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THE ROLE OF RENIN IN EXPERIMENTAL HYPERTENSION

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In 1934 Goldblatt and others discovered that partial constriction of the main renal artery produces a marked and permanent hypertension in the dog. The renal experimental hypertension caused by this or other methods in laboratory animals is similar in many respects to human essential hypertension. It is not due to excretory insufficiency of the kidney or to an increase of blood volume or cardiac output, nor is it produced through a nervous mechanism. Houssay and Fasciolo (1937a, 1937b) have shown that renal ischaemia results in hypertension through a humoral mechanism. The ischaemic kidneys of dogs with chronic hypertension were grafted into the neck of normal or nephrectomized dogs. The arterial pressure of the recipient dog rose from 30 to 80 mm. Hg in 5 to 10 minutes, while grafting a normal kidney had no such effect. The citrated plasma obtained from the veins of these ischaemic kidneys produced vasoconstriction when perfused through a Läwen-Trendelenburg preparation; normal plasma had no vasoconstrictor action (Houssay and Taquini, 1938).

Braun-Menendez and Fasciolo (1939a, 1939b) made the observation that acute partial ischaemia of the kidneys produced the same effects as chronic ischaemia: (1) if the artery of a normal kidney grafted into the neck of a dog was constricted a slight but definite increase in blood pressure was produced; (2) the blood of acutely ischaemic kidneys when injected intravenously into anaesthetized dogs produced greater increases in blood pressure than control blood; and (3) it had a vasoconstrictor action on the Läwen-Trendelenburg preparation. Braun-Menendez, Fasciolo, Leloir, and Muñoz (1939) prepared extracts from the venous blood of these kidneys and discovered a pressor substance which was named "hypertensin." They found that this substance could also be prepared *in vitro* by incubating at 37° C. blood plasma or serum with renin, a protein which is present in extracts of kidney (Braun-Menendez, Fasciolo, Leloir, and Muñoz, 1940a). It was also shown that renin is secreted by the ischaemic kidney and hypertensin is formed subsequently in the venous blood.

Our present knowledge on the mechanism of renal hypertension might be summarized as follows: The ischaemic kidney secretes renin. This protein is an enzyme which acts on a blood globulin (hypertensinogen) and gives rise to a substance (hypertensin) that has a direct vasoconstrictor action. Another enzyme, hypertensinase, which destroys hypertensin, is present in blood and tissues.

Some Properties of Renin.—In 1898 Tigerstedt and Bergman discovered that kidney extracts contained a pressor substance —renin. This substance is a protein, probably a globulin, and is inactivated by heat at 60° C. Its intravenous injection produces in the dog a gradual rise in blood pressure which lasts 10 to 30 minutes, depending on the dose. Consecutive injections of large doses at short intervals produce each time a smaller response. This phenomenon is called "tachyphylaxis," and was originally described by Tigerstedt and Bergman. Renin by itself is neither vasoconstrictor nor vasopressor; these properties are due to the hypertensin formed by the action of renin on hypertensinogen.

Methods for the Estimation of Renin.—Two general methods can be used for the detection and estimation of renin. The first consists in comparing the rise in blood pressure caused by the injection of an extract or body fluid containing renin with that of a standard solution of renin. The second is based on the formation of hypertensin when renin is mixed with hypertensinogen. The hypertensin formed can be tested for its vasoconstrictor (Page, 1940) or pressor action (Leloir, Muñoz, Braun-Menendez, and Fasciolo, 1940b). The latter method (Leloir and others 1940a, 1940b), more reliable, has been used almost exclusively in our laboratory. This method is specific for renin, very sensitive, and quantitative within the limits of a bio-assay method. A unit of renin is the amount which, incubated for 2 hours at 37° C. with an excess of hypertensinogen, forms 0.5 unit of hypertensin. Applying these methods, it has been shown that renin is liberated:

1. By Totally Ischaemic Kidneys.—The re-establishment of the renal circulation of the kidney *in situ* after total ischaemia lasting 2 to 6 hours causes a rise in arterial pressure similar to that produced by an injection of renin. The venous blood of these kidneys has pressor and vasoconstrictor properties. Using the quantitative method of Leloir and his colleagues, it has been shown (Taquini and Braun-Menendez, 1941) that very large amounts of renin may be liberated by the kidney under these conditions.

