Renin Releasing Activity of a Blood Plasma Fraction

The nature of the stimulus that causes renin release from the kidneys has not been established with certainty. It is known that the kidney rapidly releases some of its renin in various circumstances such as hemorrhage and acute renal ischemia[1-3]. The mechanism that causes kidneys to release renin is still obscure and probably more complex than formerly thought. In 1965 FASCIOLO[4] suggested the hypothesis that a plasma releasing factor may be involved.

To study humoral factors which may enhance or repress the release of renin, the technics of surviving rat kidney slices developed in our laboratory was used[2]. The slices were incubated in a Warburg flask with a modified Krebs-Ringer solution, and the renin being released during a 2 h incubation period was estimated by measuring the renin activity of the fluid bathing the slices. In this paper the effect of plasma protein fractions on the release of renin is studied.

Materials and methods. Rat kidney slices, 100 mg, including both cortex and medulla, were incubated for 2 h at 37 °C with 2.9 ml of Krebs-Ringer-phosphate-glucose-dextran solution (KRPGD) in a Warburg flask according to DE VITO et al[2]. The following plasma fractions, used in the present study, were dissolved in the KRPGD solution: Bovine serum fraction (BSF) and nephrectomized rat plasma fraction (NRSF). The bovine serum was treated according to SKEGGS et al.[6], up to the step of precipitating with 2.3 *M* ammonium sulphate, and the precipitate excluded. The supernatant was then dialyzed against running tap water and afterwards against distilled water for 72 h at 4 °C. It was then lyophilized and the dry powder of the BSF was kept at −10 °C until used.

To obtain the NRSF, 24 h after bilateral nephrectomy, the rats were bled, the heparinized blood was centrifuged and the plasma treated as explained for bovine serum. The NRSF was also fractioned by using a Sephadex G-100 chromatographic column. Rat nephrectomized plasma was taken in 5 ml, seed at the top of the column and 1 *M* NaCl, pH 8.0 (0.1 *tris*-HCl buffer) was used as eluent. Protein peaks were detected with UV-light. The second peak composed mainly by albumin with slight admitures of other small proteins was dialyzed, lyophilized and store in the cold until used.

The albumin used as control (average Mol. Wt.: 67.000, 3 × crystallized) was obtained from Mann Research Laboratories.

The Warburg flasks were gasified with oxygen, manometric readings were taken every 15 min and oxygen consumption was estimated as usual. The method for estimation of renin activity of unincubated and incubated slices and also of the medium has been described previously[2]. Renin activity was expressed in nanograms of angiotensin formed per milligram of fresh kidney tissue.

Results. Average of 20 experiments are represented in the Figure. The renin content of kidney slices shows that the average of control slices was: a) non-incubated 33.4 SE ± 4.1 ng of angiotensin/mg fresh tissue, and b) incubated with KRPGD alone 37.4 SE ± 4.2 ng/mg. In the slices incubated with the addition of plasma fractions, the renin activity was: a) with BSF (5 mg/ml) 29.9 SE ± 4.1 ng/mg, b) with NRSF (3 mg/ml) 30.2 SE ± 3.5. In both cases, the difference with unincubated slices was not significant ($p < 0.2$). However when compared with incubated control, it was significant ($p < 0.01$).

These results indicated that the renin activity of the incubated controls slices show a tendency to rise, while those with BSF and NRSF show a decrease in their renin activity.

The average renin activity released from the slices to the liquid was: a) 7.6 SE ± 0.6 ng/mg in the controls, b) 16.7 SE ± 1.2 ng per mg in the slices incubated with BSF and c) 16.4 SE ± 1.0 ng/mg in the slices with the addition of NRSF.

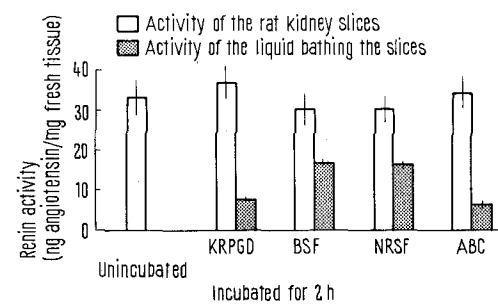
The difference between renin released in slices incubated with plasma protein fractions and controls is: 9.1 ng/mg for the kidney slices incubated with BSF ($p < 0.001$) and 8.8 ng/mg with NRSF ($p < 0.001$).

Discussion. These results show that the albumin fraction of ox and nephrectomized rat plasma show a renin releasing effect on rat kidney slices.

To exclude artifacts produced by denaturization of serum proteins by ammonium sulphate, which may cause the release of renin from the slices, nephrectomized rat plasma also fractionated by a Sephadex column was used. In this case, the usual increase in renin release was also observed. It seems safe to conclude that the active protein is already present in blood plasma.

It may be argued that the release of renin is due to some unspecific protein effect. That this is not the case is shown by experiments in which protein serum fractions were added in the same amounts and found to be devoid of any releasing effect. Albumin bovine crystallized (ABC), added to the slices for a final protein concentration similar to that of the plasma fraction used (5 mg/ml), was unable to produce any releasing effect, as shown in the Figure.

