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Inhibition of renin by sodium deoxycholate

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RENIN is an endopeptidase which splits a leucyl–leucine bond in alpha-2-globulin substrate, angiotensinogen, to yield a decapeptide, angiotensin I. Three kinds of renin inhibitors have been reported. These are the naturally occurring renin preinhibitor, lysophospholipid, reported by Sen *et al.*[1,2] and Osmond *et al.*[3] methyl or ethyl esters of synthetic tetrapeptides, Leu-Leu-Val-Tyr and Leu-Leu-Val-Phe, from our laboratory;[4] and heparin.[5]

In a previous paper[6] we demonstrated that the inhibitory active principle against renin occurred in the rabbit bile. The present study revealed that sodium deoxycholic acid, which was soluble in water, inhibited the angiotensin formation by renin *in vitro*. The inhibition of renin activity by sodium deoxycholate was competitive.

Sodium deoxycholate was obtained from Difco Laboratories, U.S.A. Renin was prepared from rabbit renal cortex using a modification of the method of Haas *et al.*,[7] followed by fractionation with ammonium sulfate between 30 and 60 per cent saturation and dialysis against physiological saline containing 2×10^{-3} M EDTA. This preparation had a renin pressor activity equivalent to 17·3 μg of synthetic Val^5-angiotensin II amide (CIBA) per ml and was diluted 10-fold with physiological saline before use for experiments. Renin substrate was prepared from the heparinized plasma of rabbits nephrectomized bilaterally 24 hr previously according to the method of Sen *et al.*[1] This preparation contained renin substrate at a concentration of 18·0 μg of angiotensin content per ml. The substrate used for experiments was diluted 3-fold with physiological saline. Protein was estimated by the method of Folin–Lowry.[8]

The reaction mixture in a total volume of 3·0 ml consisted of 0·2 ml of renin (protein amount 1·08 mg/ml), 0·5 ml of renin substrate (protein amount 3·1 mg/ml), 1·0 ml of distilled water containing sodium deoxycholate and 1·3 ml of 1/15 M phosphate buffer, pH 6·4. The mixture was incubated at 37° for 10 min and the reaction was stopped by heating the mixture in a boiling water bath for 5 min.

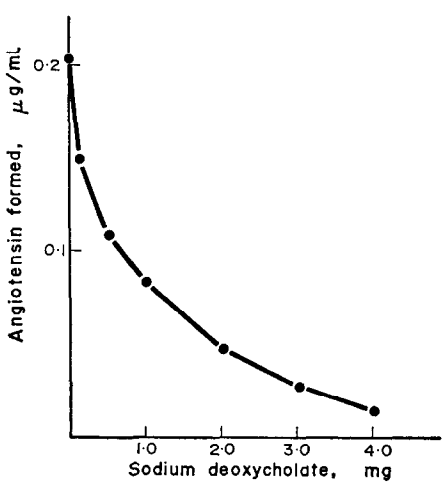

FIG. 1. Inhibition *in vitro* of the renin reaction in the presence of various amounts of sodium deoxycholate. The incubation mixture contained 0·2 ml of renin (protein amount 1·08 mg/ml), 0·5 ml of renin substrate (protein amount 3·1, mg/ml), sodium deoxycholate (0·2–4·0 mg) in 1·0 ml of distilled water and 1·3 ml of 1/15 M phosphate buffer pH, 6·4. The mixture was incubated at 37° for 10 min. To stop renin activity, the tubes were placed for 5 min in a boiling water bath.

The control assay system produced usually 0·20–0·21 μg/ml of angiotensin (total, 0·60–0·63 μg). The angiotensin that was formed was assayed by its pressor response in the anesthetized, vagotimized rat treated with pentolinium. All assays were performed by making bracket comparisons with synthetic Val^5-angiotensin II amide.

Known amounts of sodium deoxycholate ranging from 0·03 to 1·3 mg/ml (final concentration: $8{\cdot}0 \times 10^{-5}$ to $3{\cdot}2 \times 10^{-3}$ M) produced significant inhibition of angiotensin formation of renin *in vitro*. Figure 1 shows the inhibitory effects in the presence of varying amounts of sodium deoxycholate on the formation of angiotensin. The amount of angiotensin formed decreased as the amount of sodium deoxycholate added to the assay system increased.

