British Journal of Pharmacology (2001) 133, 708–712

www.nature.com/bjp

The effect of the beta$_2$ adrenoceptor gene Thr164Ile polymorphism on human adipose tissue lipolytic function

[1]Johan Hoffstedt, [2]Anastasia Iliadou, [2]Nancy L. Pedersen, [3]Martin Schalling & *,[1]Peter Arner

[1]Department of Medicine, Karolinska Institute and Research Center at CME, Huddinge Hospital, 141 86 Huddinge, Sweden; [2]Department of Medical Epidemiology, Karolinska Institute, Box 281, 171 77 Stockholm, Sweden and [3]Center for Molecular Medicine, Department of Clinical Genetics, Karolinska Institute at Karolinska Hospital, L8:00, 171 76 Stockholm, Sweden

1 A rare β_2-adrenoceptor gene polymorphism, Thr164Ile, has been described that impairs receptor function when transfected into cell lines. We investigated whether the polymorphism influences native receptor function by studying lipolysis in freshly isolated subcutaneous fat cells from 236 apparently healthy subjects.

2 Twelve subjects were heterozygous for the 164Ile variant. The fat cells of Ile carriers displayed a 6 fold increase ($P=0.02$) in the lipolytic EC_{50} of terbutaline (a selective β_2-adrenoceptor agonist), but no change in the lipolytic action of dobutamine (a selective β_1-adrenoceptor agonist), compared with the Thr carriers. Maximum adrenoceptor agonist stimulated lipolysis did not differ between Thr and Ile carriers.

3 The influence of two other polymorphisms (Arg16Gly and Gln27Glu) in the β_2-adrenoceptor gene was considered. Six 164Ile carriers also carried the 16Gly and 27Glu alleles. The latter combination occurred among 105 of the 164Thr carriers. For the 16Gly27Glu subgroup, the EC_{50} of terbutaline was about 10 fold higher in 164Ile as than in 164Thr carriers ($P=0.02$) but there was no difference between genotypes in maximum terbutaline action. There was no difference between groups in dobutamine action.

4 In conclusion, the 164Ile variant of the β_2-adrenoceptor is associated with a decreased native adipocyte receptor function, as evidenced by a marked increase in the half maximal effective concentration of the lipolytic action of a selective β_2-adrenoceptor agonist. This suggests that genetic variance in the β_2-adrenoceptor gene might be important for catecholamine function in humans, at least as far as adipocyte lipolysis is concerned.
British Journal of Pharmacology (2001) **133,** 708–712

Keywords: Adrenergic receptors; catecholamines; dobutamine; exon fat cells; lipolysis; polymorphism; terbutaline

Abbreviations: BMI, body mass index; EC_{50}, half maximal effective concentration; IA, intrinsic activity; NS, not significant; PCR, polymerase chain reaction; pD_2, negative logarithm of the half maximal effective concentration expressed as mmol l^{-1}

Introduction

Polymorphisms in the human β_2-adrenergic receptor gene might alter corresponding receptor function and thereby therapeutic responses to adrenergic drugs. Three coding variants in the β_2-adrenoceptor gene have been reported to cause amino acid variation in the receptor protein. These include the rare Thr164Ile variant and the two common Arg16Gly and Gln27Glu variants (Green *et al*., 1995). Studies on transfected cells have shown that these codon variants give β_2-adrenoceptors that show markedly different properties with respect to ligand binding, receptor signal transduction and/or internalization of receptors (Green *et al*., 1995).

However, it is less well known whether these β_2-adrenoceptor gene polymorphisms also influence native receptor function. It is quite possible that compensatory mechanisms occur in natural cells. Furthermore, the human β_2-adrenoceptor might couple differently to signal transduction systems in native cells as compared with experimental cell lines.

*Author for correspondence; E-mail: Peter.Arner@medhs.ki.se

The human fat cell is a useful, relatively easily available cell type for the study of β-adrenoceptor function (Carpene *et al*., 1998). β_1- and β_2-adrenoceptors co-exist in human fat cells, where they can fully activate lipolysis, a primary event in these cells. The Arg16Gly polymorphism appears to be lipolytically functional in man because it has been shown to be associated with variations in the lipolytic sensitivity of β_2-adrenergic agonists in human fat cells (Large *et al*., 1997). Carriers of 16Gly have been reported to show a 5 fold increased lipolytic sensitivity to selective β_2-adrenoceptor agonists (Large *et al*., 1997).

It is not known whether the β_2-adrenoceptor Thr164Ile gene polymorphism also has functional consequences in man. This form of variation in the receptor is difficult to study in its native form because it occurs at low frequency in the population. As part of a project to characterize the regulation of lipolysis in isolated subcutaneous adipocytes we identified 236 healthy subjects with a large inter-individual variation in body mass index (BMI). This material was genotyped for the Thr164Ile polymorphism and 12 subjects with 164Ile were found. The *in vitro* lipolytic effects of selective β-adrenoceptor agonists were compared between

Arg and Ile carriers. In addition, we investigated the possible influence of polymorphism in codons 16 and 27 of the β_2-adrenoceptor on lipolysis. We did not consider the β_3-adrenoceptor since this subtype has a low lipolytic activity in the human subcutaneous fat depot (Carpene *et al*., 1998; Lönnqvist *et al*., 1993).

