PHARMACOLOGY OF RENIN AND HYPERTENSIN

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Although this review concerns only the pharmacology of renin and hypertensin, a short explanatory introduction on the renin-hypertensin system was considered indispensable. The story of the discovery of this system has been told more than once. It is one of many examples in the history of science where two groups of investigators (21, 155, 170), following different approaches, have arrived simultaneously at the same discovery.

This led to a difference in nomenclature which has unfortunately persisted, creating some confusion. In the discussion which follows, the nomenclature of our group will be used exclusively, only for the sake of clarity. Hypertensinogen, hypertensin, and hypertensinase are thus synonyms of renin-substrate, angiotonin, and angiotoninase, respectively. The account of some aspects of the problem under review will be brief, since there have been previous publications on related subjects with complete bibliographies (25, 69, 167, 168, 186).

I. THE RENIN-HYPERTENSIN SYSTEM

1. Renin. Renin is a protein extracted from the kidney which interacts with hypertensinogen to give rise to the formation of hypertensin. This reaction is probably enzymatic and has an optimum pH of 7.5 to 8.5 and an optimum temperature of 37° to 39°C. The velocity of the reaction depends upon the amount of renin present, this property having been utilized for its assay (118).

Renin can be considered as a proteolytic enzyme (22, 189), although it is difficult to identify it with any of the known intracellular proteolytic enzymes.

Differences in the behaviour of renin from different animals have been observed. Renin from man, monkey, and baboon acts on the hypertensinogen of all mammals, while the renins of other animals do not act upon the hypertensin-

ogen of primates (57). Birds appear to have a renin which acts exclusively on the hypertensinogen of birds. In batrachians, reptiles, and fish, it would appear that there is no renin (8).

a. Site of formation of renin and the renin content of the kidney. There are still no satisfactory methods for the extraction and quantitative determination of renin, in spite of recent progress in this matter (83, 84). Consequently the amount of renin present, the site of its formation, and the way it is stored in the kidney are still matters of discussion.

With these reservations, two theories concerning the site of renin formation have been proposed. One of them considers that the epithelium of the proximal convoluted tubule is mainly responsible for the formation and the accumulation of renin (65); the other (76) assumes the juxta-glomerular apparatus to be responsible.

Some recent observations favour the view that renin can be inactivated by kidney tissue and transformed reversibly into an enzymatically inactive form (72). This observation may account for many of the contradictory results found in the literature concerning the renin content of the kidneys of various species and under different experimental conditions.

b. Secretion of renin by the kidney. Although some improvement has been made in the methods for the quantitative determination of renin, a reliable and sufficiently sensitive method still remains to be found for the determination of renin in blood and other body fluids. With the methods available it has been shown that acute or chronic partial occlusion and complete occlusion of the renal artery produce a liberation of renin. Renin, however, has not been demonstrated in the systemic blood of dogs, or other animals, with chronic hypertension from renal ischemia (25).

Arterial hypotension as a result of hemorrhage or shock produces a secretion of renin by the kidney, renin being detectable in the systemic blood (85, 86, 106, 204). The stimulus which produces a secretion of renin is still not known with certainty. Diminution of pulse pressure (89, 110), diminution of blood pressure (a barosensitivity of the kidney) (52), and changes resulting from renal ischemia (25) are the factors most commonly suggested.

Injected renin disappears rapidly from the blood and is either fixed, inactivated, or destroyed by tissues throughout the body. Of the abdominal organs, the kidney and the liver are the only ones that appear to be involved, to any appreciable extent, in the mechanism which causes renin to disappear (103).

2. Hypertensinogen. Hypertensinogen (renin-substrate) is the substance on which renin acts to produce hypertensin (22, 170). Up to this time hypertensinogen has been found only in plasma, where it is a component of the α-globulin fraction (191). Hypertensinogen in plasma may be measured by incubating it with an excess of renin and then determining the amount of hypertensin formed (117). One unit of hypertensinogen is that amount which gives rise to one dog unit of hypertensin. The normal amount contained per ml. of plasma is approximately 0.75 dog unit in man, 0.3 unit in dogs, and 0.45 unit in rats (25).

Hypertensinogen is formed in the liver and its concentration in plasma reflects mainly a balance between its production by the liver and the transformation

of hypertensinogen to hypertensin by renin from the kidney (119). The rate of formation of hypertensinogen by the liver is increased by adrenal cortical hormones (68), estrogens (95), and by ACTH (90, 91).

3. Hypertensinase. Hypertensin is destroyed by serum, plasma, red blood cells, and tissue extracts (kidney, liver, intestinal mucosa, etc.). This effect is due to a thermolabile non-dialyzable substance which has the properties of an enzyme, and which has been called hypertensinase (58). Most of the hypertensinase activity of tissue extracts is probably due to proteolytic enzymes, although other types of enzymes may well destroy hypertensin.

Hypertensinase activity has been found in yeast (44), snake venom (37a), several plants (73), and cultures of different fungi (11).

Hypertensinase may be assayed by measuring the amount of hypertensin destroyed after incubating a known amount of hypertensin with the hypertensinase solution to be investigated (58). The unit of hypertensinase is defined as that amount which destroys 0.5 dog unit of hypertensin in 4 hours, under certain conditions of pH and temperature (58).

4. Hypertensin. Hypertensin is the product of the interaction of renin and hypertensinogen. This same substance was called angiotonin by Page and Helmer (170). It is a polypeptide, and several attempts have been made to obtain it in a pure form (27, 28, 53, 93, 111, 182, 222). Its pressor activity is considerable, being one and one-half to three times more active, weight per weight, than noradrenaline (norepinephrine, arterenol) (181).

The identification of hypertensin depends upon biological methods, since no specific chemical reaction has been developed. Nevertheless, hypertensin may be differentiated from other vasoactive substances by its chemical properties. Hypertensin is destroyed by hypertensinase, this being a very specific reaction which permits its identification with certainty. Pitressin, which is similar in solubility, is less resistant to heating with acid and is destroyed *in vitro* by incubating with 0.01 M sodium thioglycollate (233). Pepsitensin is more resistant than hypertensin to the destructive action of hypertensinase of red blood cells (24).

Hypertensin can also be differentiated from other pressor substances by pharmacological procedures. The type, rapidity, and duration of the response may be used as a criterion to differentiate hypertensin from various other vasopressor substances. The intravenous injection of hypertensin in animals, intact, anesthetized, or with destruction of the spinal cord, causes a rise of blood pressure without tachycardia, which reaches its maximum in forty-five to sixty seconds and lasts about three minutes, without a secondary fall. The rise is less rapid than that caused by adrenaline (epinephrine), which causes tachycardia and a secondary fall, or by serotonin; and it is less prolonged than that caused by ephedrine, tyramine, and pitressin.

The pressor action of hypertensin is not influenced by sympathicolytic drugs.

II. ASSAY AND UNITAGE SYSTEM OF RENIN AND HYPERTENSIN

1. Assay of renin. Renin can be measured by a direct method, *i.e.*, by its pressor action when injected intravenously, and by indirect methods: namely,

TABLE I

Unitage system of renin

Direct method

Authors	Animal	Weight	Experimental conditions	Rise in blood pressure
				mm. Hg
Schales and Haynes (207)...	rabbit	per kg.	unanesthetized	30
Pickering and Prinzmetal (187).....	rabbit	unspecified	unanesthetized	20–30
Swingle *et al.* (225).....	dog	unspecified	nembutal	40
McEwen *et al.* (134).....	dog	unspecified	morphine-ether	30
Wakerlin and Chobot (235).	dog	unspecified	nephrectomized	40
Goldblatt *et al.* (71).....	dog	10–25 kg.	unanesthetized	30–35 in 3 min.
Hessel (99).....	dog	10 kg.	pernocton*	30

Indirect method

Authors	Substrate	Temp.	Time	pH	Hypertensin formed
		°C.	*hrs.*		
Leloir *et al.* (118).....	hypertensinase-free hypertensinogen	37	2	7.4	0.5 Buenos Aires unit
Dexter *et al.* (51).....	hypertensinase-free hypertensinogen	37	2	7.4	1 Indianapolis unit

* Sodium-5-(2-bromoallyl)-5-sec.-butylbarbiturate.

a) by measurement of the hypertensin formed *in vitro* when renin is incubated with hypertensinogen and b) by measurement of the hypertensinogen which it is capable of destroying *in vitro* in a given time.