2. By Ischaemic Kidneys of Hypertensive Dogs.—If the ischaemic kidney of a hypertensive dog is grafted into the neck of a normal dog a rise in blood pressure is produced similar to that produced by the injection of a small amount of renin (Fasciolo, Houssay, and Taquini, 1938). The venous blood of these kidneys has vasoconstrictor properties (Fasciolo, Houssay, and Taquini, 1938). Using the quantitative method of Leloir and his colleagues, renin was estimated in the renal venous blood of kidneys transplanted under the skin (Dell'Oro and Braun-Menendez, to be published). No renin was present in normal conditions, but after constriction of the renal artery, and coincident with the increase of blood pressure, variable amounts of renin (from 0.8 to 1.2 units per 12 c.cm. of plasma) were found in the renal venous blood and in the femoral blood of these dogs. All these results justify the conclusion that the ischaemic kidneys of dogs with experimental hypertension secrete renin into the blood.

3. By Kidneys in Acute Partial Ischaemia.—It has already been mentioned that a few minutes after partial constriction of the renal arteries renin can be detected in the venous blood of these kidneys.

Secretion of Renin by the Normal Kidney.—Apart from ischaemia due to constriction of the renal artery, are there other conditions which result in the secretion of renin by the kidney? Huidobro and Braun-Menendez (investigations to be published) have shown that hypotension due to haemorrhage or shock causes the liberation of renin by the intact kidney of normal anaesthetized dogs. Renin was detected and estimated in the systemic blood of these dogs. The inference is drawn that the kidney participates in the regulation of arterial blood pressure. When the blood pressure decreases the normal kidney secretes renin, which through the formation of hypertensin tends to the restoration of normal blood pressure. Renin appears to be a substance which the body uses to maintain homeostasis. Collins and Hamilton (1942) and Sapirstein, Ogden, and Southard (1941) have arrived independently at the same conclusions.

Formation and Mode of Action of Hypertensin

Enzymatic Action of Renin (Munoz et al., 1940)

The formation of hypertensin *in vitro* or *in vivo* is the result of an enzymatic reaction in which the substrate is a blood globulin that we have called "hypertensinogen," and the enzyme is renin. This affirmation is based on the following facts: (1) Renin is a protein: its activity is destroyed by heating (60° to 80° C.) and delayed or abolished at low temperatures. (2) The reaction between renin and hypertensinogen has a temperature optimum of 37° to 45° C. and a *p*H optimum of 7.5 to 8.5. (3) The yield of hypertensin is proportional to the amount of globulins. (4) If the amount of renin exceeds the optimum the maximum yield of hypertensin is not surpassed if 10 to 20 times more renin is used. (5) The time necessary for obtaining the maximum yield of hypertensin, or, in other words, the velocity of the reaction, depends on the amount of renin. (6) Hypertensinogen disappears in the reaction while renin does not.

This is the evidence which supports our assertion that renin is an enzyme. As the substrate on which renin acts is a protein, and the product of reaction is, as we shall see, probably a polypeptide, it is reasonable to classify renin as a proteolytic enzyme.

Specificity of Renin

Renin may be defined as the substance contained in kidney extracts which, when incubated with blood globulins, gives rise to hypertensin. Extracts of other organs—liver, spleen, placenta, etc.—do not contain renin. Recently Croxatto and Croxatto (1941) demonstrated that by the action of pepsin on hypertensinogen a vasoconstrictor and pressor substance is formed, the properties of which resemble those of hypertensin. These results, which have been confirmed by ourselves and by Helmer and Page (1942), constitute strong indirect evidence in favour of the enzymatic action of renin.

Renin has been found in human kidneys and in the kidneys of every mammal which has been investigated—ox, horse, dog, pig, sheep, goat, rat, rabbit, guinea-pig, etc. The incubation of renin from these animals with plasma from any of them gives rise to the formation of hypertensin. But no hypertensin is formed when any of those renins is incubated with human plasma. On the other hand, human renin is active on the plasma of any of those animals and also on human plasma. This peculiar specificity of renin led to the prediction that no pressor response should be obtained in man when pig's renin, for instance, is injected intravenously, while human renin should be active (Fasciolo, Leloir, Muñoz, and Braun-Menendez, 1940a). This prediction was verified by experiments (Battro and others, 1940). Bean (1942) found no renin in the kidneys of the species of fishes and amphibians studied by him. He found renin in the kidneys of fowl and duck which acted exclusively on blood belonging to the same class.

Hypertensinogen

Hypertensinogen, the substrate of renin in the reaction which leads to the formation of hypertensin, can be prepared from blood plasma or serum by fractional precipitation with ammonium sulphate or potassium phosphate. It is a globulin, probably a euglobulin, and is inactivated by heat at 60° C.

