In vitro effects of various metabolic inhibitors on the formation of inactive renin and the loss of renin in rabbit uterine tissue

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Abstract. A pronounced formation of renin occurs during incubation of non-pregnant uterine tissue slices in vitro. The synthesized renin appears in an enzymatically inactive form, which can be activated by acidification. Prior to incubation only a small fraction of inactive renin is present. The formation of inactive renin is blocked by puromycin and by inhibition of energy metabolism, indicating a de novo synthesis. A similar pattern of inhibition prevails the modest formation of inactive renin in post-partum uterus.

The marked loss of active renin seen during incubation of post-partum uterine tissue is partly prevented by an inhibition of energy metabolism. Potent inhibitors are iodoacetate and chloroquine. These findings are in accordance with lysosomal engagement in the inactivation of renin. Incubated kidney cortex tissue shows only a minor loss of renin during incubation. This loss is uninfluenced by attempts to block it.

The active (directly measurable) renin in a suspension of non-pregnant and particulary in pregnant and post-partum rabbit uterine tissue slices is to a certain extent lost following incubation in vitro at pH 7.4 (Jørgensen 1974, 1976a). Further experiments demonstrated (Jørgensen 1976b), that uterus also contains inactive renin. Increasing amounts of inactive renin followed incubation, most remarkable in non-pregnant uterus. No inverse correlation exists between the renin loss during incubation at pH 7.4 and the gain of inactive renin.

The present study discusses protein synthesis and energy metabolism as prerequisites for the in vitro formation of inactive renin during incubation of slices of non-pregnant and post-partum uterus at pH 7.4. Furthermore it has been investigated, whether or not the loss of active renin in post-partum uterus has the same need for a functioning protein synthesis and the same energy demand as has protein degradation in general, since this might indicate a common mechanism. The total amounts of renin (active plus inactive) have also been stated, since a conversion between active and inactive renin might take place during incubation.

Consequently, incubation was carried out with and without glucose, puromycin, DNP and iodoacetate added to the incubation medium, and both with and without aeration with oxygen during incubation. In order to investigate the direct role of lysosomes in the loss of renin, incubation was also carried out with chloroquine addition.

Similar preparations of kidney cortex incubated with and without glucose or puromycin added to the medium of incubation were compared with uterine preparations.

Materials and Methods

Animals. Uterine wall from non-pregnant and post-partum albino country rabbits supplied from Statens Seruminstitut were used. The animals were killed by a blow on the neck. In case of post-partum rabbits the execution took place 6–36 (in average 10) hours after parturition. Kidney cortex from non-pregnant rabbits of the same strain was used.
Preparation and incubation methods of tissue slices in vitro were previously described (Jørgensen 1974, 1976a). The incubation medium was as before NaCl 119 mmol/l, NaHCO₃ 17.5 mmol/l, KCl 7.0 mmol/l, CaCl₂ 2.0 mmol/l, MgSO₄ 1.2 mmol/l, NaH₂PO₄ 1.2 mmol/l and glucose 11.0 mmol/l. Neomycine 2.5 mg per ml was added to all samples prior to incubation. In some experiments glucose enrichment was omitted. In others supplementary addition took place of either puromycin dihydrochloride (Sigma) 0.2 mmol/l (final concentration), IC₃₂COONa 2.0 mmol/l, dinitrophenol (DNP, Sigma) 2.0 mmol/l or chloroquine (Sigma) 0.2 or 2.0 mmol/l prior to incubation. During the incubation for 44 h at 37°C in a Dubnoff Metabolic Shaking Incubator, the samples were aerated with 95 per cent O₂/5 per cent CO₂. In some cases this gas mixture was replaced by 95 per cent N₂/5 per cent CO₂.

**pH in the medium after incubation.** a) In non-pregnant uterus preparations pH remained about 7.4 except in samples incubated with DNP or anaerobically with a glucose containing medium, in which cases pH was about 6.5. b) In post-partum uterus preparations pH in most cases remained about 7.4 (slightly reduced in samples incubated with puromycin plus glucose). In samples incubated with DNP, anaerobically or with chloroquine plus glucose, pH was about 6.5 after incubation. c) In kidney cortex preparations pH in all cases remained about 7.4.

*Tissue extraction* (freezing and thawing 3 times, homogenization in a Potter-Elvehjem homogenizer with phosphate buffer pH 7.4 followed by extraction for 18 h at 4°C (Jørgensen 1976a), *renin radioimmunoassay* (Poulsen

![Graph showing relative amounts of inactive renin](image)

**Fig. 1**

The content of inactive renin in *non-pregnant uterus* before and after incubation at pH 7.4. The amount of inactive renin in slices plus medium is given in per cent of the initial amount of active renin. The samples were unincubated (the left column) or incubated at pH 7.4 for 44 h with or without glucose, with puromycin 2.0 mmol/l plus glucose, with DNP 2.0 mmol/l plus glucose, with iodoacetate 2.0 mmol/l plus glucose and under anaerobic conditions with glucose. The bars indicate ± 1 SEM. The numbers in the brackets indicate the number of experiments. The *P* values indicate significance in increase of inactive renin with incubation. NS: *P* > 0.05.