The finding of an extrarenal renin releasing factor does not invalidate nor exclude other mechanism[7]. Indeed it is



Effect of plasma fractions on renin activity and on renin release. Average of 20 experiments are represented. The mean renin activity released by control slices incubated with KRPGD alone was 7.6 SE ± 0.6 ng/mg, with KRPGD plus BSF was 16.7 SE ± 1.2 ng angiotensin/mg tissue and with the addition of NRSF 16.4 SE ± 1.0 ng/mg. Both plasma fractions increase significantly ($p < 0.001$) the renin being released to the liquid. In 10 experiments, albumin bovine 3-crystallized (ABC) was added to KRPGD to have a protein concentration similar to that of BSF and NRSF. Last pair of bars shows that renin release was not greater than the controls.

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a well known fact that isolated perfused kidneys will release renin when their blood flow is sharply reduced[5].

Thus, a reduction of the effective blood volume may give rise to the release of renin by two mechanism: a) by a reduction of the renal blood flow, consecutive to arterial hypotension and/or renal vasoconstriction, and b) by the release of an humoral renin releasing factor.

Résumé. L'activité de la rénine de coupes de reins de rats et celle de la rénine libérée par elles pendant une incubation de 2 heures ont été mesurées. Les coupes témoins ont libéré 22% de leur contenu original. Après adjonction d'albumine sérique, la quantité de rénine libérée était de 49%.

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Comparison of the Blocking Potency of Local Anesthetics Applied at Different pH Values

There has been some question as to whether the uncharged molecule of local anesthetic or the charged form is responsible for the nerve blocking action[1,2]. In a recent series of papers we have concluded that in the squid giant axon local anesthetics block the action potential from inside the nerve membrane in the charged form. This conclusion was reached following experiments in which the effect of pH on the action of a given concentration of tertiary and quaternary local anesthetics was studied on both internally and externally perfused axons. Additional experiments have now been completed to test our hypothesis more quantitatively.

Giant axons of the squid *Loligo pealei* were externally and internally perfused as described previously[3,4]. The maximum rate of rise of the action potential was held constant at a slightly hyperpolarized level (−60 to −75 mv) by application of a polarizing current across the nerve membrane to eliminate the effect of resting potential changes on the action potential. One tertiary derivative of lidocaine (6211–2 [N-(2-Methoxyethyl)-methylamino] 2′, 6′ Acetoxylidide · $pK_a = 6.3$) and procaine were used in the study. The purpose of the experiments was to demonstrate that if the concentration of the charged form inside is held constant the degree of block produced by the local anesthetic will be independent of total anesthetic concentration. Calculations for the changes in the pH of the solution needed to maintain the concentration of the charged form inside constant when the total concentration is altered can be made from the Henderson-Hasselbach equation, $pK_a = \text{pH} + \log [BH^+]/[B]$ where $[BH^+]$ and $[B]$ are the concentration of the charged and uncharged forms, respectively.

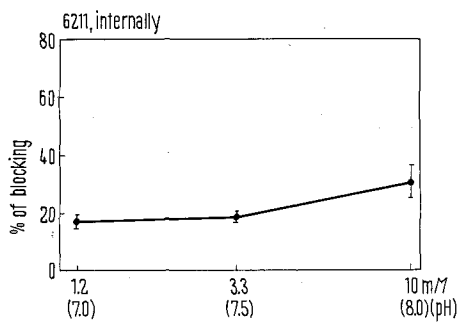

Fig. 1. The effect of changing pH on the blocking potency of different concentrations of 6211 applied inside a squid axon. Each point represents the mean of 4 experiments with the standard error indicated by the bars.

Figure 1 shows the results of experiments in which 3 concentrations of 6211 were applied inside the axon at different pH values. In these experiments the pH of the external solution was held at 8.0 by continuous perfusion. Separate experiments have shown that the pH changes alone, within the ranges used in Figure 1, had no significant effect on the maximum rate of rise of the action potential[3]. As is evident from the graph there is relatively little difference in the degree of block as you go from 1.2 to 3.3 mM if the pH is altered from 7.0 to 7.5. At 10 mM (pH 8.0), there is about a 10% increase in the percent of block as compared to the lower concentrations. There is a marked difference, however, if these results are compared

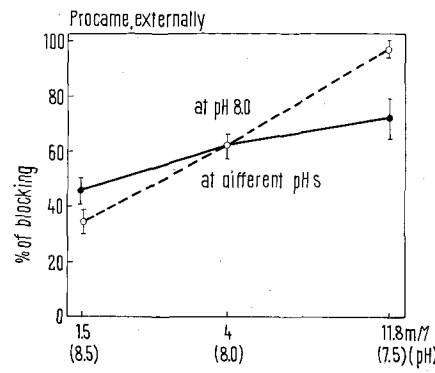

Fig. 2. The effect of pH changes on the blocking potency of externally applied procaine. The solid line represents experiments in which the concentration of procaine was increased from 1.5 to 11.8 mM while changing the pH of the solution from 8.5 to 7.5. The dashed line represents experiments in which the concentration of procaine was similarily increased while keeping the pH at 8.0. Each point is the mean of 5 experiments with the standard error.

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