Attempts to obtain hypertensinogen from other sources than blood serum have failed: liver, spleen, thymus, testes, lungs, heart or skeletal muscle, milk and egg proteins, haemoglobin, serum albumin, and some vegetable proteins did not yield hypertensin when incubated with renin (Muñoz, Braun-Menendez, Fasciolo, and Leloir, 1940). Hypertensinogen can be estimated in blood samples by the maximum amount of hypertensin it produces. A unit of hypertensinogen is that which gives rise to one unit of hypertensin under the conditions outlined.

Hypertensin

This substance, which was discovered in the venous blood of ischaemic kidneys, is the product of the enzymatic reaction between renin and hypertensinogen.

In 1938 Kohlstaedt, Helmer, and Page showed that purified preparations of renin produced no vasoconstriction when perfused with Ringer's solution through a dog's tail or rabbit's ear, but that the vasoconstrictor activity could be restored by the addition of a protein-like substance contained in blood. They designated this substance renin-activator to connote that renin was inactive as a vasoconstrictor substance without it. Subsequently they found (Page and Helmer, 1940) that from the interaction of these two substances a new substance resulted with potent vasoconstrictor and pressor actions which they called "angiotonin." It is interesting how, coming in two different ways—the Indianapolis group via the purification of renin, and that of Buenos Aires via isolation of the active substance in the venous blood of ischaemic kidneys—both groups arrived independently and simultaneously at the discovery of the substance which results from the interaction of renin and blood.

Hypertensin is very soluble in water, insoluble in ether, thermostable, and acid-resistant. In NaOH 0.15 at 100° C. it is destroyed in 10 minutes. Kept in the cold store and free from contamination, hypertensin, dried or in solution, is stable. It can be salted out by ammonium sulphate. It dialyses through cellophane. It is inactivated by incubation with pepsin and trypsin. Although Page and Helmer report that the biuret reaction is negative, the inactivation by pepsin would indicate that it is a polypeptide. Some of its chemical and physical properties (velocity of diffusion) and its proteic origin are in favour of this idea. We have not been able to obtain a pure product, and this has compelled us to adopt an arbitrary unit in order to compare the pressor action of different solutions of hypertensin. A unit of hypertensin is the amount necessary, when given intravenously, to raise the arterial pressure of a 10-kg. chloralosed dog an average of 30 mm. Hg. A standard solution was prepared which has 1 unit per c.cm.

Hypertensin does not cause tachyphylaxis. When the dose is increased the rises in pressure in mm. Hg increase as the square root of the units injected, according to the formula $X = St\sqrt{y}$, in which X and St are the blood-pressure increases in mm. Hg obtained by the unknown and by 1 c.cm. of the standard solution respectively, and y the units of hypertensin contained in the unknown (Braun-Menendez, Fasciolo, Leloir, and Muñoz, 1940b).

The Pressor Action of Renin

There is strong evidence that the action of renin *in vivo* is due to the hypertensin formed by its action on hypertensinogen:

(1) The perfusion of renin in Ringer's solution through a vascular system does not produce vasoconstriction; but if mixed with blood globulins and perfused after short incubation, marked vasoconstriction results, due to the hypertensin formed. (2) The prolonged pressor action of injected renin may be explained by the continuous formation of hypertensin: a similar pressor effect is produced by the continuous intravenous infusion of hypertensin. (3) When renin does not form hypertensin *in vitro* it has no pressor action *in vivo*. For instance, pig renin, which does not give rise to hypertensin when incubated with human serum, does not produce a pressure increase when injected intravenously into human beings. (4) The pressor action of renin is greater when the concentration of hypertensinogen in blood is increased (dogs nephrectomized 48 hours previously) and smaller when decreased (adrenal insufficiency) or exhausted (by repeated injections of renin). (5) The pharmacological actions of renin and hypertensin are identical when injected into the animal, and are influenced in the same way by diverse drugs: no change of action by

Fourneau 933, cocaine, atropine, ergotamine, etc.; potentiation by veritol or ephetonine (Braun-Menendez, Fasciolo, Leloir, and Muñoz, 1940b).

The pressor action which renin exerts through the formation of hypertensin is perhaps not the exclusive mechanism in renal hypertension. It is very possible that other phenomena play their part. Perhaps also, after hypertension is initiated by renin, some changes occur in the body which contribute to maintain the elevated blood pressure, or vice versa.

Treatment of Renal Hypertension

The better understanding of the mechanism of renal hypertension may give us some indications as to the possibilities of a specific treatment. This should aim at (Muñoz, Braun-Menendez, Fasciolo, and Leloir, 1940): (A) suppressing or diminishing the secretion of renin; (B) destroying the renin secreted by the ischaemic kidney or inhibiting its reaction with blood globulins; (C) diminishing the amount of hypertensinogen; and (D) preventing the action of hypertensin by increasing the amount or the activity of hypertensinase or by some other mechanism.