Direct method: intravenous injection. Intravenous injection in trained, normal, unanesthetized dogs (71) is probably the best direct method available because of the reproducibility of its results. Using this method, a dog unit was defined as the amount which raised the blood pressure 30 mm. Hg. This effect is apparently independent of the weight of the dog within wide limits (71, 72). The method has only two disadvantages: 1) the necessity of having several trained dogs available in order to conduct an assay, and 2) its relatively poor sensitivity.

Apart from the unanesthetized dog, many other preparations have been used for the assay of renin by its pressor action: dogs, anesthetized with chloralose or nembutal, nephrectomized, or with section of buffer nerves; anesthetized or pithed cats; anesthetized, pithed, or nephrectomized rats; unanesthetized rabbits, etc. (Table I).

Although no comparative observations have been made of the sensitivity of different test animals to the intravenous injection of renin, our experience suggests that it parallels the sensitivity to hypertensin. In other words, the pithed cat is approximately five times and the nembutalized rat fifty to one hundred times more sensitive than the unanesthetized dog.

Schales and Haynes (207) had stated earlier that renin produced the same

elevation of blood pressure in the dog as in the rabbit when the same amount was injected per kg. of body weight. This observation agrees with our own, since the weight relation between dog (10 kg.), cat (2 kg.), and rat (200 g.) is more or less the same as the relative sensitivity to renin of these animals. Perhaps the lack of correlation of weight and pressor action of renin, found in the dogs by Goldblatt and coworkers, depends on the fact that the increase of the blood pressure was proportional to the dose of renin only up to 1.2 units. In other words, their unit is too near the inflexion of the correlation line to the horizontal, and, thus, too near to the maximal pressor response to renin.

For hypertensin, as well as for renin, an international standard preparation should be adopted so as to permit comparison of potencies or units. The necessity of an international standard for renin and for hypertensin is urgent, since many investigators are working on the purification of both substances, and the degree of purification is expressed as units per weight of dry substance. As Pickering (186) expressed it, "a unit must, if possible, be defined in terms of weight of a particular substance, samples of which should be obtainable from a central source."

Indirect methods. a) Measurement of hypertensin formed *in vitro*, when renin is incubated with hypertensinogen.

This method was first devised by Leloir *et al.* (118) and has been adopted, with variations, by many authors. It consists in the incubation, for two hours at pH 7.4 and 37°C., of the renin-containing solution with an excess of hypertensinase-free hypertensinogen. An interesting variation, introduced by Sapirstein *et al.* (204a), consists in incubating at 0°C. for longer periods of time (twelve to twenty-four hours). At this temperature renin remains active, while hypertensinase is inactive; thus the previous destruction of hypertensinase by acidification can be avoided. The hypertensin formed is then assayed by its pressor action (118), or by its action on the vascular preparation of the toad (59), or on the isolated ileum of the guinea-pig (184).

Leloir *et al.* (118) defined a unit of renin (indirect method) as the amount which produces 0.5 dog pressor unit of hypertensin in two hours under the conditions described. Dexter *et al.* (51) defined a renin unit as the amount which yields one Indianapolis unit of hypertensin after two hours' incubation under the same conditions.

This indirect method is much more sensitive than the direct method. One Leloir *et al.* unit is equivalent to 0.01 Buenos Aires unit. But, by using the vascular preparation of the toad, the guinea-pig ileum, or even the blood pressure of the anesthetized rat, for the estimation of the hypertensin formed, much smaller amounts of renin can be detected. There is much confusion as to the unitage system of renin, and a precise quantitative comparison of the sensitivity of different methods for the assay of renin will be practically impossible until an international preparation of known potency is adopted.

b) Measurement of the amount of hypertensinogen which disappears. In this procedure the amount of hypertensinogen which disappears as a result of the action of renin is measured (118). In order to do this, renin is incubated with

hypertensinogen in the presence of hypertensinase. The latter destroys the hypertensin formed during the prolonged incubation. If the hypertensinogen is measured before and after incubation, there will be a difference, proportional to the amount of renin present. This method has been used for the measurement of human renin (157).

2. Assay and pressor units of hypertensin. Several methods may be used for the assay of hypertensin, but probably the best method still available, because of its specificity, consists in measuring the pressor effect of hypertensin in the dog, cat, or rat, comparing the rise of blood pressure produced by an unknown solution of hypertensin with that caused by a solution of known potency (25). The test animals may be pretreated by drugs (dibenamine (50), tetraethylammonium chloride (132), ephedrine (23)) or by different operations (section of buffer nerves (132), nephrectomy (133), destruction of central nervous system (64)), in order to increase their sensitivity or enhance the specificity of the reaction.

The relation between the amount of hypertensin injected and its pressor action has been studied (22, 23). It was concluded that, within certain limits, the following formula might be applied: $u = (d/t)^2$ in which u represents units of hypertensin in the unknown sample, d, the rise in mm.Hg of blood pressure produced by the injection of the unknown sample, and t, the rise produced by the injection of one unit of hypertensin. The same formula may be applied to the pressor action of adrenaline (23).

Using pithed rats or pithed cats as test preparations, Valle *et al.* (232) showed that within a sufficiently large zone there is a linear regression between the logarithm of the dose and the blood pressure increase produced by hypertensin. Within this range of linearity it was possible to develop a four-point assay for hypertensin following the principles used by Schild (209).

The comparison of the pressor response to hypertensin with that to other drugs, such as adrenaline (23, 51), noradrenaline (192), or tyramine (54), has some drawbacks. In the first place, the dose-effect regression curves of two different pressor drugs may not run parallel and, furthermore, some of the pressor substances mentioned may influence the response to hypertensin.

A real problem, not yet solved, is to obtain agreement among different workers as to the relative sensitivity of the test animals. No international standard for hypertensin has yet been adopted and, consequently, much confusion still arises when hypertensin units or potencies must be compared. This is due to the different definitions, conditions of test objects and types of assays used by different workers.

In the absence of an international standard, there are nearly as many different units defined as there are groups of workers in the field. (See Table II.) Comparison of different units is, for the reasons stated, very difficult. Attempts to solve this problem have been made with some success. According to Prado *et al.* (192), 1 Buenos Aires unit = 5 Indianapolis units and 1 Indianapolis unit = 0.36 Edman unit. It has been reported (232) that the sensitivity of the pithed cat and rat is of the same order of magnitude and that the precision is

TABLE II

Unitage system of hypertensin

Unit	Animal	Weight	Experimental conditions	Rise in blood pressure
				mm. Hg
Buenos Aires unit (23).....	dog	10 kg.	chloralose	20–30 (=4 μg. adrenaline)
Goldblatt unit (69).....	dog	unspecified	unanesthetized	30
Pressor unit (145).....	dog	unspecified	anesthetized	15
Indianapolis unit (190).....	cat	unspecified	pithed	30–50
Edman unit (54).....	cat	2.5–4 kg.	chloralose	50–60 (=0.15–0.25 mg. tyramine phosphate)

better with the cat. We have used the rat anesthetized with nembutal instead of the pithed rat, and have found that its sensitivity to hypertensin is much greater than that of the nembutalized cat. According to our experience, 1 dog unit corresponds approximately to 5 cat units and to 50 to 100 rat units (animals anesthetized with nembutal).

