Further evidence for reduced $beta_2$-adrenoceptor expression and modification of its density at a level beyond gene expression

Dear Sir,
Further to a recent report on impaired $beta_2$-adrenergic responses in women with central obesity [1] we would like to draw your attention to a comparable reduced $beta_2$-adrenoceptor ($beta_2$-AR) expression [2] in a syndrome also related to insulin resistance [3], namely salt sensitivity. We have recently published that cultured fibroblasts of young normotensive males express after several passages $beta_2$-AR ranging from 22 to 298 fmol/mg interindividually. This heterogenous expression of $beta_2$-AR is independent of passage number, at least between the 6^{th} and 10^{th} passage. The expressed $beta_2$-AR are functionally active, demonstrated by a linear relation between $beta_2$-AR number and cyclic AMP production. Since salt sensitivity is a precursor of essential hypertension [4] and since essential hypertension is associated with insulin resis-

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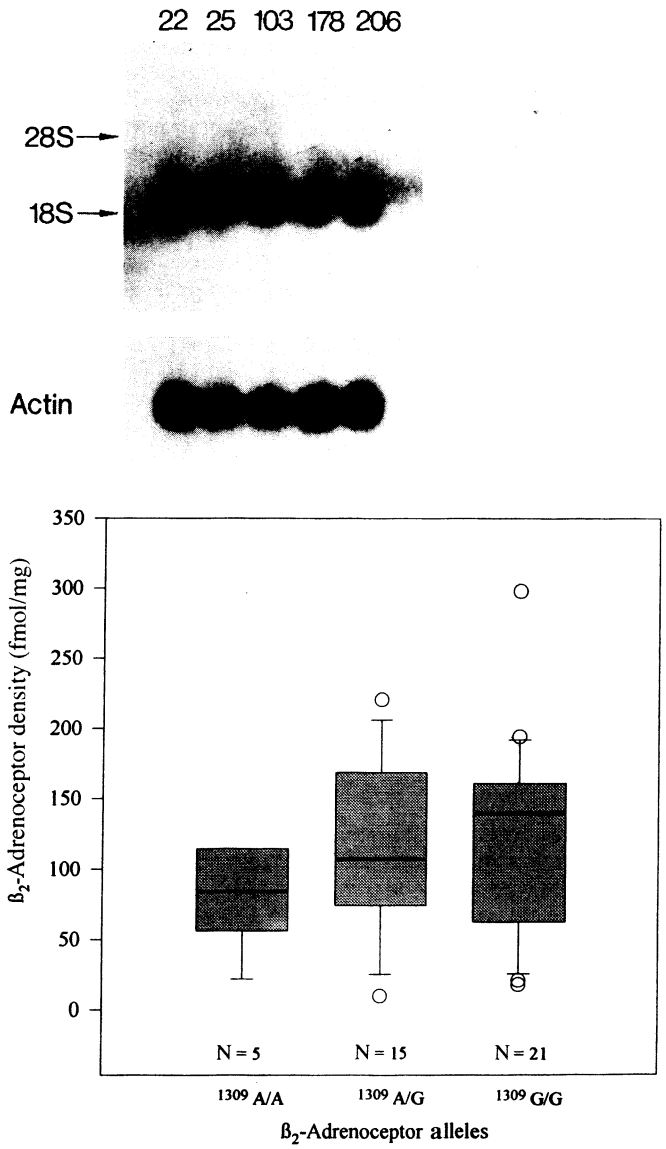

Fig. 1. *Top panel:* mRNA signals of five subjects with widely varying beta$_2$-AR expression in fibroblast culture (respective densities given at top). The signal of β-actin mRNA is shown for comparison. As can be seen, despite the wide variation of beta$_2$-AR expression there are no detectable differences in the mRNA signal. *Bottom panel:* Box Whisker plot of beta$_2$-AR densities in unrelated subjects with the ^{1309}A/A allele (n = 5), with the ^{1309}A/G allele (n = 15), and with the ^{1309}G/G allele (n = 21). Beta$_2$-AR density and affinity was determined by duplicate six-point analysis of (–)-3-^{125}I-iodocyanopindolol binding as previously described [2]. As can be seen, homozygosity for one of the alleles influences the amount of beta$_2$-AR associated with the membrane fraction

tance [5], our findings fit nicely with those reported by Reynisdottir et al. [1].

