successively at pH 5, 6, 7.3, and 8.5. The potassium contracture method of measuring changes in mechanical threshold of muscle fibres has been extensively employed to illustrate this aspect of caffeine activation in vertebrate[16], crustacean[15] and insect[17] skeletal muscles. The results of typical experiments are shown in Figure 2. In these experiments, the muscle fibre preparations were perfused for 10 sec with normal crab ringer at the experimental pH, and the perfusate was then replaced by 200 m*M* KCl crab ringer at this pH. It is clear that reduction in pH noticeably lowers the rate of rise of the KCl contracture tension, but there was no significant effect of pH on the maximal contracture tension achieved. From these results we may rule out any effect of changes in pH on the actual mechanical threshold. Changes in pH would thus appear to have little effect on the quinine interaction with the excitation-contraction coupling process at the muscle fibre periphery.

Changes in pH do have an important effect on the physical state of some alkaloids in solution. Caffeine contractures increase in size with increasing pH of the medium[18], and it has been suggested that the variability of caffeine action may be due to the greater proportion of 'aggregated' caffeine in solution at low pH values[19], the uncharged (more active) form of caffeine being predominant in solution at high pH values. The proportion of uncharged (highly active) to total quinine in solution is also affected by pH, the uncharged portion increasing as the pH is raised[3]. It is possible that enhancement of quinine contracture tension with elevation of pH may be due to greater availability of uncharged quinine in solution at these high pH values. However, this view is difficult to reconcile with the observation that changes in pH had little effect on the duration of the quinine contracture of crab skeletal muscle. In addition, lowering of the pH of crab ringer did not cause any noticeable precipitation of the quinine, an event often observed with caffeine[19,20], even though a quinine concentration of 5 m*M* is near saturation. A pH interaction with the excitation-contraction coupling mechanism would seem to be a more likely reason for pH modification of contracture tension. It is clear that any modification of the quinine interaction with the excitation-contraction coupling mechanism by changing the pH does not involve a change in the main two peripheral parameters of membrane potential and mechanical threshold. Since the speed of pH-induced tension changes of quinine contractures suggests an extracellular site of action, but not at the fibre periphery, the transverse tubular system would seem the most likely site of pH-induced action on the excitation-contraction coupling.

One possibility is that elevation of pH may sensitize the transverse tubular system to any depolarizing action of quinine and potassium, thus accelerating the inward transmission of the surface depolarization and leading to a greater than normal release of calcium from the sarcoplasmic reticulum. This view would explain the increase in the rate of rise of contracture tension with increasing pH value. Alternatively, a high pH value may facilitate a rapid and greater than normal calcium release from the dyadic junctions between the transverse tubular system and the sarcoplasmic reticulum[21], and a low pH may inhibit calcium release. In this case, pH changes would affect the calcium control system of the dyad itself from an extracellular site, but without noticeable effect on the transverse tubules, thus short-circuiting the normal initial stages of the excitation-contraction coupling mechanism. Either view would account for the striking alterations seen in the rising phase of both potassium-induced and quinine-induced contractures as the pH is altered.

Résumé. Les contractures des muscles squelettique du cancre *Carcinus maenas* déclanchées par la quinine sont augmentées si on soulève le pH du milieu extracellulair et se diminue par la réduction du pH. On propose que le lieu d'action du pH sur ces contractures est le système des tubules transversales ou le changement du pH peu modifier la mobilisation du calcium.

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Antihypertensive Effect of Purified Enzyme Showing Angiotensinase Activity from Rabbit Red Cells in Rats

Our previous report has described a purification method and properties of the enzyme showing angiotensinase activity from rabbit red cell hemolysate[1]. The present study was undertaken to observe the effect of s.c. injection of the enzyme on the blood pressure levels in experimental renal hypertensive rats. The experiment was also designed to follow the changes in the levels of plasma renin and angiotensinase activities in acute hypertensive rats.

Materials and methods. The enzyme was prepared from rabbit red cell hemolysate by the fractionation with ammonium sulfate and DEAE cellulose column chromatography[1]. The enzyme, which inactivated completely 300 µg of Val^5-angiotensin-II-amide (Ciba) during 1 h incubation, was injected s.c. in a dose of 5 mg of protein per rat daily. The preparation did not show any depressor effect in rats when injected i.v.

Experiment 1. 17 rats with Goldblatt-type hypertension were divided into the following 2 groups. The experimental 9 rats were injected the enzyme solution (0.5 ml) during 5 weeks period following nephrectomy. The control 8 rats received the injection of phosphate buffer solution (0.1 *M*, pH 6.8). Blood pressure was measured

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periodically by plethysmography with a tail cuff, using the apparatus of Natsume Instrument Co., Tokyo.