3. Other methods of assay of hypertensin. The Laewen-Trendelenburg vascular preparation of the toad (*Bufo arenarum* Hensel (59) or *Bufo ictericus* Spix (184, 192)), has been used for the assay of hypertensin. With this preparation it is possible to detect amounts of hypertensin of 1/200 to 1/500 Buenos Aires unit per ml. or of 1/40 to 1/20 Indianapolis unit. The main disadvantage of this preparation, apart from being less specific and quantitative than the pressor assay, lies in the fact that one has to wait a long time (about ten to twenty minutes) between successive tests. During the interval the sensitivity of the preparation may vary, thus interfering with the precision of the observation.

Bioassay of hypertensin using the isolated ileum of the guinea-pig was first used by Collins and Hamilton (31), and has been developed and studied by the Sao Paulo group of investigators (184, 193). Its sensitivity is approximately the same as that of the vascular preparation of the toad (it can detect between 1/40 to 1/20 Indianapolis unit); it has many advantages, such as simplicity of preparation, economy of time, and applicability to a four-point assay. Its only disadvantage, apart from its doubtful specificity, lies in the unpredictable variation of sensitivity of the preparation during the course of an assay. Unfortunately, none of the methods of assay is absolutely free from this objection.

The isolated rat uterus has also been used for assay of hypertensin (211), recording the time between the addition of the sample to the bath and the maximum contraction. The smallest amount which can be estimated in a bath of 10 ml. capacity varies from 1/10 to 1/20 dog unit.

III. PHARMACOLOGY OF RENIN AND HYPERTENSIN

1. Renin pressor action. a. Time course of pressor action. Renin was discovered by its pressor action (229). This property has been amply confirmed by many investigators. The intravenous injection of one unit (Goldblatt unit) of renin

into a trained unanesthetized dog produces a rise in blood pressure of about 30 mm.Hg, which becomes manifest in ten to twenty seconds, reaches its maximum in two minutes, and returns to normal in about thirty minutes (25, 72). The blood pressure elevation is proportional to the amount of renin injected up to a rise of 35 mm.Hg (1.2 units) (72). For larger doses the elevation of blood pressure is no longer proportional but is sustained much longer. The areas under the curves, which represent the increment of pressure as a function of time, are directly proportional to the doses between 1 and 3 units (72).

Renin elicits also a pressor response when injected intraperitoneally in anesthetized rats (45, 62). Subcutaneous injection of 14 to 28 Goldblatt units of renin into rats, maintained on 1 per cent sodium chloride solution, causes a slight sustained rise in blood pressure. This effect is potentiated by bilateral nephrectomy (38) and, in unilaterally nephrectomized rats, by pretreatment with sodium chloride and desoxycorticosterone acetate (146). Subcutaneously injected renin does not increase the blood pressure in normal dogs (70). However, it has a prolonged pressor action in dogs in which the sensitivity to renin has been enhanced by section of the carotid sinus and aortic depressor nerves and by premedication with tetraethylammonium (132). By intramuscular injection renin has proved ineffective in raising the blood pressure of dogs or cats (214), probably due to insufficient dosage or other interfering circumstances, such as anesthesia, etc.

b. Tachyphylaxis. When repeated intravenous injections of renin are made at short intervals, the pressor response becomes successively less; finally, no response is obtained despite the injection of large doses. This phenomenon, improperly called tachyphylaxis, has been studied by numerous investigators (97, 99, 109, 112, 116, 134, 163, 171, 187, 229, 236).

Tachyphylaxis is not an exclusive property of renin. It is also observed with other drugs, such as pitressin, amphetamine, methamphetamine, ephedrine, etc. (241). Evidence from many sources indicates that the so-called tachyphylaxis to a pressor drug occurs: 1) when the substance is not rapidly destroyed in the organism; 2) when the receptors (*i.e.*, smooth muscle cells) are in a state of maximal response to the substance. The latter condition may be present in spite of a restoration of the blood pressure to its original level, due to the intervention of homeostatic mechanisms (66).

Tachyphylaxis to renin has some special characteristics. As we have seen, its pressor action is due to the formation of hypertensin from hypertensinogen. Successive injections of renin produce a progressive diminution of hypertensinogen in the plasma. When the latter becomes exhausted, a new injection of renin produces no effect (163). It has been shown that renin remains in the blood stream of a dog for at least thirty minutes after the injection of a large dose (80 to 120 dog pressor units) (103). It may be concluded that "renin tachyphylaxis is due to the persistence of renin in the blood and to the exhaustion of hypertensinogen" (25). In a more recent study, using renin of high purity, Goldblatt *et al.* (72) conclude that "for a relatively constant elevation of blood pressure to be maintained as the result of the injection of renin, two conditions

must be satisfied: 1) there must be an adequate supply of hypertensinogen in the blood; 2) the arterioles must be capable of responding adequately to further stimulation by hypertensin".

These two conditions determine generally the pressor response to the injection of renin in various experimental conditions. But, to interpret correctly some cases, it must be borne in mind that the intact kidney is able to secrete renin into the blood, when it is injured (196), or when its blood supply diminishes due to arterial hypotension, shock, etc. (106). This renal secretion of renin may influence the pressor action of injected renin by acting upon the two aforementioned conditions: the hypertensinogen concentration in the blood and the reactivity of the arterioles to hypertensin. Due to the disregard of these two factors (and especially of the secretion of renin by the intact kidney), many contradictory results have been reported on the effect of different experimental conditions upon the pressor response to injected renin.

c. Experimental conditions which modify the pressor action of renin. In considering the experimental conditions which modify the pressor action of renin, apart from the factors just mentioned, other important factors should be taken into account, which refer to other pressor substances as well. The blood pressure response is the complex result of the direct action of the agent on the target organs and of the homeostatic mechanisms which may come into play to counteract the pressor effect. The response of the different target organs, as well as the regulator mechanisms involved, may vary independently under the different conditions encountered in experimentation. Therefore, it cannot be expected that a given drug will always elicit the same response. The state of affairs which is present when one injects a pressor drug, and which will determine the magnitude of the response, may be termed "cardiovascular reactivity", and has been defined as "the degree with which the heart and peripheral vascular bed respond to quantitative stimuli . . ." (174). It is highly variable, not only from animal to animal of the same species, but in the same animal from time to time (174). This great variability should make us very cautious in concluding that a change in magnitude of the pressor response is directly related to the factor which has been experimentally introduced or suppressed.

In order to clarify the problem, we will divide the conditions which may modify the pressor action of renin into two groups: 1) those which affect the *in vivo* formation of hypertensin, and 2) those which affect the sensitivity to hypertensin.

1) The *in vivo* formation of hypertensin, when renin is injected into the blood stream or gains access to the blood through other routes, is still incompletely understood, due to the lack of sufficiently sensitive and quantitative methods for studying the concentration of hypertensin in plasma. However, indirect evidence shows that the amount of hypertensin formed is closely related to the concentration of hypertensinogen in the blood. Hypertensinogen is formed in the liver, and its rate of formation appears to be under the influence of adrenal cortical hormones; its concentration in the blood depends on the rate of its formation by the liver and upon its destruction by endogenous or exogenous renin. Experimentally it increases after nephrectomy (32, 117, 156, 171), after

treatment with ACTH (90, 91), stilbestrol, and other estrogens (95, 96), and in hypertension with renal insufficiency. It becomes reduced following the injection of renin, following adrenalectomy, and in hypotension or shock (25).

