INDUCTION OF PLASMA CARBOXYCATHEPSIN (ANGIOTENSIN I CONVERTING ENZYME) OF NORMOTENSIVE AND HYPERTENSIVE RATS IN RESPONSE TO A SINGLE DOSE OF CAPTOPRIL

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Carboxycathepsin (angiotensin I converting enzyme, kininase II, peptidyl-dipeptidase A, EC 3.4.15.1) catalyzes the conversion of physiologically inactive angiotensin I into pressor angiotensin II and destroys depressor bradykinin (Bk), and is thus a key enzyme of the renin-angiotensin (RAS) and kallikrein-kinin systems [1]. Carboxycathepsin (Cc) is present in the body in two forms: membrane-bound, located on the surface of the endothelium of all the vessels, and free, present in plasma and other biological fluids. Plasma Cc is synthesized by endothelial cells and is secreted by them into the blood stream. The properties and amino acid composition of these forms of the enzyme are identical, but the plasma enzyme contains about three times as much sialic acid [8]. In its effect on the blood pressure (BP) the plasma enzyme plays a substantially less important role than the membrane-bound enzyme. Cc present in the plasma evidently participates in the realization of the compensatory reactions of the body aimed at maintaining BP within the physiological limits. In dogs in extremal states the writers have observed that when BP falls Cc activity rises, and vice versa [2]. If teprotide (SQ 20881), a specific inhibitor of Cc, is injected by intravenous drip into dogs in the early stage of resuscitation, despite injection of the inhibitor, activity of the enzyme, after an initial fall, began to rise gradually in the course of 1.5-3 h, and by 3 h after the beginning of injection it had reached its original level [3]. In the present investigation the effect of inhibition of Cc activity by the specific inhibitor captopril on its plasma concentration was studied.

EXPERIMENTAL METHOD

Four groups of unanesthetized female rats weighing 180-200 g were used. The six rats of group 1 were normotensive and were kept on a standard diet (with a daily Na^+ intake of 0.084 g); the eight rats of group 2 were normotensive and were kept on a low sodium diet (0.024 g Na^+ daily); the rats of groups 3 and 4, with six in each group, were hypertensive and were kept on a standard diet. A model of bilateral renovascular hypertension was produced by constricting the left renal artery by applying a nichrome wire coil after one-stage removal of the contralateral kidney [6]. In the rats of group 4, starting with the first week after the operation, plasma Cc activity and BP were determined once a week for 8 weeks, starting 1 week after the operation. The animals of the first three groups received captopril by gastric tube in a dose of 10 mg/kg. In the control, instead of captopril the rats were given physiological saline (groups 1 and 3) or distilled water (group 2). Blood was taken from the caudal artery at different time intervals (from 1 to 6 h) after administration of captopril. Captopril was given once a week and a single blood sample was taken. Parallel measurements were made of BP by a bloodless method in the caudal artery, using the KN-209 instrument (Natume, Japan). Cc activity was measured in heparinized plasma by a fluorometric method, based on removal of the His-Leu dipeptide from the C-terminal fragment of angiotensin I: Cbz-Phe-His-Leu [2].

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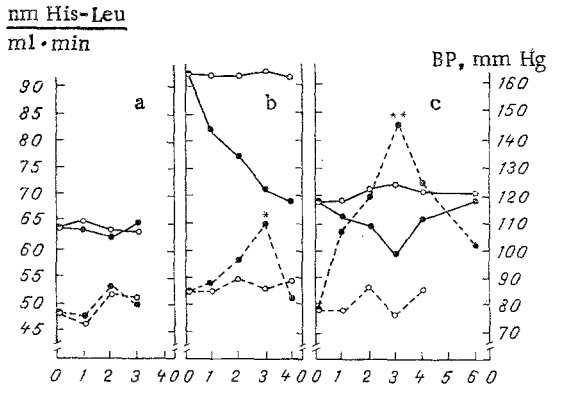

Fig. 1

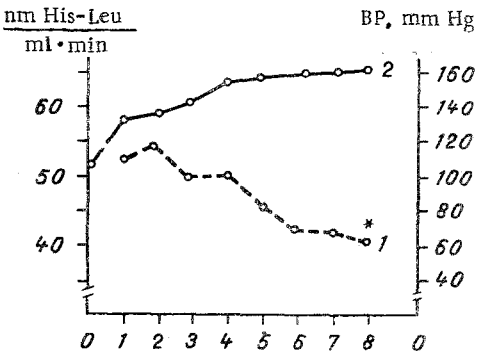

Fig. 2

Fig. 1. Changes in plasma Cc concentration and BP of rats at different times after injection of captopril: a) normotensive (group 1); b) hypertensive (group 3) rats on standard diet; c) normotensive rats on low salt diet (group 2). Abscissa, time (in h). Continuous line — BP; broken line — Cc. Filled circles — after injection of captopril; empty circles — after injection of physiological saline or water. *P = 0.05, **P < 0.001.