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The content of inactive renin in post-partum uterus before and after incubation at pH 7.4. Symbols as in Fig. 1, note change of scale. The samples were furthermore incubated with chloroquine 2.0 or 0.2 mmol/l plus glucose.

Results

Inactive renin

Non-pregnant uterus

A significant \((P < 0.001)\) gain in enzymatic activity of renin was demonstrated when the unincubated preparations with a content of from 0.5 to 3.0 GU active renin per g tissue (mean 1.2, \(n = 14\)) were acidified at pH 4.8. The amounts of inactive renin were \(40 \pm 3.0\) per cent of the active renin (Fig. 1). Incubation for 44 h of the tissue slices in a glucose containing buffer at pH 7.4 gave a more than tenfold rise of inactive renin. Incubation without glucose gave less than half of this rise. As previously (Jørgensen 1976b) a minor loss of active renin was seen with incubation (not depicted).

Statistical evaluation. Mean values are given \(\pm 1\) SEM. Student’s paired \(t\)-test was used to compare groups.
The content of \textit{active renin} in \textit{post-partum uterus} before and after incubation at pH 7.4. Symbols as in Fig. 1. The \( P \) values indicate significance in inhibition of decrease in active renin as compared to incubation with glucose alone for 44 h.

NS: \( P < 0.05 \).

In average 30 per cent (range 20–40) of the inactive renin was found in the medium before incubation. An increase to about 90 per cent followed the incubation, and this was the case, whether or not the medium contained glucose.

Whenever puromycin, DNP or iodoacetate was added to the samples or when anaerobic incubation was performed, a blockage of the pronounced increase in inactive renin that used to follow incubation at pH 7.4 for 44 h took place, and this was independent of the presence of glucose (Fig. 1, the results are only depicted for the glucose containing samples).

In these cases mean 70 per cent (range 35–90) of the inactive renin was found in the medium following incubation.

\textbf{Post-partum uterus}

The basic level of active renin before incubation varied between 2.4 and 35.4 (mean 11.4) GU per g tissue. Acidification of the unincubated samples showed relatively small amounts of inactive renin. Only 21 ± 2.9 per cent (\( P < 0.001, n = 9 \)) of the initial amounts of active renin could be demonstrated. A small gain of this basic content was seen following incubation for 44 h at pH 7.4 and independent of the presence of glucose (Fig. 2).

This minor gain again was inhibited by puromycin, DNP or iodoacetate or by anaerobic incubation (Fig. 2). Admixture of chloroquine did not provoke any inhibition, in contrast an increase was seen (\( P \sim 0.05 \)). Omitting glucose altered in no cases the results (not depicted).

Following incubation the majority of the inactive renin (range 32–91 per cent) in all cases was left in the medium, the fraction was only about 35 per cent prior to incubation.
The loss of renin

Post-partum uterus

A loss of about 80 per cent of the active renin was shown subsequent to incubation in glucose enriched medium at pH 7.4 for 44 h. Sample incubation without glucose resulted in a smaller reduction of active renin (Fig. 3). Due to the small increment of inactive renin with incubation, the loss of total (active plus inactive) renin (Fig. 4) is less pronounced than the loss of active renin, although still significant \( P < 0.01 \).

Before incubation in average 14 per cent (range 10–17) of the active renin was present in the medium. After incubation, with glucose enriched medium, mean 30 per cent (range 19–39), and without glucose mean 20 per cent (range 10–50) of the remaining active renin was found in the medium.

Addition of puromycin to glucose containing samples caused a smaller loss of active renin compared to corresponding standard experiments (Fig. 3). A more pronounced inhibition effect could be demonstrated with DNP and anaerobic incubation, and the most potent inhibitors were iodoacetate and chloroquine (Fig. 3). In all cases omission of glucose addition gave similar results (not depicted). The loss of total renin (Fig. 4) increased when

Fig. 4.

The content of total (active plus inactive) renin in post-partum uterus before and after incubation at pH 7.4. The amounts of total renin in slices plus medium are given in per cent of the initial amounts of active renin. Symbols as in Fig. 1. The \( P \) values indicate significance in inhibition of decrease in total renin as compared to incubation with glucose alone for 44 h. NS: \( P < 0.05 \).
puromycin was added to the samples \( (P < 0.05) \). In all other cases a substandard loss of total renin was documented. Again independence of the presence of glucose was observed.

In most cases after addition of inhibitors between 55 and 80 per cent of the remaining active renin was in the medium. Only from 30 to 55 and from 17 to 46 per cent, respectively, of the remaining active renin was present in the medium when puromycin without glucose and chloroquine 0.2 mmol/l with and without glucose was added.

**Kidney cortex**

As in previous work (Jørgensen 1976a) a loss of 27 ± 2.2 per cent \( (P < 0.01, n = 5) \) of the original renin content (10–27 GU per g tissue) was observed during 44 h incubation at pH 7.4 in presence of glucose. A similar loss was found during incubation without glucose \( (n = 2) \). In no cases an activation was seen upon acidification at pH 4.8. Addition of puromycin to the incubation medium did not change the above mentioned minor loss of renin during incubation at pH 7.4. This stable condition persisted whether glucose was present in the incubation medium \( (33.2 ± 3.8 \text{ per cent}, P > 0.1, n = 5) \) or not \( (n = 2) \).