Nephrectomy increases the sensitivity to renin: the magnitude and duration of its pressor action are increased. This fact, first observed by Tigerstedt and Bergman (229), was confirmed later by many investigators. The increased sensitivity appears only after several hours, and is maximal at approximately forty-five hours after bilateral nephrectomy in the dog (104, 133). The greater and more prolonged pressor response to renin of nephrectomized dogs is due probably to a combination of factors: a) the persistence of renin in the blood for a greater length of time (103); b) an increase of hypertensinogen in plasma (156) and c) a hypersensitivity of the vessels to hypertensin (104, 133, 171, 201). The sensitivity to renin of animals injected with ACTH or estrogens has not been explored.

In experimental renal hypertension sensitivity to renin seems to be normal in the benign phase (164), but may increase in the malignant phase (172).

When plasma hypertensinogen becomes reduced, sensitivity to the pressor action of renin is also reduced. Such is the case after a previous injection of renin, a condition already discussed under tachyphylaxis. In adrenalectomy and shock the sensitivity to renin is reduced, probably due to the reduction of plasma hypertensinogen and the reduced sensitivity of the blood vessels to hypertensin, which are present in both experimental conditions. It is known that when the blood pressure is low, as in adrenal insufficiency or in shock, the normal kidney secretes renin into the blood stream. Although the relation between sensitivity to the pressor action of renin, concentration of hypertensinogen in plasma, and concentration of renin in plasma has not been systematically studied in these experimental conditions, indirect evidence points out that such a relation exists.

2) Experimental conditions which affect the sensitivity of the blood vessels to hypertensin affect also, and in the same sense, the sensitivity to renin. (See Table III.)

d. Influence of drugs. Drugs may influence the sensitivity to the pressor action of renin because of 1) an influence on the rate of formation of hypertensin, 2) a fall in blood pressure causing the kidney to secrete renin, thus reducing the sensitivity to injected renin, and 3) an influence on the sensitivity of the blood vessels to hypertensin.

1) No drug is known to accelerate or inhibit *in vivo* or *in vitro* the reaction hypertensinogen → hypertensin. But, as has been mentioned, several substances, such as ACTH, stilbestrol, and other estrogens, may cause an increase in the concentration of plasma hypertensinogen. The action of these substances on the sensitivity to the pressor action of injected renin has not been studied.

2) Drugs which cause a fall in blood pressure may produce a reduction in the sensitivity to injected renin because the normal kidney is secreting renin into the blood. In order to eliminate this factor, the action of such drugs should be studied in nephrectomized animals. As most, if not all, of the investigations have

been carried out in normal animals, the conclusions derived from them should be considered provisional. Ether, urethan, and nembutal anesthesia apparently diminish the pressor action of renin in rabbits and dogs.

3) Drugs having an influence on the sensitivity to hypertensin have a parallel action on the sensitivity to renin. (See Table IV.)

e. Prolonged hypertension with renin. Acceptance of the hypothesis that arterial hypertension due to renal ischemia is caused by renal secretion of renin needed the demonstration of a sustained hypertension by the continuous intravenous infusion of renin. Previous attempts had given unsatisfactory results. But, in more recent experiments, it has been shown that by the constant infusion of adequate amounts of renin a moderate hypertension can be maintained in unanesthetized rabbits (14) or dogs (72) during varying periods of up to twenty-three days (14).

2. Hypertensin pressor action. a. Type of blood pressure elevation. Intravenous route. The intravenous injection of one unit of hypertensin (dog unit of Goldblatt) into a trained unanesthetized dog produces a rise of blood pressure of about 30 mm.Hg. The curve reaches a maximum in forty-five to sixty seconds and the pressure returns to normal within three minutes without any secondary fall. The same type of curve is obtained in other mammals (cat, rabbit, rat) anesthetized with chloralose, nembutal, ether, or other substances.

By increasing the dose a direct proportionality between hypertensin concentration and blood pressure rise is obtained only up to 0.8 to 1 unit (72, 226). For amounts greater than 1 unit the formula $u = (d/t)^2$ may be applied, as described p. 30.

The duration of the blood pressure elevation is related to the dose of hypertensin. The areas under the curves of blood pressure are, in the unanesthetized dog, nearly directly proportional to the dose of hypertensin up to 5 units (72).

Other routes of administration. No rises of pressure have been obtained by the administration of hypertensin subcutaneously, intramuscularly, or intraperitoneally, possibly due to insufficient doses or a too slow absorption (25).

b. Response to repeated injections. As long as the experimental conditions remain constant (level of blood pressure, degree of anesthesia, respiratory rate, temperature, and so forth), the injection of a given amount of hypertensin produces, in mammals, the same response, even though the interval between injections is short (three to five minutes) over a period of two to six hours. But tachyphylaxis occurs in the toad and serpent (8), probably due to the fact that, in these animals, the destruction of hypertensin takes place very slowly, because hypertensinase has a slow action at 15–18°C.

c. Continuous injection. If hypertensin is injected intravenously into an animal continuously for thirty or more minutes, a rise in blood pressure is produced and is maintained throughout the infusion (22, 168).

d. Conditions which modify the reaction to hypertensin. The previous injection of large amounts of renin reduces, or even abolishes, the pressor response to the injection of hypertensin (72). The same occurs when the kidney secretes relatively large amounts of renin into the blood, as may happen after total renal

ischemia, after lowering of the blood pressure by hypotensive drugs, anesthetics, serious operations, shock, etc. Most investigators have not taken into account this complicating factor and, consequently, their results should be considered with reservations. Furthermore, the same considerations previously made for renin should apply to the case of hypertensin: the great variability in the so-called cardio-vascular reactivity from animal to animal, and in the same animal from time to time, should make us cautious in concluding that a change in magnitude of the pressor response is directly related to the factor which has been experimentally introduced or suppressed.

In Table III are listed some of the conditions which allegedly modify the reaction to hypertensin.

Renin-hypertensin and the baroceptor reflexes. Reflexes arising in the carotid sinus and aortic baroceptors tend to counteract the pressor responses to injected drugs. Acute section of the carotid sinus and aortic depressor nerves increases the pressor action of renin and hypertensin (55, 132). On the other hand, Thomas and McLean (228) found that responses to hypertensin were unchanged in chronic neurogenic hypertensive dogs, while Page *et al.* (175) found increased pressor responses to both renin and hypertensin, but not to noradrenaline. By perfusing the carotid sinus by a pump at a steady pressure and sectioning the other three buffer nerves, Page *et al.* (175) also showed enhanced responses to noradrenaline and hypertensin.

The role of the baroceptor reflexes in the regulation of blood pressure and in the establishment and maintenance of hypertension is one of the riddles of experimental medicine. The humoral agent of hypertension, whatever it may be, must exert its action against those powerful regulators, or else influence the neural mechanisms in such a way as to set them at a new level of sensitivity (18). It is a fact that renin by constant infusion may produce a long maintained hypertension (14), while noradrenaline and adrenaline do not. It has also been shown that during infusion of hypertensin or renin the pressor response to carotid artery occlusion is increased, while adrenaline causes a decrease and tyramine no change (9).

The important work of Heymans and coworkers (101) has shown that the distensibility and resistance to stretch of the arterial wall where the pressoceptors are located is an important factor in their mode of action and may be modified by the local action of drugs. Unfortunately, the local effect of hypertensin on the activity of carotid pressoceptors has not yet been studied.

e. Influence of drugs. Early in the pharmacological study of hypertensin it was found that its pressor action is not prevented, nor substantially modified, by the previous injection of sympathicolytic (or adrenolytic) or parasympathicolytic substances. On the other hand, the previous injection of renin diminishes, or even abolishes, the pressor action of hypertensin. If the action of the latter substance is maximal on its specific receptors, these will not respond to further stimulation.

