Angiotensin Tachyphylaxis

Repeated injections of small or medium doses of angiotensin (0.01 to 2 µg./Kg.) always elicit the same blood-pressure reaction in the conscious dog (fig. 2). Occasionally, the pressor effect of a small dose of this order of magnitude may decrease following the first one or two injections, remaining subsequently constant.

A high dose of angiotensin (25 to 50 µg./Kg.), eliciting a maximum response, is followed by a diminished response to subsequent small or medium doses of angiotensin. It is only after a certain period (45 to 90 minutes) that the sensitivity to angiotensin slowly returns (figs. 2 and 3). The restoration of the responsiveness to angiotensin is not delayed by repeated injections of small doses of angiotensin. The response to small doses of norepinephrine is not changed. Furthermore, a high dose of norepinephrine is not followed by reduced sensitivity to smaller doses of either norepinephrine or angiotensin.

The intravenous infusion of angiotensin (fig. 4) at a dose of 0.1 µg./Kg./min. provokes a constant elevation of the blood pressure for as long as the infusion is continued. With the medium dose of 1 µg./Kg./min. angiotensin, after a steep initial rise, in spite of continued infusion at the same rate, blood pressure returns to a somewhat lower but still elevated level, maintained during the further duration of the infusion. The same occurs during infusion of the high dose of 15 µg./Kg./min. of angiotensin, but the fall of blood pressure after the initial rise is even more pronounced.

Furthermore, it is evident that, by the infusion of increasing amounts of angiotensin, the pressor response to a subsequent single injection of 1 µg./Kg. angiotensin is more diminished after the higher than after the lower dose. In contrast, the effect of a single dose of norepinephrine is the same after the infusion of angiotensin as before (fig. 4). These blood-pressure changes during and after an infusion of angiotensin again suggest the development of a dose-dependent tachyphylaxis.

Renin Tachyphylaxis

Within a certain range, increasing doses of renin elicit a linear dose-dependent pressor response, provided that one waits for the return of blood pressure to the initial value before giving the following dose (fig. 5). Repeated injections of small doses of renin (100 to 500 µg./Kg.) always elicit the same blood-pressure response. In contrast, repeated injections of medium doses of renin (1 to 2 mg./Kg.) produce a typical tachyphylaxis, unless blood pressure has completely returned to the initial values at the moment when the subsequent injection is given. After the injection of small or medium doses of renin, blood pressure returns to its initial values within 10 to 30 minutes.
A single high dose of renin (5 to 10 μg./Kg.) leads to the well-known tachyphylaxis, so that a small or medium dose of renin injected afterwards remains without effect on the blood pressure (fig. 6). The initial responsiveness returns gradually within about one hour. The duration of the hypertensive response to a high dose of renin seems to be shorter than that obtained by lower doses; the pressure in the inferior vena cava runs parallel to the arterial pressure (fig. 6).

The intravenous infusion of renin at several different rates (fig. 7) elicits a blood pressure response similar to that of angiotensin: the low dose of 50 to 100 μg./Kg./min. increases the pressure to a higher level, which is maintained during the duration of the infusion, whereas, after the high dose of 5 μg./Kg./min., the steep initial rise is followed by a decrease to nearly normal values, in spite of the continuation of the infusion at the same rate.

**Effect of Renin on Angiotensin Response**

Whereas, after the administration of a small dose of renin, the response to angiotensin is not altered, higher doses of renin, given as a single injection or in the form of an infusion, reduce the sensitivity to angiotensin (figs. 5 and 7). Sensitivity to angiotensin is restored together with that to small doses of renin within 45 to 60 minutes. The responsiveness to norepinephrine is not influenced by renin, whether it be given in small or large doses.

**Effect of Angiotensin on Renin Response**

A high single dose (25 to 50 μg./Kg.) of angiotensin reduces the pressor response to a subsequent injection of renin in the same
way as it does to angiotensin (figs. 3 and 5). Again, after about 50 to 80 minutes, the previous responsiveness to renin is restored together with that to angiotensin.

Discussion

Comparing the dose-response curves for renin and for angiotensin we find a linear dose-dependent pressor response over a range of 2 powers of 10. With regard to renin, our results are similar to those of Blacket et al., but differ from those reported by Goldblatt, who found a flattening of the curve at relatively low dose levels, a fact which might be due to the different renin preparation employed. The remarkable result of our experiments is, however, that the dose-response curves of renin and angiotensin in the dog run almost parallel. On a weight basis, 1 mg. of our crude renin preparation liberates about 0.0002 mg. of angiotensin at the time of the maximum pressor response. This ratio was also found in other experiments on which we will report separately.

The results of our experiments have confirmed that it is possible to obtain a tachyphylactic effect with renin. We furthermore confirmed the finding of Goldblatt et al. that the time interval between the injections is of importance for the intensity of this phenomenon. In addition, we found that small doses of renin which lead to a moderate increase of blood pressure do not produce tachyphylaxis, even if they are injected rather rapidly one after the other. Furthermore, the steady infusion of low doses of renin is followed by an elevation of blood pressure to a constant level without any signs of tachyphylaxis. These findings are comparable to the reactions obtained after repeated injections of small or medium doses of angiotensin, which also lead to a reproducible blood-pressure response without tachyphylaxis. As with other pressor agents, increasing doses of renin not only reinforce the intensity but also prolong the duration of the hypertensive response. In the case of renin, however, this is only true within a certain dose range, which, if exceeded, results in a decrease in the duration of the hypertensive response. With angiotensin, this effect is less distinct because of its shorter duration of action.

