IMPAIRED REGULATION OF $\beta$-ADRENOCEPTORS IN PATIENTS ON MAINTENANCE HAEMODIALYSIS

A E Daul, A M Khalifa, N Graven, O-E Brodde
Medizinische Klinik and Poliklinik, University of Essen, FRG

Summary

In patients on maintenance haemodialysis the number of lymphocyte $\beta_2$-adrenoceptors (determined by $\pm$-125Iodoxyanopindolol binding) was not different from that in healthy controls; lymphocyte cyclic AMP responses to (-)-isoprenaline ($10^{-8} - 10^{-4}$M) or NaF (10 and 50mM), however, were significantly reduced. Dynamic exercise on a bicycle (80% of maximum heart rate) for 15 minutes caused in 10 healthy volunteers a fourfold increase in plasma catecholamines; concomitantly lymphocyte $\beta_2$-adrenoceptor number increased by about 55 per cent. In contrast, in patients on maintenance haemodialysis exercise induced only a twofold increase in plasma catecholamines and did not affect $\beta_2$-adrenoceptor number. It is concluded that in chronic uraemia regulation and responsiveness of $\beta$-adrenoceptors is impaired.

Introduction

Several signs of reduced sympathetic activity have been observed in patients on maintenance haemodialysis treatment. A defective function of sweat glands, reduced elevation of blood pressure in response to sustained hand-grip exercise, non-volume responsive chronic hypotension [1], elevated plasma norepinephrine (NE) and reduced responsiveness to NE-infusion [2] have been reported. In order to find out whether these disturbances may be due to an impairment of the $\beta$-adrenoceptor ($\beta$-R)/adenylate cyclase system we determined in lymphocytes of chronic haemodialysis patients $\beta_2$-receptor number (by $\pm$-125Iodocyanopindolol (ICYP) binding) and responsiveness (by cyclic AMP responses to isoprenaline stimulation). In addition, acute regulation of lymphocyte $\beta_2$-receptor was assessed by the effects of dynamic exercise (15 minutes on a bicycle at 80 per cent of maximal heart rate) on $\beta_2$-receptor number and responsiveness.
Subjects and methods

Twenty-eight patients on maintenance haemodialysis treatment (9 females, 19 males, mean age 48 ± 2 (24–68) years) and 41 healthy volunteers (12 females, 29 males, mean age 36 ± 2.8 (20–81) years) participated in the study after having given informed written consent. All subjects were not on antihypertensive therapy. Blood samples were always taken between 9.00 and 10.00 am, i.e., 10–20 hours after the previous haemodialysis treatment. After 30 minutes of rest 20ml venous blood was drawn with the subjects in a sitting position and anticoagulated with 500 IU heparin/10ml blood. Lymphocytes were isolated and β2-receptor density was determined by ICYP binding as recently described [3]. Changes in intracellular cyclic AMP in response to isoprenaline or NaF stimulation were measured as described by Dillon et al [4].

Exercise protocol

Seven male patients on maintenance haemodialysis (mean age 30.1 ± 3.2 (20–40) years) and 10 male healthy controls (mean age 28.5 ± 1.7 (23–36) years) participated in this study. Exercise was carried out in a quiet air-conditioned room always between 10 and 12 am. Subjects assumed a supine position and a cannula was inserted into an antecubital vein. After one hour of rest exercise was performed on a bicycle ergometer (Bosh, Berlin, FRG) in a supine position starting with an initial work load of 25W (in haemodialysis patients) or 50W (in controls). Work load was increased by 25W every two minutes until 80 per cent of the maximal heart rate (200-age) was reached. This final work load (75–125W in the haemodialysis patients, 100–150W in the controls, respectively) was kept constant until a total exercising time of 15 minutes was reached.

Blood samples were obtained immediately prior to exercise, at the end of exercise and one hour after exercise for determination of lymphocyte β2-receptor number and responsiveness and for radioenzymatic measurement of plasma catecholamines [5].

Blood pressure and heart rate were recorded automatically by a Tonomed® (Speidel & Keller, Jungingen, FRG) and by an electrocardiogram.

Statistical evaluations

The experimental data given in the text and in the figures are means ± SEM on N experiments. The maximal number of ICYP binding sites and the equilibrium dissociation constant (Kd) were calculated from plots according to Scatchard [6]. The significance of differences was calculated by Student’s ‘t’ test. A p-value less than 0.05 was considered to be significant.

Results

The mean number of β2-receptors in lymphocytes of patients on maintenance haemodialysis (956 ± 81 specific ICYP binding sites/cell, n=28) was not significantly different from that in an age-matched control group (774 ± 49 ICYP
binding sites/cell, n=41). The β-receptor agonist isoprenaline (10^-8 - 10^-4 M) produced in both groups a concentration-dependent increase in cyclic AMP content. In haemodialysis patients, however, this response was significantly attenuated at each concentration (Figure 1). In addition, NaF (10 and 50 mM, respectively) caused at both concentrations in lymphocytes of healthy controls an increase in cyclic AMP content, which was nearly three times as high as in lymphocytes of haemodialysis patients (Figure 1). Exercise on a bicycle in a supine position (at 80% of the maximal heart rate for 15 minutes) led to an increase in systolic blood pressure from 120 mmHg to 190 mmHg in both groups (Figures 2 and 3). Immediately after exercise in controls plasma catecholamine levels were increased about fourfold (0.47 ± 0.11 ng/ml to 1.83 ± 0.23 ng/ml, Figure 2), while in haemodialysis patients this increase was significantly less (0.79 ± 0.21 to 1.65 ± 0.31 ng/ml, Figure 3). In controls, lymphocyte β2-receptor number increased significantly from 463 ± 36 prior to exercise to 720 ± 64 following exercise. This increase in β2-receptor was accompanied by an exaggerated response of the lymphocyte cyclic AMP system to isoprenaline (10 μM) stimulation (Figure 2).