It is universally accepted today that hypertensin has a direct vasoconstrictor action which is not mediated by the sympathetic system nor by sympathicomimetic substances.

TABLE III

References concerning the influence of experimental conditions upon the pressor action of renin and hypertensin

Experimental condition	Hypertensin			Renin		
	Decrease	No effect	Increase	Decrease	No effect	Increase
A. Nervous system						
Sharp trauma to brain	168			55		
Section of spinal cord			168, 172		150	
Spinal anesthesia			79			55
Total sympathectomy			172			
Section vagal nerves		21, 23, 132			229, 163, 156	
Same and occlusion carotid sinus		175				
Section of buffer nerves (acute)		23	55, 175			55, 175
B. Hypotension and shock						
Hemorrhagic shock	151			151		
Hemorrhagic hypotension and nephrectomy			175			
Shock	166					
Shock by acute section of spinal cord				131		
C. Extirpation of organs						
Hypophysectomy					150, 104	
Thyroidectomy	168					
Adrenalectomy (untreated)	23		223	224, 47, 99, 104		
Adrenalectomy acute		170, 221		221	150	
Hepatectomy			23			
Total abdominal evisceration			23			
Nephrectomy (24–48 hours)		201	23, 172, 104		201	229, 150, 234, 235, 170, 62, 104, 185, 77, 172, 80
D. Other experimental conditions						
Renal hypertension		164, 178			108, 178, 77, 172	240, 108, 170, 164, 107, 116, 185
Neurogenic hypertension		228, 172	175			175
Ligature of renal pedicle		43			43	
Ligature of hepatic artery		43			43	

TABLE IV

References concerning the influence of drugs upon the pressor action of renin and hypertensin

Drug	Hypertensin			Renin		
	Decrease	No effect	Increase	Decrease	No effect	Increase
A. Pressor drugs						
Noradrenaline infusion		175				
Veritol*			21, 23			
Ephetonine			21, 23			
Tyramine			21, 23			
Pitressin	21, 23					
Adrenaline		21, 23				
B. Anesthetics						
Ether				205, 187		
Urethan				187		
Nembutal				187		
C. Sympatholytic and adrenolytic agents						
Ergotamine		97		239	97, 99, 64	
Ergotoxine				55		
Dihydroergocornin		15				
Hydergin		15				
Fourneau 933		21, 23, 170			108, 156	
Yohimbine		243				
Dibenamine		244				
Tetraethylammonium			177			132
D. Other substances						
Cocaine		97, 170	23		99, 163, 64	239, 21, 163
Procaine			55			55
Atropine		21, 23, 170			99, 64, 163, 156	
Nicotine					100	
Sodium thyocyanate				45		
Dimercaprol (BAL)		169			169	
Desoxycorticosterone—acute		220, 219		220		221
Desoxycorticosterone—chronic		148 (dog)	81, 148 (rat)		148 (dog)	148, 80 (rat)
Cysteine		135				
Amine oxidase				210		
Benadryl	169					

* p-(2-Methylaminopropyl)phenol

In Table IV are listed some of the substances which may influence the magnitude of the pressor action of hypertensin. As has already been recalled, in order to conclude that a given drug or experimental condition increases or decreases the pressor action of hypertensin, a series of methodological and interpretative problems must be taken into account. In most cases the experiments mentioned in the literature have not been conducted with the necessary rigor and the conclusions derived are therefore only tentative. We believe that a systematic study of this problem is worthwhile and would probably shed light on the mechanism of action of hypertensin and incidentally on that of renin.

Two important problems are still unsolved which refer not only to the pressor action of hypertensin but also to other pressor substances. They are: a) the influence of the initial level of blood pressure on the magnitude of pressor response to a given dose, and b) the mechanism of the potentiating or inhibiting action of certain drugs on the pressor effect of a given dose.

3. Action of hypertensin on smooth muscles. Ludueña (130), in a comprehensive study of the action of hypertensin on smooth muscles, concluded that it produced contraction of almost all organs investigated, although the sensitivity of different organs varied. He studied the action of hypertensin on the uterus of rat, rabbit, guinea-pig, dog, and cat, the intestine of dog, rat, guinea-pig, and cat, strips of urinary bladder, gall-bladder, bronchial rings, vasa deferentia, isolated ureter, retractor penis of the dog, dorsal muscle of the leech, cat pupil and nictitating membrane.

His analysis of the action of hypertensin indicates that it may be classed among the musculotropic drugs, since it contracts the uterus of the rat and the intestine of dog, rabbit, and guinea-pig, which are relaxed by adrenaline. It also contracts the intestine and strips of bladder made insensitive to acetylcholine by atropine. Its motor action parallels neither sympathetic nor parasympathetic action.

Later studies have endeavoured to use the action of hypertensin in isolated organs for its assay, or for its differentiation from other substances.

Many of the published results have been conducted with preparations of hypertensin of varying degrees of purity; consequently many of the conclusions drawn from them are provisional and new studies should be made with purer preparations of hypertensin.

a. Action on the intestine. Intestinal peristalsis. Both angiotonin and renin injections produce in the rabbit (2) vigorous peristalsis of the intestine, lasting for about two to three minutes. Intravenous injection of hypertensin in the dog causes a marked increase in both tone and peristaltic activity of the duodenum and contraction of the gall bladder (88).

Isolated intestine. Page and Helmer (170) first showed that the isolated intestine does not contract when renin or hypertensinogen is added separately to the bath. But a mixture of both (with formation of hypertensin) causes powerful contraction. This action of hypertensin has been confirmed by many other workers (29, 31, 130) and has been utilized for the bioassay of hypertensin (29, 184, 193). Collins (30) has shown that tetraethylammonium bromide in

concentration of 1/13,000 to 1/40,000 increased the response of the isolated intestine to hypertensin.

b. Action on blood vessels. Hypertensin, whether exogenous or formed by the action of renin on hypertensinogen, *in vitro* or *in vivo*, has a direct vasoconstrictor action. Renin by itself has no such action.

The vasoconstrictor action of hypertensin has been studied by different methods. In isolated vascular preparations perfused with salt solution, such as the Laewen-Trendelenburg preparations of the toad (22), the rabbit ear (165, 170, 171), and the leg or tail of the dog, hypertensin has a vasoconstrictor action which is roughly proportional to the amount injected.

Direct observation of blood vessels *in vivo* has shown that intravenous injection of renin or hypertensin causes constriction of the arterioles in the ear (1), mesentery, and intestine (2) of normal rabbits. Capillaries did not constrict, whereas venules of the mesentery (2) and ear (1) exhibited a moderate constriction.

Hypertensin causes contraction of isolated rings of rabbit aorta (94), as described by Furchgott and Bhadrakom (67). The motor receptors with which hypertensin reacts appear to be different from those with which adrenaline or 5-hydroxytryptamine or histamine react (66). Still further work with purified preparations of hypertensin is necessary in order to understand the mechanism of action of hypertensin on the vascular smooth muscle.

Tachyphylaxis to hypertensin in vascular preparations. Page and Helmer (171) reported that they had observed tachyphylaxis to hypertensin when injected intravenously in dogs and cats. This has not been confirmed (*vide supra*). They also observed that the injection of hypertensin in Ringer solution produced intense vasoconstriction when perfused through the rabbit ear. The second injection had less effect, and the third, none. The addition of normal blood, or of certain extracts of blood, to the perfusing fluid restored the vasoconstrictor action of hypertensin. The substance responsible for this effect was called angiotonin-activator or co-substance (171). The authors concluded that angiotonin-activator was necessary for the action of hypertensin. In the toad the phenomenon observed by Page and Helmer does not occur (23): in the Laewen-Trendelenburg preparation, perfused with Ringer solution, the action of hypertensin is consistently vasoconstrictor, even if the injections are repeated many times (23, 59).