Experiment 2. 60 rats received the operation for Goldblatt-type hypertension were divided into the following 2 groups. 1 group was treated with the subcutaneous injection of the enzyme and the other group treated with phosphate buffer as a control for 3 weeks following nephrectomy. The levels of plasma renin activity (PRA)

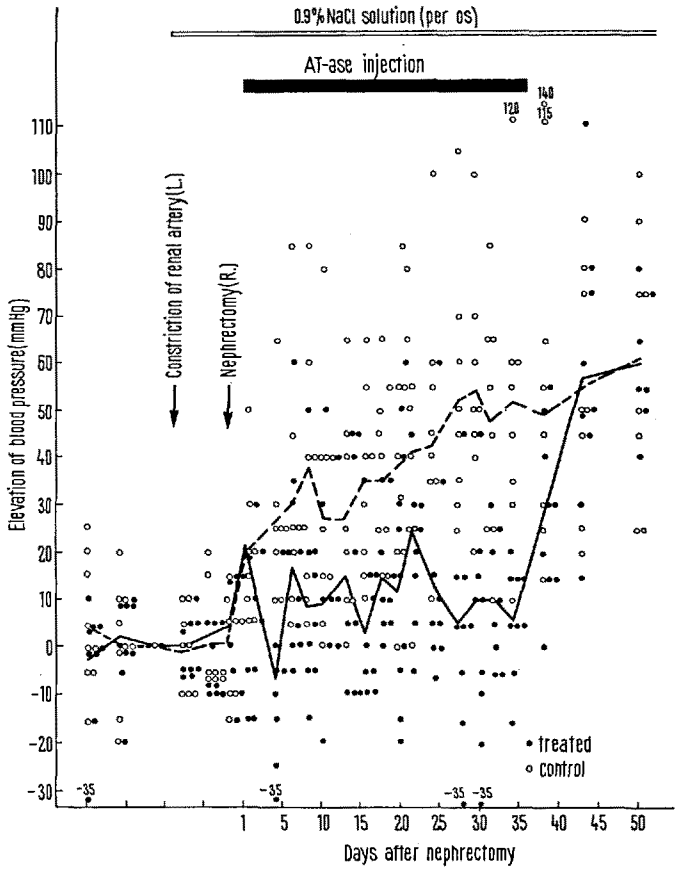

Fig. 1. Effect of ATase injection on blood pressure levels in acute stage of experimental renal hypertensive rats. ---, average of blood pressure elevation of 8 control rats. —, average of blood pressure elevation of 9 treated rats.

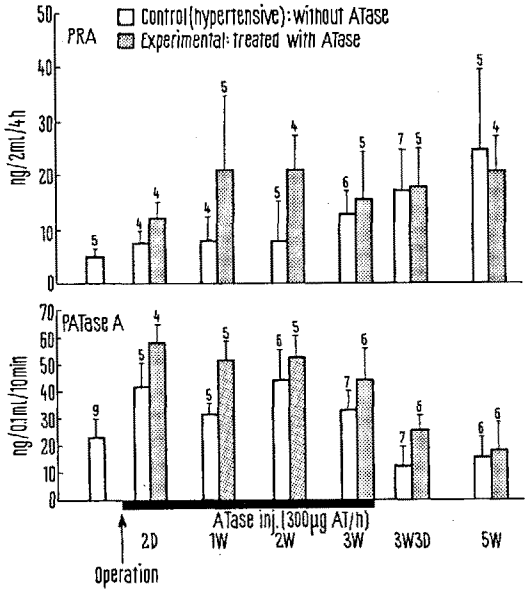

Fig. 2. Plasma renin activity (PRA) and plasma angiotensinase activity (PATase A) in acute hypertensive rats before, during and after treatment with ATase. □, average of PRA or PATase A with S.D. and number of rats in control group. ■, average of PRA or PATase A with S.D. and number of rats in treated group with ATase.

and angiotensinase activity (PATase A) were estimated before, during and after the 3 week injection period. PRA was measured according to the method of JONG[2] and was expressed as ng of angiotensin-II equivalent produced/2 ml of plasma/4 h. PATase A was expressed as ng of inactivated angiotensin-II/0.1 ml of plasma/10 min.

Results and discussion. Experiment 1. The effect of s.c. injection of the enzyme on the blood pressure levels in Goldblatt-type hypertensive rats is shown in Figure 1. The development of hypertension in the treated group was significantly inhibited during the injected period as compared with that in the control group. The difference of the blood pressure levels between the both groups was significant at 1% significant level. After cessation of the enzyme injection, the blood pressure levels rose to that of control rats within a few weeks. No reduction of blood pressure was observed in the chronic hypertensive rats upon the injection of the enzyme.