During the 44 h incubation the renin content in the incubation medium increased from about 2 to about 40 per cent independent of the presence of glucose and puromycin.

**Discussion**

**Inactive renin**

**Non-pregnant uterus**

Inactive renin in amniotic fluid has previously been demonstrated by Lumbers (1971), Day et al. (1975) and Skinner et al. (1975). In the present study as well as previously (Jørgensen 1976b), a marked increase in the amount of inactive renin followed incubation of non-pregnant uterine tissue slices in a buffer at pH 7.4. This expected increase was completely inhibited by puromycin (an inhibitor of protein synthesis) and by inhibitors of cellular energy metabolism which also inhibit protein synthesis, leading to the conclusion that the increase was an effect of a de novo synthesis of renin. The de novo synthesized renin appears exclusively in an enzymatically inactive form, in contrast to a simultaneous minor reduction of active renin (Jørgensen 1976b). The renin compound, which has a slightly higher molecular weight than active renin (Jørgensen 1979a), may be a proenzyme of renin as suggested by Poulosen et al. (1979) who investigated mouse submaxillary renin. Another possibility is a linkage of renin to an inhibitor as suggested by Boyd (1974) and Leckie & Mc McConnell (1975) in kidney experiments. If the latter is the case, the accumulation of inactive renin with incubation is caused by a conversion of de novo synthesized active renin to inactive renin by binding to an inhibitor.

The marked formation of inactive renin in non-pregnant uterus makes a discussion of the small loss of active renin with incubation (Jørgensen 1976b) inconclusive.

**Post-partum uterus**

The small increase in inactive renin that follows incubation was influenced by inhibitors in a similar way as was the non-pregnant uterus. In this tissue, where no increase in total (active plus inactive) renin is seen with incubation, the increase in inactive renin could be caused by either de novo synthesis of a proenzyme or conversion of active to inactive renin.

**The loss of renin**

**Post-partum uterus**

A marked loss of active renin with incubation is the predominant process in post-partum uterus. If we are confronted to a proenzyme of renin, the loss (degradation) is measured by the decrease in active renin. The measured loss is a minimum loss, since some of the new formed prorenin could be converted to active renin during incubation. On these assumptions, the inactivation of renin is found to be inhibited by omitting glucose in the medium, or adding puromycin, DNP or iodoacetate or by anaerobic incubation. Of these iodoacetate is most potent. This is in accordance with the accepted requirements of metabolic energy for proteolytic degradation of enzymes and other proteins (Simpson 1953; Steinberg & Vaughan 1956; Schinke et al. 1965; Brostrom & Jeffay 1970; Hershko & Tomkins 1971). Impairment of protein synthesis has been found either to inhibit (Woodside 1976) or not to affect (Ahn & Rosenberg 1967).
degradation. Complete inhibition has been shown when an augmented inactivation is studied during incubation of hepatoma cells under starving conditions (Hershko & Tomkins 1971; Epstein et al. 1975). A marked inhibition of the inactivation of renin followed addition of chloroquine, which accumulates in lysosomes and inhibits cathepsin B and perhaps also other cathepsins (De Duve et al. 1974; Poole & Wibo 1973). As cathepsin B is a sulphhydryl protease and a high degree of inhibition was obtained with iodoacetate, an involvement of lysosomal enzymes in the inactivation of renin (as proposed for other proteins (Holzmann 1976)) is a reasonable approach. Eskildsen (1973) demonstrated a high diffusion rate of uterine renin to the extracellular space. Jørgensen (1974, 1976a,b) also found relatively high amounts of renin in the incubation medium. In most cases higher amounts were present when metabolic inhibitors had been added. The present experiments permit no conclusions whether this is due to an increased release of renin into the medium or to a decreased uptake from the medium during incubation. However pinocytosis might be important for the transport of renin into the cells, where inactivation probably takes place. This leaves another explanation of the demonstrated inhibition, since pinocytosis requires metabolic energy (Jacques 1975) and also can be inhibited by chloroquine (Fedorko et al. 1968).

Suppose inactive renin to be renin bound to inhibitor. Then the loss of renin is represented by the difference in total (active plus inactive) pre- and post-experimental amounts, because a conversion of active to inactive renin during incubation is possible. Again the measured loss is a minimum loss, because a simultaneous formation of renin cannot be excluded. This does not invalidate the above adopted pattern of inhibition, except for puromycin. Addition of puromycin actually caused an increased loss. This is hardly caused by increased degradation activity, since puromycin is believed either to inhibit or to have no effect on degradation. A blocked simultaneous formation of renin is a reasonable interpretation.

Kidney cortex

Jørgensen (1976b) gave evidence for the lack of de novo synthesis and of inactive renin during kidney tissue incubation; only a small loss of renin was observed. The results were unchanged when omitting glucose or additioning puromycin. Thus kidney renin probably has no rapid turnover.

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References


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