In order to elucidate further the regulation of beta$_2$-AR expression we measured the steady-state content of beta$_2$-AR mRNA in five unrelated subjects covering a wide range of beta$_2$-AR densities of 22, 25, 103, 178, and 206 fmol/mg, respectively, by Northern blot. A ^{32}P labelled SmaI/SmaI fragment (1277–1714) of the beta$_2$- AR was used (10^6 cpm/ml) as a probe. Hybridisation was performed at 42 °C in the presence of 50 % formamide. The amount of beta$_2$-AR mRNA was related to the amount of mRNA for the "house keeping" gene 3-actin.

No differences in the amount or in the size of beta$_2$-AR mRNA could be detected between the five subjects, irrespective of the beta$_2$-AR densities (Fig. 1, top panel). The 2.2 kilo base size of the beta$_2$-AR mRNA was identical to that previously reported [6]. These findings point towards a regulation of the beta$_2$-AR expression beyond gene expression, which is consistent with the results of Reynisdottir et al. [1].

It is interesting to note that silent base substitutions and substitutions leading to changes of the beta$_2$-AR primary structure (^{1309}G → A, causing Gly^{16} → Arg; ^{1685}A → C and ^{1686}C → A causing Typ^{141} → Ser) have been reported [7]. The beta$_2$-AR is N-glycated at Asn^6 and Asn^{15}. The Gly^{16} → Arg mutation resembles the *X* within the Asn*X* Ser/Thr glycation consensus sequence. Glycation-deficient beta$_2$-AR expressed in COS-7 cells showed a 50 % decrease on the level of accumulation on the cell surface, demonstrating that glycation is important for correct trafficking of the beta$_2$-AR protein through the cell [8].

We have determined the frequency of the ^{1309}A/G alleles in 41 subjects and related these to the expression of beta$_2$-AR in cultured fibroblasts. As can be seen from Figure 1 (bottom panel) there is a reduced expression of beta$_2$-AR in the homozygote ^{1309}A/A allele, with intermediate expression in the ^{1309}A/G allele. This finding points towards a possible influence of the Gly^{16} → Arg amino acid change in the glycation process and hence an impaired accumulation of the beta$_2$-AR at the cell surface.

We are not aware of any other reports showing an altered subcellular distribution of the Gly^{16} → Arg mutant beta$_2$-AR protein. It would be interesting to further characterize the genetics and the glycation patterns of the beta$_2$-AR in the population studied by Reynisdottir et al. [1].

Yours sincerely,
P. Kotanko, O. Höglinger, A. Binder, F. Skrabal

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Response from the authors

Dear Sir,
Dr. Kotanko and his colleagues are to be thanked for their most interesting comments and results.

It is true that data regarding $beta_2$-adrenoceptor expression are comparable in salt-hypertension [1, 2] and abdominal obesity [3], since both conditions are associated with the insulin-resistance (metabolic) syndrome [4]. The data from Dr. Kotanko et al. suggest that genetic defects may cause post-mRNA alterations of $beta_2$-receptor expression in certain cell types. Whether this is true for fat cells remains to be established. The findings by Dr. Kotanko and his colleagues are based on experiments with cultured fibroblasts. These cells are investigated after several passages when they presumably are free of environmental influence. Unfortunately, such experiments cannot be performed on mature adipocytes, since the latter cells do not divide.

However, the results of recent studies on fat cells from subjects with the fully-developed insulin resistance syndrome lend further support to the theory that defects in $beta_2$-adrenoceptor expression beyond gene expression are of pathophysiological significance. Abdominal subcutaneous fat cells from elderly males with abdominal obesity, glucose intolerance, insulin resistance, hyperinsulinaemia and (in most cases) hypertension were investigated [5]. A marked reduction in cell surface $beta_2$-adrenoceptor expression was observed in spite of normal $beta_2$-receptor mRNA; $beta_1$-receptor number and mRNA were also normal. The defect in $beta_2$-adrenoceptor expression correlated significantly ($r = 0.67$) with the degree of insulin resistance in the individual experiments.

It is quite possible that the $Gly^{16} \rightarrow Arg$ mutation observed in the studies by Kotanko et al. is causing impaired cell surface expression of $beta_2$-adrenoceptors. As the authors point out this mutation alters receptor glycation and thereby transport of the $beta_2$-receptor protein through various subcellular compartments.

When all the data discussed above are put together it is tempting to assume that genetic defects in $beta_2$-adrenoceptor expression; which may be linked to the $Gly^{16} \rightarrow Arg$ mutation, could be involved in the aetiology of several insulin resistant conditions. It is important to test this hypothesis further by performing genetic and glycation studies of $beta_2$-adrenoceptors on the different populations investigated by Dr. Reynisdottir and her colleagues [2, 4].

Yours sincerely,
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