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Species Specificity in Reaction between Renin and Angiotensinogen

Angiotensin, the most potent pressor substance known, strongly suspected as a cause of renal hypertension, is an octapeptide formed from angiotensinogen by the action of renin. It has long been thought that only primate renins are capable of acting on human angiotensinogen as reviewed by Braun-Menéndez *et al.*[1].

Contamination of angiotensinase, which readily destroys the product, has always been a trouble in the *in vitro* formation of angiotensin. However, 'Dowex 50*W*-*X*2' (NH_4^+ form), introduced by Boucher *et al.*[2] into the reaction to protect it, has made the yield of human angiotensin quite high. This seemed to us more promising than previous means such as acid[3] or charcoal treatments[4]. In fact, our application of this method in the use of different animal materials has revealed the apparent formation of angiotensin in every case tested in the present experiments.

Crude renins were prepared by Haas's method[5] from autopsied human kidneys taken within 3–24 h after death and animal (bovine, hog and rat) kidneys taken within 3 h after being killed. Lyophilized human serum protein fraction IV by Cohn's fractionation[6], which contains most of the serum α_2-globulin, served as human angiotensinogen.

Incubation of each 5 ml. of aqueous renin, derived from 50 g of kidney, and 0·5 g of lyophilized human angiotensinogen or 72 ml. of animal plasmas containing the equivalent amounts of α_2-globulin was carried out with 2·5 ml. 'Dowex 50*W*-*X*2' at *p*H 6·0 and 37° C for 3 h. After the reaction, the resin was collected on a sintered-glass filter and treated by Boucher's procedure[2]. The crude preparation, dissolved in saline, was assayed by blood pressure using a female Wistar rat weighing 200–250 g.

The pressor activity of each preparation was found in the identical R_F value with that of a synthetic angiotensin-II in paper chromatography (*n*-butanol : acetic acid : water = 4 : 1 : 5). Hydrolysis of the preparations by 6 N hydrochloric acid at 100° C or by pronase at 37° C for 1 h resulted in a complete loss of the pressor activity. Control experiments without either renin or the substrate did not produce any pressor materials.

The amounts of the formed angiotensin in duplicate experiments were averaged and expressed in nanograms of synthetic isoleucine[5]–angiotensin octapeptide activity[7] as shown in Table 1. The results clearly show that, contrary to the previous concept[1], not only human but also bovine, hog and rat renins can act on human angiotensinogen, although at different rate and to lesser extent than on their own substrates.

We assume, therefore, that the previous lack of sufficient protection may have caused the inactivation of the angiotensin which misleadingly indicated the species specificity in this reaction. Further application of the method to other animal materials, together with the chemical analyses of the formed angiotensins, may help to elucidate a common chemical feature, regardless of the species difference, in the binding site of the enzyme-substrate complex of the reaction.

Table 1. *In vitro* Formation of Angiotensin from Heterogeneous Materials as compared with Homologous Materials

The amounts of product per 1 g of kidney are expressed in nanograms of synthetic isoleucine[5]–angiotensin-II pressor activity

Species of renin	Species of substrate	Amounts of formed angiotensin (ng)
Human	Human	52
Bovine	Human	8
,,	Bovine	46
Hog	Human	12
,,	Hog	20
Rat	Human	20
,,	Rat	78

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Direct Action of Adrenocorticotrophic Hormone on Lipase in the Adrenal Gland of the Rat

Many investigations have demonstrated that the adrenal gland of the rat is extremely rich in lipids[1], and cholesterol esters and triglycerides together represent approximately 90–95 per cent of the total adrenal lipids[2]. A large amount of histological work has demonstrated that stress, or the administration of adrenocorticotrophin, causes depletion of fats in the adrenal glands and that there is probably a close but unknown relation between adrenal fats and the functional activity of the gland[3–5]. It is clear that changes in the adrenal lipids under varying conditions of stimulation are of considerable importance. Pigafetta and Macchitella[6] investigated the adrenal glands of rats by histological and histochemical methods and demonstrated that depletion of lipids in the adrenal cortex takes place in consequence of the action of the adrenal lipase which is activated by ACTH administered *in vivo* over a long period before the experiments.

The present experiments were designed to investigate the direct effect of ACTH, added *in vitro*, on the activity of lipase in the whole adrenal homogenate of rats.

Adult male Wistar rats were used. They were killed by decapitation and the adrenal glands were quickly removed and homogenized in ice-cold 0·25 M sucrose with 0·001 M EDTA. 0·4 ml. of the homogenate (approximately 16 mg of fresh tissue) was added to the incubation medium and incubated for 2 h at 37° C in a Dubnoff metabolic shaker in air. The incubation medium contained 0·04 ml. EDIOL (Shenlabs), 0·6 ml. of 20 per cent lyophilized human albumin and 1·0 ml. Krebs–Ringer phosphate buffer, *p*H 8·5. Adrenocorticotrophic hormone (Léčiva Prague, lot No. 250360) was added in the amounts of 0·01, 0·1 and 10 milliunits per flask, diluted in 0·1 per cent human albumin. At the end of the incubation period the free fatty acids (FFA) were extracted and determined by the method of Dole[7]. The activity of lipase was calculated