(A) *Suppression or Diminution of the Secretion of Renin.*—In Goldblatt dogs, release of the constriction of the renal artery, removal of the clamp, or nephrectomy results in a drop to normal of the elevated blood pressure. In man the existence of hypertension with unilateral renal disease has been recognized. In many of these cases excision of the diseased kidney resulted in a return of blood pressure to normal with disappearance of other symptoms and complications. The development of collateral circulation in the ischaemic kidneys of Goldblatt dogs—whether spontaneous or promoted by decapsulation and implantation of omentum or spleen—results in a drop to normal of the elevated blood pressure. The promotion of collateral circulation has also been attempted in man by grafts of omentum or muscle in renal tissue with varying results.

(B) *Destroying the Renin secreted by the Ischaemic Kidney.*—It has been shown, first by Fasciolo (1938) and then by others, that the normal kidney exerts some sort of protective action against hypertension of renal origin. Grollman, Williams, and Harrison (1940) on a basis of indirect evidence believed that this action was due to an antipressor substance secreted by the normal kidney. They injected extracts of kidney to hypertensive rats and dogs, and obtained a decline in blood pressure. These extracts were active even on oral administration. Page and co-workers (1941) have also prepared different kinds of extracts of kidney, muscle, and lung which, when injected into dogs or rats with experimental hypertension, caused the blood pressure to fall to normal levels. They have obtained the same results in human beings with malignant hypertension. These studies are very important and promising, but for the moment it cannot be stated whether those extracts contain specific neutralizing or antipressor substances or whether their benefit arises from the introduction of non-specific foreign elements into the body. The attempts of Wakerlin and Johnson (1941) to neutralize the action of renin by immunological means have apparently been unsuccessful. Schroeder and Adams (1941) reduced the blood pressure of hypertensive human beings, rats, and dogs by the injection of tyrosinase. They reported that this enzyme inactivates renin *in vitro* in the presence of catechol and hypertensin in the presence of serum. Further evidence is necessary to judge these results.

(C) *Diminution of the Amount of Hypertensinogen.*—Very little, if anything, is known about the exact nature of hypertensinogen, its site of formation, etc. It is nevertheless theoretically possible to lower the blood pressure in renal hypertension by diminishing the amount of hypertensinogen. For the moment we know of only one way to obtain this result—that is, by the injection of large amounts of renin.

(D) *Increase of the Amount of Hypertensinase.*—Serum, plasma, or red cells, and extracts of many organs (kidney, liver, spleen, etc.) are active in destroying hypertensin, owing to the presence of a substance which we have designated "hypertensinase." This substance is destroyed by heating and has a *p*H optimum between 7.5 and 8.5. It can therefore be considered as an enzyme. Its action is not suppressed by anaerobiosis, cyanide, octyl alcohol, chloroform, thymol, toluol, fluoride, iodo-acetate, pyrogallol, etc. It is precipitated with ammonium sulphate between 0.3 and 0.6 saturation. It is inactivated in 20 minutes at 25° C. at *p*H 3.9. A unit of hypertensinase is the amount which destroys 0.5 unit of hypertensin in 4 hours in a volume of 10 c.cm. with an initial concentration of 1 unit of hypertensin (Fasciolo, Leloir, Muñoz, and Braun-Menendez, 1940b). Fasciolo and others (1940a, 1940b) have studied the distribution of hypertensinase in saline extracts of different organs. The highest content was found in the kidney and intestinal mucosa, the lowest in plasma or serum. Haemolysed blood cells contain a large amount of hypertensinase. We have made some preliminary essays injecting kidney and liver extracts rich in hypertensinase into hypertensive dogs. The results, though promising, have not been very clear. The fact that hypertensinase is a most unstable substance renders difficult its isolation in tissue extracts. These difficulties have hampered progress in this direction. Page now ascribes the antipressor action of his renal extracts to the presence in them of hypertensinase.

Conclusion

We believe that the therapeutic problem of renal hypertension has not yet been solved; but we are confident that the solution will be attained by a patient and thorough investigation of the underlying mechanisms which are involved in the production of renal experimental and human hypertension. In spite of the enormous progress of the past few years we still know very little about them. Let us hope that in the near future research will give medicine a new and powerful weapon to fight arterial hypertension, this most death-dealing malady to-day.