Experiment 2. Changes in the levels of PRA and PATase A in acute hypertensive rats treated with the purified angiotensinase are illustrated in Figure 2. PRA in normal rats were 5.2 ng in average. In the control group, PRA increased gradually to an average of 21.5 ng after 5 weeks following the operation with blood pressure elevation.

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However, the levels of PRA in the experimental group rose to 21 ng already in the 1st or 2nd week after the injection.

PATase A in the control animals rose to 40–45 ng immediately after the operation from a preoperative average of 23.4 ng. The high level was maintained for 2 weeks; then it decreased gradually in the 3rd week. In the 5th week, PATase A became lower than the preoperative level. In the experimental group, during the enzyme injection period in combination with suppression on blood pressure elevation, PATase A were usually higher than those in the control group, and became normal 2 weeks after cessation on the injection.

The results suggested that the renin angiotensin system could play an important role in the acute phase of experimental hypertension. The reasons for this conclusion are as follows; 1. the development of hypertension was suppressed by the ATase injection in acute stage, 2. the increase of PRA in the treated group suggested the existence of some feed back mechanism by the treatment with ATase in acute phase.

Zusammenfassung. In der akuten Phase der Goldblatt-Hypertonie der Ratte verhinderte die Injektion von reiner Angiotensinase ein Aussteigen des Blutdruckes. Die Behandlung war unwirksam bei chronischem Hochdruck.

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Activation of Rat Stomach Histidine Decarboxylase After Inhibition of Acid Secretion with 2-Phenyl-2-(2-Pyridyl)-Thioacetamide (SC-15396)

Gastric histidine decarboxylase in the rat is an adaptive enzyme, its activity being dependent upon the functional state of the stomach[1,2]. Thus, the activity is low after prolongated fasting and is markedly increased after gastrin (pentagastrin) administration, vagal excitation or feeding. The enzyme-activating effect of vagal excitation and feeding – but not that of pentagastrin – is abolished by antrectomy[3–5], indicating that endogenous gastrin is one important factor in the regulation of the enzyme activity. Gastrin release is markedly dependent upon antral pH, a low pH being inhibitory[6]. Recently, vagal denervation and treatment with atropine were found to cause a powerful activation of gastric histidine decarboxylase[5,7]. Since both vagotomy and atropine treatment abolished basal and pentagastrin-stimulated acid secretion[5,7] it was suggested that the resulting high antral pH would stimulate gastrin release, thus activating the histidine decarboxylase. The following concept was advanced[5,7]. All agents (except pentagastrin) and all experimental conditions, which stimulate acid secretion through a direct action on the parietal cell and which do not have a gastrin-releasing effect, will suppress the activity of gastric histidine decarboxylase by lowering the antral pH, thus inhibiting gastrin release. All agents and experimental conditions, which inhibit acid secretion without inhibiting gastrin release, will activate the enzyme by increasing the antral pH, thus stimulating gastrin release.

2-Phenyl-2-(2-pyridyl)-thioacetamide (SC-15396) is an inhibitor of gastric acid secretion. Having no anticholinergic effects, it was introduced as a gastrin antagonist ('anti-gastrin')[8–11]. In the rat and dog, SC-15396 also reduces the secretory response following treatment with histamine, cholinergic drugs and vagal excitation[12–17] and should consequently be regarded as a general inhibitor of gastric acid secretion. From the concept presented above, treatment with SC-15396 should cause activation of gastric histidine decarboxylase as a consequence of the resulting elevation of antral pH. This assumption was tested in the present study.

SC-15396 (Searle, Chicago) was dissolved in dimethylsulfoxide (10 mg/ml) and given s.c. in a dose of 50 mg/kg to rats, fasted for 48 h prior to injection. Control rats (also fasted for 48 h) received DMSO alone (5 ml/kg). All rats were killed 3 or 6 h after the injections. The mucosa of the oxyntic gland area was scraped off the stomach wall and homogenized in 0.1 M phosphate buffer, pH 6.9, to a final concentration of 100 mg (wet weight) per ml. After centrifugation at $10.000 \times g$ for 15 min in a refrigerated centrifuge, aliquots of the supernatant (usually 0.4 ml) were incubated with carboxyl-labelled ^{14}C-L-histidine ($4 \times 10^{-4} M$; 1.3 mC/mmol, New England Nuclear) in the presence of pyridoxal-5-phosphate ($10^{-5} M$) and glutathione ($4 \times 10^{-4} M$) in a total volume of 0.5 ml. The samples were incubated under nitrogen at 37 °C for 1 h under continuous agitation in a metabolic shaker. The histidine

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