In contrast to the findings of other investigators and also to those previously reported by us using small doses of angiotensin, we were able to demonstrate that there is a definite tachyphylaxis to angiotensin, provided that sufficiently high doses (e.g., 25 to 50 µg./Kg.) are given. Angiotensin in high doses also induces tachyphylaxis toward renin. Consequently, we may state that, in addition to the known fact that renin by itself exhibits tachyphylaxis and that it provokes a tachyphylaxis toward angiotensin, there is complete cross tachyphylaxis, since angiotensin too is capable of having a similar influence on the response to renin. With both substances the tachyphylactic phenomena are dose-dependent, i.e., they are only demonstrable above a certain dose level.
Repeated injections of increasing doses of renin (R). After a large dose of angiotensin (H), the effect of a small or medium dose of angiotensin or renin is reduced. All doses in micrograms per kilogram. Upper trace: heart rate; lower trace: blood pressure.

Figure 5

Effect of a high dose of renin (10 mg./Kg.) on venous pressure (above), heart rate (middle), and arterial pressure (below) of the conscious dog. The reaction to a medium dose of renin (1 mg./Kg.) is abolished, although blood pressure has returned to its initial value.

Figure 6

Hill and Pickering reported earlier on the effects of continuous infusions of renin in the rabbit. From their results it is apparent that with high doses of renin the blood pressure begins to return toward the control values after the initial high values were reached, indicating a tachyphylactic reaction comparable to that observed by us in our experiments of shorter duration. Also in agreement with our results is the lack of tachyphylaxis during the infusion of low doses of renin.

These results lead us to consider the dynamic relationship between the liberating enzyme and the polypeptide. If, in vivo, angiotensin were really the reaction product of renin, the observations reported in this paper could all be explained on the basis of the tachyphylaxis to angiotensin. Angiotensin by itself, if given in high doses, produces tachyphylaxis towards angiotensin as well as towards renin, which acts by liberating angiotensin. Correspondingly, high doses of renin which liberate high doses of angiotensin produce tachyphylaxis towards angiotensin, whether given directly or liberated by subsequent doses of renin. The longer duration of the effect of renin would then have to be explained by the fact that it liberates angiotensin as long as it is present in the plasma. We have, however, always to bear in mind that all the reactions described in this paper and reported by other investigators deal with the effects of intravenously injected renin or angiotensin, i.e., with systemic actions of a humoral factor which perhaps under normal conditions may never enter the systemic cir-
culation. Hence, it is in no way proved that the liberation of angiotensin by renin and the tachyphylaxis which develops to angiotensin play any physiological role.

The mechanism of the tachyphylaxis which develops to renin and angiotensin is not clear. Certainly, it is not due to a continued state of contraction of smooth muscle in the arterioles. If it were the case, it would be necessary to assume that a change in the distribution of local vascular resistance occurs, so that areas sensitive to angiotensin remain constricted while others develop compensatory dilatation permitting the subsequent fall of blood pressure or at least preventing a further rise. Such an explanation does not recommend itself to us. Based on the observation that the cardiac output, as well as the blood pressure, can decrease during an infusion of renin, Langford discussed the possibility that the decrease in cardiac output could be involved in the development of the tachyphylaxis. In our experiments the venous pressure in the vena cava was found to run parallel to the arterial pressure after injection or infusion of both renin and angiotensin at all dose levels, thus excluding at least right ventricular failure as a cause of the tachyphylactic response. Furthermore, the fact that the response to norepinephrine is not diminished during the time when tachyphylaxis to angiotensin or renin is established suggests that the heart is not the responsible factor.

In addition, Langford claims that a hypothetical “substance x” liberated by renin simultaneously with angiotensin acts as a competitive antagonist to angiotensin. If, as is strongly suggested by our experiments, renin tachyphylaxis is due to angiotensin tachyphylaxis, the factor x of Langford is not necessary to explain the phenomena observed.

There remains the factor of enzymatic de-
struction of either renin or angiotensin. Angiotensinase is available in abundance in the blood and in various tissues, but little is known about the activity and reaction kinetics of this enzyme. In experiments which will be described elsewhere, we did not observe a more rapid destruction of angiotensin by the plasma of animals showing tachyphylaxis to angiotensin following a high dose, and therefore a variation in angiotensinase activity seems unlikely to be responsible.

We, therefore, conclude that this tachyphylactic phenomenon is due to a transitory decrease in sensitivity of the receptor which reacts to angiotensin, although we are aware that this is only a circumlocution, and we do not really understand the underlying mechanism.

Summary

In the conscious dog, synthetic angiotensin and crude hog renin produce dose-response curves which run strictly parallel if the maximum blood pressure attained is taken as the parameter. Repeated injections of small or medium doses of angiotensin or of small doses of renin do not produce tachyphylaxis. In contrast, high doses of renin, as well as of angiotensin, diminish or abolish the pressor response to subsequent injections of the same substances. Furthermore, cross tachyphylaxis between renin and angiotensin can be demonstrated. Infusions of renin and angiotensin in various dosages elicit characteristic blood pressure changes, which are very similar for both substances and also display tachyphylaxis at sufficiently high doses. It is concluded that so-called renin tachyphylaxis is, in fact, due to a diminished response to angiotensin. This might be taken as an indirect proof that renin liberates angiotensin not only in vitro but also in the organism.

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K. D. BOCK and F. GROSS

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