REFERENCES

Battro, A., Braun-Menendez, E., Lanari, A., and Leloir, L. F. (1940). *Rev. Soc. argent. Biol.*, **16**, 376.
Bean, J. W. (1942). *Federation Proc.*, **1**, 6.
Braun-Menendez, E., and Fasciolo, J. C. (1939a). *Rev. Soc. argent. Biol.*, **15**, 161; *C. r. Soc. Biol.*, Paris, 1940, **133**, 324.
—— —— (1939b). *Rev. Soc. argent. Biol.*, **15**, 401; *C. r. Soc. Biol.*, Paris, 1940, **133**, 728.
—— —— Leloir, L. F., and Munoz, J. M. (1939). *Rev. Soc. argent. Biol.*, **15**, 420; *C. r. Soc. Biol.*, Paris, 1940, **133**, 731.
—— —— —— —— (1940a). *J. Physiol.*, **98**, 283.
—— —— —— —— (1940b). *Rev. Soc. argent. Biol.*, **16**, 398.
Collins, D. A., and Hamilton, A. S. (1942). *Federation Proc.*, **1**, 16.
Croxatto, H., and Croxatto, R. (1941). *Rev. Soc. argent. Biol.*, **17**, 439.
Dell'Oro, R., and Braun-Menendez, E. Ibid. (to be published).
Fasciolo, J. C. (1938). Ibid., **14**, 15; *C. r. Soc. Biol.*, Paris, 1938, **128**, 1129.
—— Houssay, B. A., and Taquini, A. C. (1938). *J. Physiol.*, **94**, 281.
—— Leloir, L. F., Munoz, J. M., and Braun-Menendez, E. (1940a). *Science*, **92**, 554.
—— —— —— (1940b). *Rev. Soc. argent. Biol.*, **16**, 643.
Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W. (1934). *J. exp. Med.*, **59**, 347.
Grollman, A., Williams, J. R., and Harrison, T. R. (1940). *J. Amer. med. Ass.*, **115**, 1169.
Helmer, O. M., and Page, I. H. (1942). *Proc. Soc. exp. Biol.*, N.Y., **49**, 389.
Houssay, B. A., and Fasciolo, J. C. (1937a). *Bol. Acad. Med. B. Aires*, Sept., 342.
—— —— (1937b). *Rev. Soc. argent. Biol.*, **13**, 284; *C. r. Soc. Biol.*, Paris, 1938, **127**, 147.
—— and Taquini, A. C. (1938). *Rev. Soc. argent. Biol.*, **14**, 5; *C. r. Soc. Biol.*, Paris, 1938, **128**, 1125.
Kohlstaedt, K. G., Helmer, O. M., and Page, I. H. (1938). *Proc. Soc. exp. Biol.*, N.Y., **39**, 214.
Leloir, L. F., Munoz, J. M., Braun-Menendez, E., and Fasciolo, J. C. (1940a). *Rev. Soc. argent. Biol.*, **16**, 75; *C. r. Soc. Biol.*, Paris, 1940, **134**, 487.
—— —— —— —— (1940b). *Rev. Soc. argent. Biol.*, **16**, 635.
Munoz, J. M., Braun-Menendez, E., Fasciolo, J. C., and Leloir, L. F. (1940). *Amer. J. med. Sci.*, **200**, 608.
Page, I. H. (1940). *Amer. J. Physiol.*, **130**, 22.
—— and Helmer, O. M. (1940). *J. exp. Med.*, **71**, 29.
—— —— Kohlstaedt, K. G., Fouts, P. J., and Kempf, G. F. (1941). Ibid., **73**, 7.
Sapirstein, L. A., Ogden, E., and Southard, F. D. (1941). *Proc. Soc. exp. Biol.*, N.Y., **48**, 505.
Schroeder, H. A., and Adams, M. H. (1941). *J. exp. Med.*, **73**, 531.
Taquini, A. C., and Braun-Menendez, E. (1941). *Rev. Soc. argent. Biol.*, **17**, 465.
Tigerstedt, R., and Bergman, P. G. (1898). *Skand. Arch. Physiol.*, **8**, 223.
Wakerlin, G. E., and Johnson, C. A. (1941). *J. Amer. med Ass.*, **117**, 416.

H. D. Hahn (*Amer. J. Syph. Gon. ven. Dis.*, 1941, **25**, 200) reviews the literature of second infection in congenital syphilis, and comes to the conclusion that up till now no indisputable second infection in a congenital syphilitic patient has been reported. He records, however, two personal cases of reinfection in adequately treated congenital syphilis. In the first case there were thirteen years of clinical and serological negativity between the congenital and the acquired infection. In the second, though the serological test for syphilis never became entirely negative, there were at least eight years of clinical negativity, including a negative cerebrospinal fluid between the congenital and the acquired infection.