In haemodialysis patients, on the contrary, dynamic exercise failed to produce any significant changes in lymphocyte β2-receptor number. Accordingly, isoprenaline evoked increases in intracellular cyclic AMP content were not changed (Figure 3).

One hour after exercise systolic blood pressure, β2-receptor number and plasma catecholamine had reached pre-exercise values in both groups.

Figure 1. Effects of (-)-isoprenaline (A) and NaF (B) on the intracellular cyclic AMP in healthy volunteers and in patients on maintenance haemodialysis.
Figure 2. Effects of dynamic exercise (15 min on a bicycle at 80% of maximum heart rate) on lymphocyte β₂-adrenoceptor density, plasma catecholamine concentrations, lymphocyte cyclic AMP production evoked by 10μM (−)-isoprenaline and systolic blood pressure in 10 healthy volunteers. Ordinates (from top to bottom): β₂-adrenoceptor density in ICYP binding sites/cell; plasma catecholamines in ng/ml; increase in cyclic AMP content induced by 10μM (−)-isoprenaline in pmoles cyclic AMP/10⁶ cells and systolic blood pressure in mmHg. Abscissa: time in minutes. Given are means ± SEM. Solid horizontal lines and broken lines: means of pre-exercise values ± SEM assessed after one hour of rest in a supine position.
Discussion

In the present study, isoprenaline (via $\beta_2$-receptor stimulation) produced in lymphocytes of patients on maintenance haemodialysis significantly less increases in intracellular cyclic AMP content than in controls. These results favour the idea that in patients undergoing chronic haemodialysis treatment the responsiveness of $\beta_2$-receptor is reduced. Since the number of $\beta_2$-receptors in haemodialysis patients was not different from controls, the decreased responsiveness of the $\beta$-adrenergic system seems to be due to a post-receptor defect. In haemodialysis patients the stimulatory effect of NaF was markedly attenuated. Since NaF is known to activate the adenylate cyclase directly, i.e. non-receptor mediated [7], it may be concluded that in chronic haemodialysis
patients reduced responsiveness of β2-receptor is due to an impairment of the
adenylate cyclase activity.

In healthy subjects dynamic exercise (80% of maximal heart rate of 15 min)
led to an increase (about fourfold) in plasma catecholamine and lymphocyte
β2-receptor number (approximately 55%). The increase in β2-receptor number
was accompanied by an elevation of isoprenaline-evoked cyclic AMP production
indicating an enhanced β2-receptor responsiveness. The mechanism of this con-
comitant rise in plasma catecholamines and β2-receptor number and responsive-
ness is not yet clarified. In rat reticulocyte ghosts, however, it has been shown
that stimulation of β-receptor by catecholamines increases enzymatic methyla-
tion of phospholipids and that enhanced phospholipid methylation may unmask
cryptic β-receptor resulting in an increasing receptor number. It may be possible,
therefore, that in healthy subjects exaggerated release of endogenous catechol-
amines evoked by dynamic exercise may unmask cryptic β2-receptor by a similar
mechanism. Taking this into consideration, the lack of effect of dynamic exer-
cise on β2-receptor number in haemodialysis patients may further support the
view that the responsiveness of the β-adrenergic system is reduced.

Conclusions

In patients undergoing maintenance haemodialysis treatment the responsiveness
of lymphocyte β2-receptor is diminished. This reduced responsiveness might be
due to a post-receptor defect, most likely to an impairment of the adenylate
cyclase activity. Since the properties of lymphocyte β2-receptor are very similar
to β2-receptor in other tissues [9], including human heart [10], it may be
concluded that the responsiveness of peripheral β-receptor is also reduced in
chronic uraemia. Such a reduced β-receptor responsiveness may be the cause
of end-organ resistance to adrenergic stimulation in haemodialysis patients.

Acknowledgments

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References

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Open Discussion

WIZEMANN (Giessen) Firstly, did you incubate lymphocytes from healthy persons with uraemic serum and secondly is there evidence that adrenoceptors on platelets are representative for the heart and the vasculature?

DAUL At the moment we can't say what changes in lymphocyte β-adrenoceptors mean in patients. In normotensive subjects the properties of lymphocyte β₂-receptors are very similar to the properties of receptors in other tissue including heart and lung.

RITZ (Chairman) May I ask two questions. The first relates to the mechanism of the putative post-receptor defect? Some years ago there was a paper from the Hopital Necker measuring the isoproterenol heart rate response showing it was impaired in uraemic patients*. This was reversible with parathyroidectomy. Do you have any information on this?

DAUL No, but let me say that we have two patients with chronic hypertension which was reversible after dialysis.

RITZ The second question relates to your findings with sodium chloride which are suggestive of a coupling protein defect. Did you test other hormones such as PTH or glucogen to see whether they had a similar attenuated cyclic AMP response?

DAUL No.

ZOCCALI (Reggio, Calabria) Your results are at variance with a previous paper by Galeazzi† who found that the fall in heart rate caused by the β-adrenoceptor blocking drug Pindolol is higher in dialysis patients than in normal subjects. Is there any way to reconcile these results with yours?

DAUL I think it is very difficult to compare these two studies.

†Galeazzi RL, Gugger M, Weidmann P. Kidney Int 1979; 15: 661