In a previous review on this subject (25), these findings were interpreted by us in the light of Morton and Tainter's (154) observations on the action of tyramine and ephedrine on the perfused hind legs of the cat. When perfused with Locke solution the vasoconstrictor action of these substances diminished gradually or even disappeared. The action reappeared if the leg was perfused with defibrinated blood or heparinized plasma, or if adrenaline was added to the Locke solution. From these observations Morton and Tainter (154) concluded that the vasoconstrictor action of amines in the group of tyramine and ephedrine was due, at least in part, to blocking of the local mechanisms which inactivate adrenaline. It is probable, however, that the state of the vessels, and therefore

their reactivity, plays a role, since the addition of 0.5 per cent gelatin to Locke solution was sufficient to increase considerably the activity of these drugs (154).

More recent work has brought this problem again to the attention of investigators in this field. Mylon and Heller (158, 159, 160, 161), in perfusion experiments on the rabbit ear, have shown that several vasoinactive renin-containing fractions of hog kidney (161), or protein fractions of other organs (159), especially liver, became vasoconstrictor when the perfusion fluid was supplemented by minute amounts of adrenaline (159) or of plasma (158). These authors showed then that hypertensin had no effect on the vessels of the perfused rabbit ear, but became strongly vasoconstrictor when the perfusing fluid was supplemented with traces of adrenaline or fresh plasma (160). They conclude that constriction of the blood vessels of the rabbit ear is dependent to a large degree upon traces of adrenaline and tyrosine containing compounds.

More recently Helmer (94) also found a diminishing action of hypertensin with time when tested on a rabbit aorta preparation as described by Furchgott and Bhadrakom (67). The ability of hypertensin to cause contraction could be restored by the addition of human, dog, and rabbit plasma, which by themselves had no effect on the strip.

More work is necessary to determine the nature and the mechanism of action of the so-called hypertensin-activator. The observation that two forms of hypertensin exist (222), which may be converted one into the other by incubation with plasma, and that a marked heterogeneity of hypertensin preparations can be demonstrated by means of countercurrent distribution (Paladini and Braun-Menéndez, 1955[1]), may have some bearing on this problem.

c. Seminal vesicles and vasa deferentia. The motility of the male accessory genital organs may be modified by the hormonal conditions of the donor animals (137). Hypertensin causes no response, even of a tonic nature, in the isolated seminal vesicle, or vasa deferentia, of normal rats, but the same organs of castrated rats respond with rhythmic contractions without residual increase in tone (183).

d. Isolated gall bladder. Hypertensin in relatively large amounts causes strong contraction of the isolated gall bladder of the guinea-pig (88) and the dog (130).

4. Other actions of renin and hypertensin. a. Action of renin and hypertensin in man. The injection of pig renin does not produce hypertension when injected intravenously in man (6, 231), due to the species specificity described (*vide supra*). Renin prepared from human kidneys does produce a rise in pressure in man similar to that which occurs in other mammals (6, 208). The rise is proportional to the amount injected and affects both systolic and diastolic pressures; a reflex bradycardia, a slight diminution of blood flow to the forearm, and an increase in venous pressure, but no change in the cardiac output have been observed (208).

The injection of hypertensin in man reproduces systolic and diastolic hypertension with the same characteristics as in the experimental animal. The action is rapid and varies with the dose (6). The blood pressure returns to its normal value at the end of five to nine minutes (7, 34, 79). No significant difference

[1] Biochim. biophys. Acta, 18: 580–581, 1955.

was observed between normotensive and hypertensive subjects in their response to hypertensin (7). A slight bradycardia occurs at the height of the pressor curve (6, 7, 16, 227, 238), which is reflex in nature and abolished by atropine (7, 238). No significant changes have been reported in the electrocardiogram (7).

The stroke volume was decreased by hypertensin according to some authors (227, 238), while others (16) found the cardiac output reduced, chiefly as the result of bradycardia, with little change in stroke volume.

An increase in venous pressure occurs which, according to Wilkins and Duncan (238), is accompanied by an increase in cardiac size, which was not observed by Bradley and Parker (16). Wilkins and Duncan (238) also found prolongation in circulation time and diminution of blood flow to the extremities (plethysmograph and skin temperature). Perhaps some of these findings, which the latter authors consider as evidence of "myocardial failure", may be due to untoward symptoms, such as nausea, vomiting, and precordial pain, which are encountered sometimes when hypertensin is injected into human beings. It must be kept in mind that these studies were made when hypertensin was still a rather impure preparation.

b. Action on the heart. Intravenous injection of small doses of renin or hypertensin in mammals causes no consistent variations of pulse rate (229); but more potent doses result in a moderate cardiac slowing (152), probably reflex in origin (87). In dogs with neurogenic hypertension, hypertensin elicits marked tachycardia (228), probably due to a stimulation of the cardioaccelerator mechanism.

The systolic discharge is not greatly changed by small doses; but it is reduced by doses causing a rise in pressure of more than 30 mm.Hg. The venous pressure and the diastolic volume are also increased (152).

In the heart-lung preparation of the cat (102), renin and hypertensin cause an increase in cardiac output (against a constant peripheral resistance), no change in heart rate, and no significant electrocardiographic changes (102). These observations suggest that the amplitude and vigor of ventricular systole are enhanced. This seems to be contradicted by observations of Middleton and Wiggers (152), who worked in entire dogs with open thorax and anesthetized with morphine-nembutal. On the isolated heart, perfused with blood according to the method of Moe and Visscher (153), hypertensin produced transient coronary vasoconstriction, decrease in diastolic volume, and a marked and extended increase in oxygen consumption, work performance, and efficiency (124, 125). On myocardiograms recorded from heart strips, mechanically isolated *in vivo* from the right ventricle of open-chest dogs, renin or hypertensin intravenously administered cause a marked increase in the force of contraction (126, 128).

On the isolated heart, perfused with Ringer-Locke solution, renin, because of the absence of hypertensinogen, is inactive (99, 100, 102, 229). Hypertensin causes no significant change in heart rate, an increase in the amplitude of the beats, and a decrease in coronary flow (102, 230).

On isolated auricles of the guinea-pig, or isolated strips of the coronary arteries of cattle, renin has no effect (99, 100). On isolated papillary muscle of the cat

fatigued by rhythmic electrical stimulation, hypertensin causes an increase of isometric contractions (126, 127). Renin has little or no action (129).

c. Action on peripheral blood flow. Landis *et al.* (113) found in rabbits that renin was unusual among the other pressor substances (adrenaline, tyramine, pitressin, pituitrin, ergot derivatives, guanidine) in elevating arterial pressure without consistent reduction of skin temperature (107) (which was accepted as a rough measure of peripheral flow). Corcoran and Page (37) found no consistent change in skin temperature of trained normal dogs when hypertensin was infused. Both renin and hypertensin produce a transient decrease, followed by a more prolonged increase, in blood flow in the femoral artery (98).

No significant variations in flow or protein content of lymph was observed after infusion of renin in anesthetized dogs (92).

d. Pulmonary pressure. Intravenous injection of renin was reported to cause a rise in pulmonary arterial pressure in the dog (26, 63). This effect was not confirmed for the dog, but was found in the cat and rabbit after injecting renin or hypertensin (195). The rise in pressure cannot be attributed solely to back pressure on the pulmonary circuit due to the rise in systemic pressure (63), since a similar rise in systemic pressure, caused by occluding the aorta, caused only insignificant changes in pulmonary pressure (195). It cannot be attributed to an action of renin or hypertensin on cardiac output, since a rise in perfusion pressure was found in isolated lungs, perfused with blood, after injection of renin (10, 56). It is probably due to a direct vasoconstriction of the arterioles of the pulmonary circuit. A study of the effect of hypertensin on the systemic and pulmonary arterial and capillary pressures in man (162) showed that intravenous injection of hypertensin raised both pulmonary arterial and capillary pressures, presumably due to constriction of pulmonary arterioles and venules.

e. Action on the kidney. The action of renin on the kidney and its mechanism have been the subject of much controversy. The purity of the kidney extracts injected, the route of administration, dosage, animal species, state of hydration of the animal, and other experimental conditions should be taken into account when considering the literature.

