

PART XXI

NEPHROLOGY POSTERS

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IMPROVEMENT OF INSULIN BINDING TO ERYTHROCYTE INSULIN RECEPTORS IN URAEMIA BY HAEMODIALYSIS

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Summary

Insulin binding to erythrocytes obtained from uraemic patients was determined using a radioreceptor assay. The binding was reduced by 50 per cent in 20 non-diabetic uraemic patients in comparison with 20 controls (4.7 ± 1.79 vs 9.37 ± 1.30 (mean \pm SD) $p < 0.01$). During the course of haemodialysis insulin binding steadily increased in a time dependent manner in proportion to the efficiency of haemodialysis as assessed by relative decrease in plasma urea, uric acid or creatinine. Incubation of healthy donors' erythrocytes with uraemic plasma resulted in a dose dependent inhibition of insulin binding with a maximum of 40 per cent. These data indicate the presence of dialysable inhibitors of insulin binding in uraemic plasma.

Introduction

Uraemia is associated with reduced glucose tolerance and decreased sensitivity of peripheral tissues to the action of insulin [1]. The molecular mechanisms responsible for altered insulin sensitivity are not known, but a number of chemically diverse substances accumulate in uraemia [2] and they may influence the binding of insulin to its receptors or alter any post receptor steps in the action of the hormone. Taking advantage of the gradual decrease in concentration of uraemic toxins which occurs during the course of a haemodialysis we studied the effects of uraemic intoxication on the degree of binding of insulin to its receptor.

Patients and methods

We studied 20 uraemic patients aged 22–59 years regularly dialysed three times weekly with a disposable capillary kidney. Dialysate contained no glucose. Stable non-obese non-diabetic outpatients were selected for the study. The

control group consisted of 20 healthy non-obese subjects without evidence of glucose intolerance.

Erythrocytes were purified by a two step gradient according to the method of Gambhir et al [3] from blood collected after an overnight fast. Insulin receptors on erythrocytes were studied using ^{125}I -insulin as described previously [3,4]. In some experiments cross incubations were performed where normal erythrocytes were exposed for 18 hours at 4°C to uraemic plasma or uraemic erythrocytes to normal plasma. Specific receptor binding was expressed as a fraction of total radioactivity.

Results

Erythrocytes obtained from uraemic patients before haemodialysis bound 50 per cent less insulin than controls with means (\pm SD) of 4.70 ± 1.79 and 9.37 ± 1.30 for uraemics and controls respectively ($p < 0.01$). After five hours of treatment by haemodialysis insulin binding improved in most patients ($p < 0.01$) and reached the normal range in some (Figure 1). A closer look at changes in insulin binding during the course of haemodialysis in two selected uraemic patients

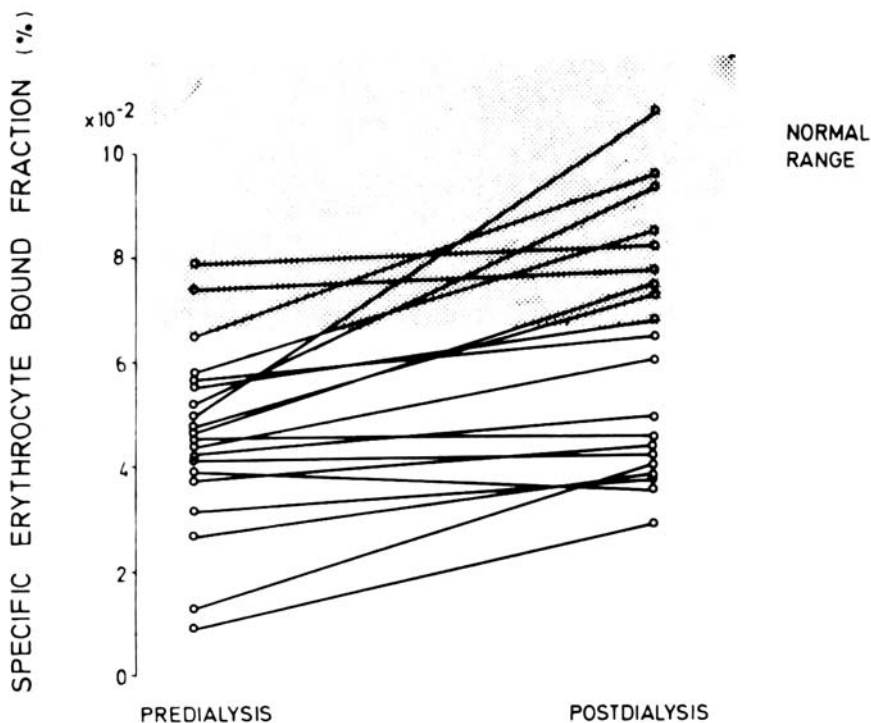


Figure 1. ^{125}I -insulin binding to erythrocytes from 20 uraemic patients before and after haemodialysis