Fig. 2. Changes of Cc activity (1) and BP (2) during development of renovascular hypertension after operation. Abscissa, time (in weeks). *P = 0.05.

EXPERIMENTAL RESULTS

After administration of captopril to the animals plasma Cc activity falls [7]. However, the complex of captopril with Cc, like captopril itself, is unstable and easily breaks down. Under these circumstances the serum Cc activity gradually rises [5-13]. To determine the plasma Cc concentration, Cc activity was measured under conditions facilitating breakdown of its complex with captopril: high dilution of the plasma (by 720 times) and relatively prolonged incubation of the samples (2 h). If under these conditions the complex of the enzyme with captopril broke down incompletely during determination of its activity, a gradual increase in Cc activity ought to be observed in the plasma during keeping. It was shown, for example, that after keeping for 3 h in ice, activity of the enzyme was 10% higher than initially, and after keeping the samples for 2-3 weeks at −20°C, according to data given by various workers [5, 13], it was increased by between 10 and 40 times. In the present experiments, however, activity of the enzyme, determined on the first day, was the same as on the 2nd day, and also on all successive days until the 14th day of keeping of the plasma. This stability of the results is evidence in support of complete breakdown of the complex under the conditions used.

In the rats of group 2, Cc activity and BP were virtually indistinguishable from normal values. In the rats of group 3, Cc activity also was within normal limits, but BP averaged 165 mm Hg (fluctuating from 155 to 186 mm Hg). A single dose of captopril caused the BP of the animals of these groups to fall (Fig. 1b, c), but did not change BP in the rats of group 1 (Fig. 1a). Against the background of the fall of BP in the animals of groups 2 and 3 an increase in the plasma Cc concentration was observed, whereas in the rats of group 1, in which BP remained unchanged, the Cc concentration did not increase. An increase in the plasma Cc concentration was observed as early as 1 h after injection of captopril and it reached a maximum after 3 h (Fig. 1b, c). A more marked and prolonged increase in the Cc level was observed in rats kept on a low salt diet (group 2). The increase in the plasma Cc concentration in response to administration of captopril against the background of lowering of BP was thus observed only in rats with an activated RAS. No such response was present in normotensive rats.

The absence of a fall of BP after a single dose of captopril taken by normotensive persons and animals has been observed by a number of investigators [7]. Administration of captopril against the background of diuretics or of a low salt diet, however, causes BP to fall, just as in most hypertensive patients [7]. The increase in the plasma Cc concentration is evidently a compensatory reaction of the body to inhibition of its activity by the inhibitor. It is also possible that it reflects the state of the RAS in the body. Whatever the case, in rats with more marked activation of RAS (group 2) a greater increase in the Cc concentra-

tion also was observed. In all probability biosynthesis of the enzyme was increased. For instance, an increase in the Cc concentration in the serum and in the plasma membranes of the lungs was observed by Fyhrquist et al. [11] after prolonged administration of captopril to rats, and by Forslund et al. [10] after administration of enalapril, another powerful Cc inhibitor. Most probably secretion of the enzyme by the endothelium is increased, although, of course, the possibility cannot be ruled out that enzyme present on the surface of the vascular endothelium is released into the blood stream. The time of synthesis of secretory proteins is about 20-40 min [14], and of membrane proteins 1-3 h [4]. In the present experiments the increase in Cc activity began about 1 h after administration of the inhibitor. Whereas captopril appears in the blood 15-30 min after peroral administration [9], its secretion begins to increase on average at the end of the first hour after appearance of the preparation in the blood.

Evidence that in this case there is a compensatory response of the body is given by the results of the writers' previous experiments on animals under conditions of resuscitation [2, 3] and also by data showing a fall of plasma Cc activity in spontaneously hypertensive rats [12].

We studied relations between plasma Cc activity and BP in animals during the formation of hypertension (group 4), starting with the first week after the operation. As will be clear from Fig. 2, during a gradual increase in BP and its stabilization at a relatively high level, plasma Cc activity began to fall in the 4th week.

The body thus responds to administration of a single dose of the Cc inhibitor captopril by a fall of BP and a simultaneous increase in the plasma Cc concentration in animals with an activated RAS. In normotensive rats, kept on a standard diet with a normal sodium content, no such response was observed. Consequently, during changes in BP, under both acute (after a single dose of captopril, during resuscitation [2]) and chronic (long-term administration of captopril [11] or enalapril [10], the formation of renovascular hypertension) conditions, opposite changes in activity and concentration of Cc in the plasma are observed. It can be tentatively suggested that the plasma Cc to a certain degree reflects the functional state of the RAS.

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