The intravenous injection of renin elicits an increase in kidney volume (12, 64, 150). The renal blood flow, as measured by the thermostromuhr (224), diminishes after injection of renin or hypertensin (98, 100). Increased renal volume in association with decreased renal blood flow is usually interpreted as the result of efferent glomerular artery constriction (200).

Renal clearances. Renal clearance studies, made by Corcoran and Page (36, 37) in dogs with explanted kidneys, show that slow intravenous infusion of renin or hypertensin results in most instances in decreased renal plasma flow (calculated from phenol red plasma clearance divided by renal extraction percentage) and increased renal extraction of inulin from blood (increase in glomerular filtration). They found no correlation between proportion of inulin extracted from blood by the kidneys and rise in arterial pressure, while the former was directly proportional to the degree of reduction of plasma flow. They therefore conclude that both the reduced blood flow and the increase in the filtration rate are the result of constriction of the glomerular efferent arterioles.

Pickering and Prinzmetal (188) measured the creatinine clearance in anesthetized rabbits before and after the intravenous injection of renin. Results differed according to the experimental conditions. In rabbits, in which renin was injected during the subsidence of water diuresis, creatinine clearance determinations, made in the half hour periods before and after injecting renin, showed no significant changes. On the other hand, when the creatinine clearance rates were measured during two to ten minute periods before the injection of renin, and for one period of 10 and one of 15 minutes afterwards, a profound fall in the creatinine clearance rates occurred during the short phase of reduced urine flow immediately following renin injection.

Brandt and Gruhn (17) measured creatinine and PAH clearances in unanesthetized rabbits before and after the intravenous injection of renin, at the peak of the renin induced diuresis which usually occurred about twenty minutes after the renin injection. They found no significant changes in glomerular filtration rate and a slight decrease in renal plasma flow, indicating a rise in the filtration fraction and efferent arteriolar constriction. Sellers *et al.* (213) showed that no significant changes in creatinine clearance occurred in rats injected intraperitoneally with renin, in spite of the increased urine output produced by this substance; if anything, there was a slight reduction in creatinine clearance which was measured during a period of twenty minutes, starting 20 minutes after the renin injection. In anesthetized rabbits, intravenous injection of renin had no significant effect on creatinine clearance (202). Lippman *et al.* (123) measured the creatinine clearance for fifteen minutes after the intravenous injection of a large dose (4 dog units) of renin in the rat and found a significant drop. Hughes-Jones *et al.* (105) measured the inulin and diodone clearance over half hour periods before, immediately after, and an hour or more after the intravenous injection of renin in the unanesthetized rabbit. Inulin clearance did not change significantly, but diodone clearance was always reduced in the first half hour period, the filtration fraction being conspicuously raised. Glucose Tm did not change significantly. Whitney *et al.* (237) found a fall of creatinine clearance and PAH clearance (40.8 per cent) following intravenous injection of renin into the anesthetized dog. The clearance periods were of 15 minute duration. Renin has no effect on tubular transport of PAH (202).

A more or less detailed account has been given of the studies made on this subject by clearance methods to show that, due to the different experimental designs used, the results may seem contradictory. An analysis of the data shows, however, that renin causes a decrease in plasma flow, a finding which confirms results obtained with the thermostromuhr. More confusing is the situation when considering the changes in glomerular filtration. Apparently the fall in glomerular filtration, reported by some authors, is transitory, occurring only during the first minutes following renin or hypertensin injection; this is followed by a period of normal, or increased, glomerular filtration rate. For this reason, if long clearance periods are conducted, the decrease in glomerular filtration may not be detected. The decreased blood flow determines an increased filtration fraction, which speaks for a constriction of the efferent arterioles.

Urine flow. The action of renin on urine flow was first observed by Bingel and Claus (12), who described an increase following the intravenous injection of renal extracts in lightly anesthetized rabbits. Hessel (99) reported that subcutaneous injection of renin in unanesthetized rabbits or rats caused no change in the course of water diuresis; by intravenous injection it caused an increase in urine flow. Hessel points out that, in spite of the diuretic effect, the chloride concentration in urine did not diminish. He also concludes that the occurrence of diuresis in spite of the blood vessel constriction may be explained by an increase in filtration pressure. In 1940, Pickering and Prinzmetal (188) studied more closely the effect of renin on urine flow in the unanesthetized rabbit. After intravenous injection the flow of urine was reduced during the first ten minutes and then rose to very high levels. Together with the diuresis, the excretion of chloride and sodium increased markedly. The diuretic action of renin has been repeatedly confirmed in the rabbit (17, 105) and the rat (39, 49, 139, 213). In the dog, renin has a purely antidiuretic effect (188, 237), even in animals with transection of the pituitary stalk (237). Nevertheless, it has been reported that renin has a natriuretic and diuretic effect in dogs with sectioned carotid sinus and aortic depressor nerves (78).

The question arises as to what is the nature of the action of renin on the kidney. Immediately following the injection of renin there is, in the rabbit (105, 188) and the rat (Uranga, unpublished observations), a short phase in which urine flow is diminished. This antidiuretic action of renin coincides with a marked rise in arterial pressure and a fall in the rate of glomerular filtration (105, 188), indicating constriction of the afferent glomerular arteries or diminution of the number of open glomerular capillaries. That both mechanisms may occur is shown by the serial angiographic studies of Daniel *et al.* (46). The intravenous injection of renin in anesthetized rabbits was followed by a constriction of the distal segments of the interlobar arteries, which persisted for twenty or more minutes, even after the blood pressure had returned to normal. The angiograms did not show individual vessels as small as the afferent and efferent glomerular arteries, but demonstrated that at least part of the effect of renin occurs proximal to the glomerulus. These findings are concordant with the observation that the renal blood flow falls after the injection of renin, and that this fall may outlast the rise in blood pressure. But, before concluding that the antidiuretic phase of the renin action is due to a constriction of the afferent renal vessels, it should be pointed out that the consequent ischemia affected, in half of the angiographic experiments, chiefly, or even solely, the peripheral cortex. It is thus possible that, together with the arteriolar constriction, a redistribution of blood occurs, directing a good part of the renal circulation to the juxta-medullary glomeruli and the vasa recta. In any case, the transient antidiuretic effect seems due exclusively to a diminished glomerular filtration rate. The diuretic phase that follows, at least in the rabbit and the rat, coincides with a raised blood pressure, a normal, or not significantly altered, glomerular filtration rate, and a diminished renal blood flow.

The conclusion arises that the diuresis, chloruresis, and natriuresis produced

by renin are due to a diminished tubular reabsorption of water, chloride, and sodium. But, again, it is possible that the tubular action of renin has a vascular basis, and that a diversion of blood from the cortical nephrons to the juxtamedullary nephrons, or away from some part of the tubules, may play a part.

Some authors (39) believe that it has not been sufficiently proved that the renal effects of renin are mediated by hypertensin, because in their experiments on rats the intraperitoneal or subcutaneous injection of hypertensin did not show a diuretic effect. Nevertheless, the experiments of Hughes-Jones *et al.* (105) and of ourselves (Uranga, unpublished) show that, when injected intravenously in adequate doses, hypertensin has an effect upon the kidney identical with that of renin. Both have a short antidiuretic action, followed by an increased urinary output (Uranga, unpublished), both increase sodium and chloride excretion, both affect the inulin and diodone clearance in the same way (105). Furthermore, in rats, highly purified pig renin produces effects on diuresis, albuminuria, and natriuresis which are quantitatively similar to those elicited by an equipressor amount of crude renin (40).