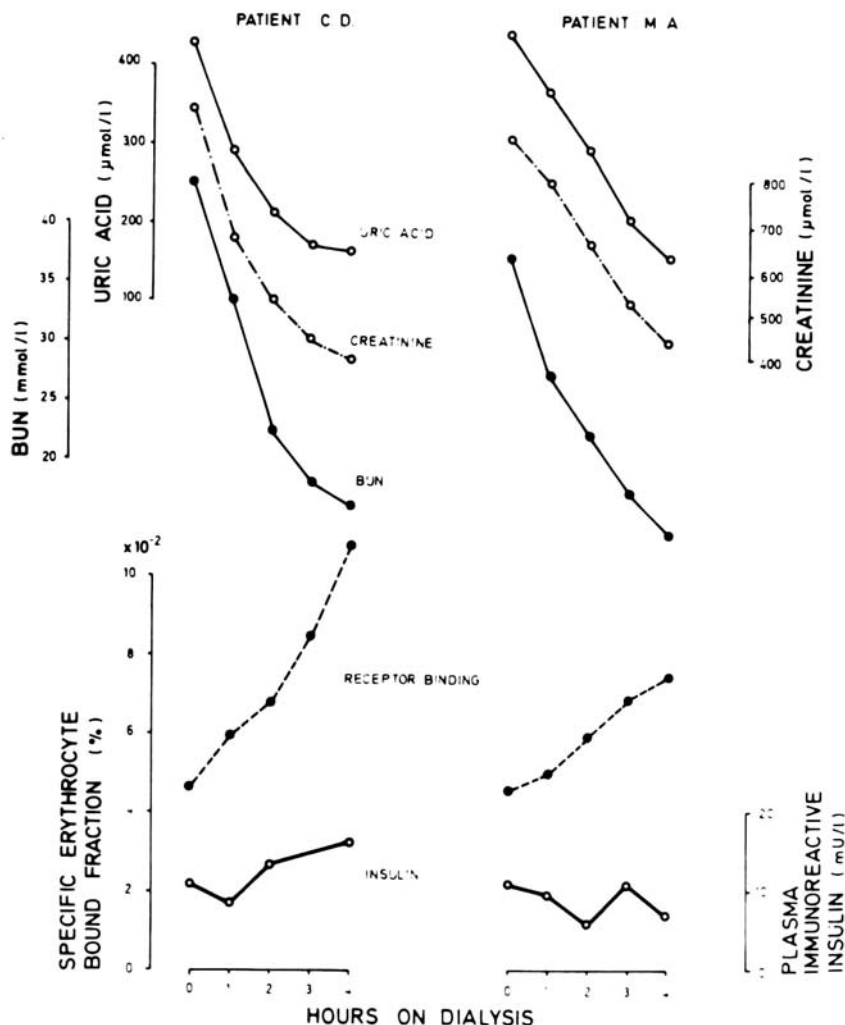


Figure 2. The changes in insulin binding, plasma urea, uric acid, creatinine and immunoreactive insulin during the course of haemodialysis in two patients

revealed a steady, time dependent increase in binding parallel with decrease in plasma creatinine, urea or uric acid (Figure 2). During the same time plasma immunoreactive insulin concentrations did not change appreciably. A strong negative correlation was found between the magnitude of receptor binding at different time points during haemodialysis and corresponding values of plasma urea, creatinine or uric acid ($p < 0.01$, not shown). This indicated that restoring of insulin binding depended on efficiency of extraction of uraemic waste products. Cross incubation tests of normal erythrocytes with uraemic plasma