When the mixture of 0·3 μg of synthetic Val^5-angiotensin II amide and 4 mg of sodium deoxycholate was incubated at 37° for 30 min, the pressor response of the mixture in the rat was the same as that of synthetic angiotensin II amide used. The result indicated that sodium deoxycholate neither modified the physiological pressor activity of angiotensin, nor showed hypotensive effect when injected intravenously. Next, the following experiments were made to know whether sodium deoxycholate had a destroying effect to renin substrate and renin activity or not. One-half ml of renin substrate was

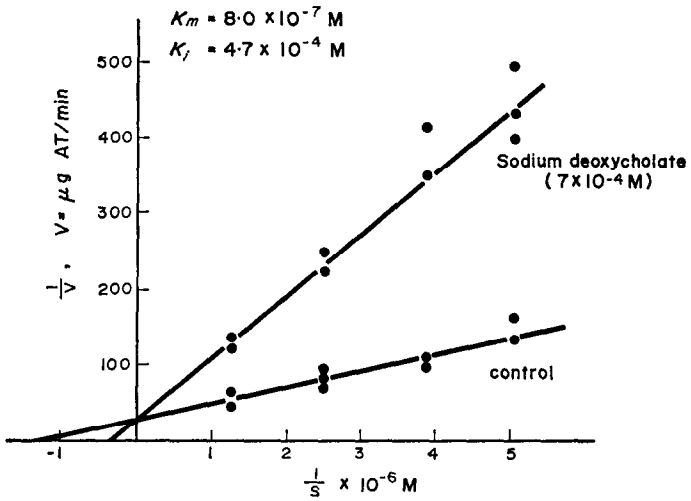

FIG. 2. Lineweaver–Burk plot of the relationship between the reciprocals of substrate concentrations and reaction velocity. In each experiment, 0·2 ml of renin was incubated with varying amounts of substrate (0·1, 0·13, 0·2 and 0·4 ml). The concentration of sodium deoxycholate was $7{\cdot}0 \times 10^{-4}$ M. The incubation was performed at 37°, pH 6·4, for 5 min. It was assumed that 1 mole of substrate produced 1 mole of angiotensin.

incubated with 4 mg of sodium deoxycholate at pH 6·4, 37° for 2 hr. After the incubation, the concentration of renin substrate was measured. The concentration of renin substrate was estimated by incubation of the substrate with excess renin according to a modification of the method of Pickens *et al.*[9] Incubation of renin substrate with sodium deoxycholate was sustained without any reduction of the amount of angiotensin formed as compared to control experiment. Sodium deoxycholate, therefore, does not inactivate renin substrate under the conditions of the experiment. One-fifth ml of renin was incubated with 4 mg of sodium deoxycholate at pH 6·4, 37° for 90 min. The preincubation of renin with sodium deoxycholate resulted in no reduction of enzyme activity *in vivo* (pressor activity) and *in vitro* (angiotensin formation). In these experiments, sodium deoxycholate was removed from the incubation mixtures by dialysis against distilled water before the estimation of the amount of renin substrate or renin activity. The results obtained suggested that sodium deoxycholate inhibited the reaction of renin with substrate.

The inhibitory mechanism of sodium deoxycholate was investigated; the Lineweaver–Burk method[10] of plotting was used for distinguishing types of inhibition. Inhibition of a competitive nature was observed (Fig. 2).

Besides sodium deoxycholate, inhibitory effects of renin by several other related compounds such as sodium cholate, taurocholic acid and glycocholic acid are under investigation.

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Third Department of Medicine,
Osaka University Hospital,
Fukushima-ku, Osaka, Japan

KUNIO HIWADA
TATSUO KOKUBU
YUICHI YAMAMURA

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Alteration of glucose and glycogen in specific regions of mouse central nervous system by L-3,4-dihydroxyphenylalanine (levodopa)*

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THE IMPLICATION of discrete structures of the central nervous system (CNS) in the syndrome of Parkinsonism[1,2] and the known increase of dopamine in the CNS by L-3,4-dihydroxyphenylalanine (levodopa),[3] an agent effective in the treatment of Parkinsonism,[4] has led to a search for a regional impact of levodopa on the intermediary metabolism of the CNS. When used in combination with a monoamine oxidase inhibitor, levodopa increased blood and brain glucose and lowered brain glycogen.[5] In combination with reserpine, however, DL-3,4-dihydroxyphenylalanine (*dl*-DOPA) did not increase brain glucose.[6]

This report presents evidence that a high dosage of levodopa alone indeed alters levels of glucose and glycogen of mouse CNS. Moreover, the temporary lowering of glycogen resides at the lower levels of the neuraxis studied.

Groups of adult male mice (17–23 g) from Carworth Farms were fasted overnight and then injected intraperitoneally with 0·1 ml/10 g body wt. of a suspension of levodopa in 0·9% saline (400 mg/kg). Control animals received an equal volume of 0·9% saline. At various times, thereafter, whole animals were rapidly frozen in Freon 12 (CCl_2F_2) chilled to −150° with liquid N_2,[7] and stored at −80° until dissection.

Samples of cerebral cortex, caudate nucleus, brain stem (ponsmedulla) and spinal cord were dissected and weighed in a cold room at −15°. Approximately 15 mg of each region of four animals from either control or drug-treated groups, respectively, was combined for the preparation of precipitated

* A preliminary report of these results was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Palo Alto, Calif. (August 1970).