Croxatto and coworkers (38, 39, 41) have studied the diuretic effect of intraperitoneal renin in rats under different experimental conditions. They have shown that the diuretic action of renin depends on various factors, such as route of administration, ingestion of sodium chloride, and the normal functioning of the adrenals. Diuresis, induced by renin, is more intense in rats drinking 1 per cent sodium chloride instead of tap water; adrenalectomy abolishes the diuretic effect of renin in rats drinking 1 per cent sodium chloride solution (41). The incapacity of the adrenalectomized animals is corrected by the administration of desoxycorticosterone acetate or cortisone (38). According to these authors, the polyuric action of renin is not due to a direct or indirect stimulation of the adrenal cortex, nor to a temporary inhibition of the neurohypophysis. From their observations they conclude that the polyuric action of renin is conditioned by the body stores of Na^+ and Cl^-. When the amount of sodium chloride available is diminished (adrenalectomy, hypophysectomy, etc.) the diuretic action of renin is impaired; on the other hand, an excess of sodium chloride raises the sensitivity of the animals to the diuretic action of renin (38).

Proteinuria. Injection of renin is followed by proteinuria in the rabbit (17, 105, 188), the rat (3, 4, 5, 139, 214), and in dogs with chronic buffer nerve section (78). The proteinuria is attributed in part to increased permeability of glomerular capillaries (123, 214). Intravenous injection of renin produces increased renal excretion of intravenously administered hemoglobin (142), or Evans' blue labelled plasma protein (214). This is due partly to increased glomerular permeability and partly to diminished tubular reabsorption of proteins.

Normally, the small amount of urinary protein has two components with electrophoretic mobilities similar to α- and β-globulin. In renin treated rats, urinary proteins have components corresponding to each of the normal serum proteins: albumin, α-, β-, and γ-globulin (120). The relative percentage of albumin increases with increased proteinuria (120, 214).

Adrenalectomy impairs renin proteinuria (4) and inhibits the proteinuria of

normal male rats. Adrenal cortical extracts, partially, and cortisone or desoxycorticosterone, wholly, restore these functions (5, 145). Hypophysectomy also impairs the proteinuric response to renin (145), while administration of 1 per cent sodium chloride (145) or adrenocorticotrophin (74, 136) potentiates the proteinuric effects of renin. Hypertensin, in the dose used (about 247 dog pressor units in twelve hours given in divided doses intraperitoneally every thirty minutes), increased both urine flow and proteinuria (143).

Renal compensatory hypertrophy. Subcutaneous or intraperitoneal administration of renin in high doses inhibits the increase in weight of the remaining kidney twenty-four to forty-eight hours after unilateral nephrectomy in rats (206). This fact has led the authors to suggest that secretion of renin may act as a self-regulating mechanism in the control of kidney growth.

f. Action on the adrenal glands. Relatively small doses of hog renin injected intravenously in normal rats cause adrenal ascorbic acid depletion. This effect is not obtained in hypophysectomized rats and is partially blocked by adrenergic blocking agents (215, 216). It may be assumed that renin causes the release of adrenocorticotrophin by a direct action or through the secretion of catechols by the adrenal medulla. The latter possibility cannot be discarded, since it was shown that injection of hypertensin causes the discharge of adrenaline even by denervated adrenals (23). It has also been shown that subcutaneous injections of renin, in rats, result in marked enlargement of the zona glomerulosa of the adrenal cortex (49).

g. Vasculo-toxic action. Winternitz *et al.* (242) showed that intravenous injection of renin, in bilaterally nephrectomized dogs, caused "edema, hemorrhage and necrosis of muscle, including heart muscle, smooth muscle of blood vessel walls, hollow viscera, and diaphragmatic muscle". These effects were not demonstrable in normal dogs. The vascular lesions thus elicited resemble those which occur in the malignant form of experimental hypertension, and can also be observed after injecting renin intravenously in renal hypertensive dogs with renal excretory insufficiency (115, 116).

Renin injected subcutaneously also causes acute, widespread, necrotizing vascular lesions in bilaterally nephrectomized dogs given 1 per cent sodium chloride as drinking fluid (149). In rats, under similar experimental conditions, the arteriolar lesions elicited by subcutaneously administered renin are less severe and frequent than in dogs (147).

Subcutaneous injection of renin into uninephrectomized rats, pretreated with desoxycorticosterone acetate and 1 per cent sodium chloride as drinking fluid, precipitates an "eclampsia-like" syndrome, characterized by massive edema, oliguria, hypertension (140), and widespread visceral hemorrhages and diffuse vascular lesions resembling those of malignant hypertension (142). Pretreatment with cortisone, or hydrocortisone (144), under identical experimental conditions, similarly sensitizes the animals to the vasculo-toxic action of renin, while the simultaneous administration of Apresoline (hydralazine hydrochloride, 1-hydrazinophthalazine hydrochloride) prevents the appearance of the "eclampsia-like" syndrome (199).

The fact that the subcutaneous route of administration of renin is as effective as the intravenous or intraperitoneal route, raises the question of whether renin acts as a foreign protein or through the formation of hypertensin. The latter proposition is more likely, since 1) the pressor action of subcutaneously administered renin has been proved (138, 146); 2) hourly intraperitoneal administration of hypertensin to rats, pretreated with desoxycorticosterone acetate and salt, duplicates the syndrome elicited by the subcutaneous administration of renin (143); 3) the vasculo-toxic action and the pressor action of kidney extracts containing renin cannot be separated; and 4) the pressor and necrotizing effects are lost when the extracts are heated to 70°C. or destroyed by peptic or tryptic digestion (242).

The mechanism of this effect of renin (or hypertensin) is still a matter for speculation. But there are several reasons for accepting the suggestion that it is associated with a) abnormalities in the electrolyte concentration of intracellular and extracellular fluids, b) the state of hydration, c) elevated blood pressure and, perhaps, e) alterations in vascular and cellular permeability (20). The following facts favour such a view. Subcutaneous or intraperitoneal administration of renin into normal rats elicits polyuria, natriuresis, and chloruresis, increased consumption of water and 2 per cent sodium chloride solution, and an increased specific appetite for sodium (19). In rats pretreated with desoxycorticosterone, cortisone, or hydrocortisone, plus salt, it produces edema, a positive fluid balance with increased fluid intake. In nephrectomized rats receiving renin and sodium chloride 1 per cent, large pleural effusions, decreased blood volume, and hypoproteinemia occur which, together with the proteinuria, seem to be associated with an alteration in vascular permeability (147). In dogs anesthetized with chloralose, intravenous injection of renin or hypertensin induces a pronounced though transient rise in potassium and a greater and more prolonged rise of glucose in the blood (61).

CONCLUSIONS

From the present review one important conclusion arises, *i.e.*, that the pharmacological effects of renin are mediated through the *in vivo* or *in vitro* formation of hypertensin. Most, if not all, of the pharmacological actions of renin may be duplicated by the administration of hypertensin, if proper dosage and mode of administration are used. On the other hand, renin has no specific pharmacological action in the absence of its substrate.

In spite of the fair amount of work reviewed, the pharmacology of both renin and hypertensin still requires much further study. Two aspects should receive special consideration: 1) a revision of many pharmacological reactions should be made with better methods and with purer preparations of renin and hypertensin; and 2) a definition of a unit of potency for both substances should be agreed upon by all workers in the field and there should be an international standard of a stable preparation obtainable from a central source.

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