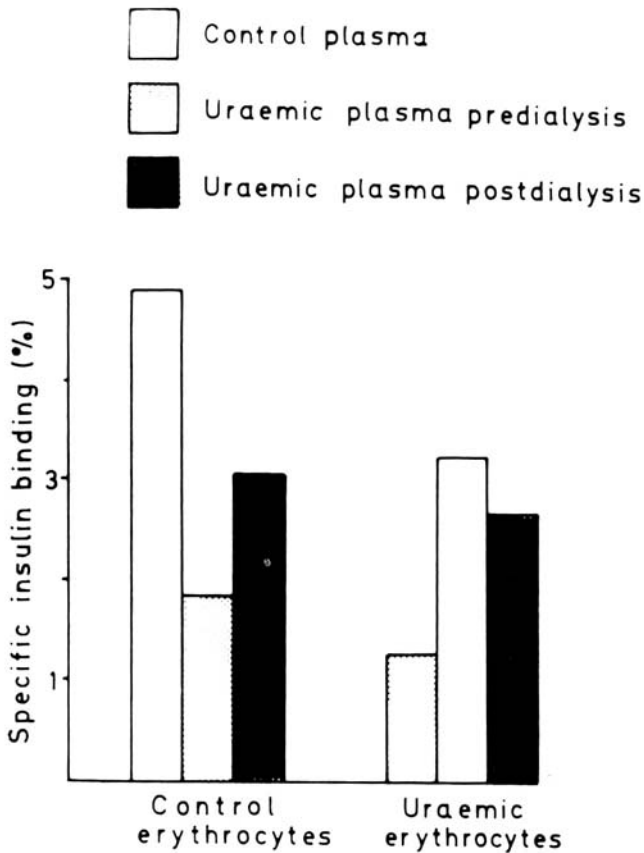


Figure 3. Cross incubation experiments on uraemic and normal erythrocytes with normal and uraemic plasma

resulted in 60 per cent decrease in binding (Figure 3). This demonstrated clearly that the disorder in insulin binding is induced by a dialysable plasma factor. The alteration in binding is reversible since incubation of uraemic erythrocytes with normal plasma restored insulin binding towards normal values (Figure 3). Progressive diluting of uraemic plasma showed a striking dose related kinetics of inhibition when tested on normal erythrocytes (Figure 4).

Discussion

The precise mechanism of alteration in specific insulin binding in uraemia is not known, but the reversible nature of inhibition and the time course of increase in binding during haemodialysis suggests the presence of circulating inhibitors in uraemic plasma which are readily dialysable. This is supported by the finding that restoration of insulin binding depends on efficiency of haemodialysis, by cross incubation experiments and by evidence of a dose related inhibitory effect.

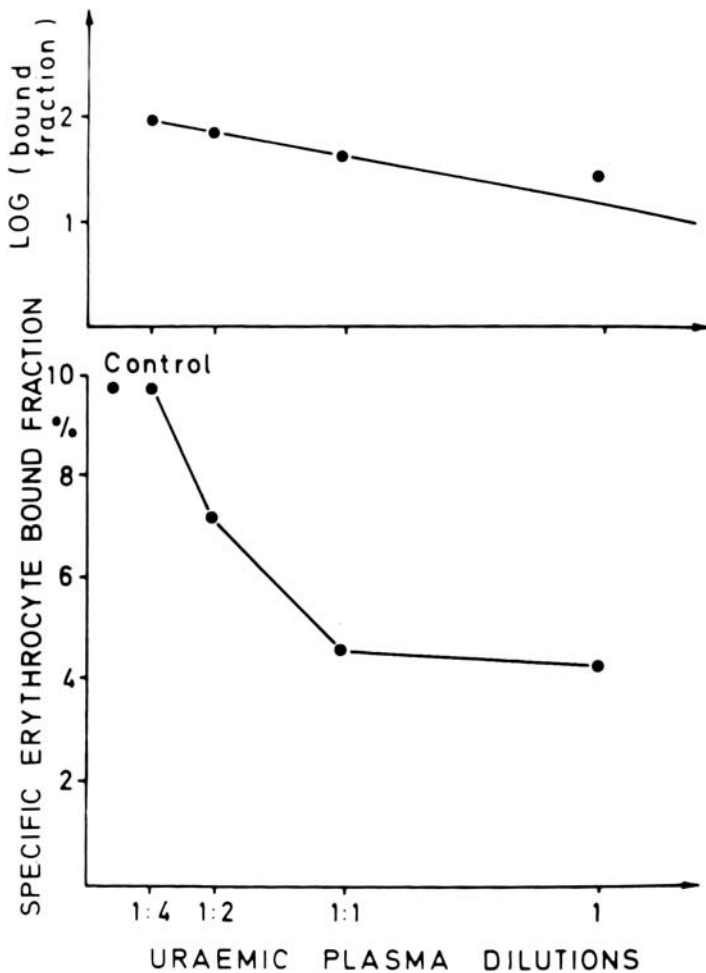


Figure 4. Inhibition of insulin binding to healthy donors' erythrocytes at different dilutions of uraemic plasma

Marked variability in binding noticed in our patients before haemodialysis and differences in relative increase in binding after haemodialysis (Figure 1) could be explained by individual differences in concentration of plasma inhibitors.

Similar decrease in insulin binding in uraemia was recently reported [5] but no attempt was made to study directly the effects of haemodialysis.

Small molecules such as beta-hydroxybutyrate or biguanide metformin [6,7] can significantly alter the interaction between insulin and its receptor in vitro. A number of chemically related compounds such as methylguanidine or guanidine are known to accumulate in uraemia [8], but at present it is impossible to say whether these or other substances mediate haemodialysis induced changes in insulin binding.

Contrary to the finding in other insulin resistant states, such as obesity with hyperinsulinaemia, in uraemia there was no correlation between changes in plasma immunoreactive insulin and insulin binding.

References

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