Studies in recombinant cells have shown that the 164Ile variant of the β_2-adrenoceptor has decreased receptor affinity, impaired coupling to adenylyl cyclase and impaired agonist-promoted sequestration of the receptor compared with the wild form of the receptor (Green *et al*., 1993). We hypothesized that if similar alterations occur in native human fat cells then they would be accompanied by a decreased lipolytic sensitivity to selective β_2-adrenoceptor agonists.

Methods

Subjects

We investigated 236 subjects of Scandinavian origin who were consecutively recruited to a study on adrenergic regulation of lipolysis in subcutaneous fat cells. The study comprised both non-obese ($n=124$) and obese ($n=112$) otherwise healthy volunteers who were referred to our obesity unit for treatment of over weight. None of the subjects was either completely sedentary or involved in sporting activities. None had undertaken a slimming diet during the 6 months prior to study. Obesity was defined using WHO criteria (body mass index, BMI >30 kg $\times$ m^{-2}). None of the subjects was on regular medication. Sixty-one men and 175 women were included. The ages ranged from 20 to 70 years. The BMI of the obese subjects was in the range 30.4 – 53 kg m^{-2}; in the non-obese the BMI was in the range 18 – 29.6 kg m^{-2}. Twenty women were menopausal.

The subjects came to the laboratory in the morning after an overnight fast. Height and weight were determined and a subcutaneous fat biopsy (about 0.5 to 1 g in lean and 1 to 2 g in obese subjects) was obtained under local anaesthesia from the umbilical region (Kolaczynski *et al*., 1994). The study was approved by the hospital ethics committee. The procedure was initially explained to each subject and his or her consent was obtained.

Fat cell experiments

The methods to perform and analyse fat cell experiments have been described in detail elsewhere (Lönnqvist *et al*., 1993). In brief, isolated fat cells were prepared and their average size, volume and weight determined. Dilute fat cell suspensions (2%, vv^{-1}) were incubated in duplicate for 2 h at 37°C in a Krebs – Henseleit phosphate buffer (pH 7.4) containing bovine albumin (20 g l^{-1}), glucose (1 mg ml^{-1}) and ascorbic acid (0.1 mg ml^{-1}). At the end of the incubation, an aliquot of the medium was removed for glycerol analysis (lipolysis index) and the amount of glycerol release related to the number of incubated fat cells.

The following agents were added in increasing concentration (10^{-10} – 10^{-5} mol l^{-1}, dependent on the type of agent) to the incubation medium at the start of the experiment; dobutamine (a selective β_1-adrenoceptor agonist), terbutaline (a selective β_2-adrenoceptor agonist) and isoprenaline (a non-selective β_1/β_2-adrenoceptor agonist). The basal condition was defined as that with no agonist present.

Since human subcutaneous fat cells have functional spare β-adrenergic receptors (Arner *et al*., 1988) it is possible to use classical pharmacological methods to evaluate receptor function (Kenakin, 1990). The individual concentration-response curves were linearized by log-logit transformation and analysed for pD_2 (negative logarithm of half maximum effective concentration, EC_{50}). Furthermore, responsiveness (glycerol release at maximum effective drug concentration) was determined from the original curves. The intrinsic activity (IA) of dobutamine and terbutaline was also determined according to the formula: (lipolysis at maximum effective dobutamine/terbutaline concentration) – (basal lipolysis)/(lipolysis at maximum effective isoprenaline concentration) – (basal lipolysis). Changes in pD_2, IA and responsiveness reflect receptor and post-receptor related events as discussed (Kenakin, 1990). We have previously demonstrated the selectivity of dobutamine and terbutaline on β-adrenoceptor mediated lipolysis in isolated human fat cells (Lönnqvist *et al*., 1993). A plateau of response was reached at the highest concentrations in all cases and with all drugs. Thus it was possible to obtain an accurate measure of pD_2, IA and responsiveness of the different drugs in each of the subject that was investigated.

Genotyping

DNA was prepared from frozen (−20°C) venous blood. The Arg16Gly, Gln27Glu and Thr164Ile polymorphisms in the β_2 adrenoceptor gene were determined as described previously (Large *et al*., 1997). A combination of polymerase chain reaction (PCR) and digestion of the PCR product with restriction enzymes (to obtain restriction fragments with variations in length) was used. The restriction enzyme, Mnl1 was used for the Thr164Ile polymorphism, Ita1 for the Gln27Glu polymorphism and BsrD1 for the Arg16Gly polymorphism.

Drugs and chemicals

Bovine serum albumin (fraction V) was from Sigma (St. Louis, MO, U.S.A.), (−)-Isoprenaline hydrochloride from Hässle (Mölndal, Sweden), terbutaline sulphate from Draco (Lund, Sweden), dobutamine hydrochloride from Lilly (Indianapolis, IN, U.S.A.), *Thermophylus aqualicus* (Taq) polymerase from Perkin Elmer-Cetus (Emeryville, CA, U.S.A.), BsrD1 and Ila1 from New England Biolabs Inc. (Beverly, MA, U.S.A.) and Mnl1 from Boehringer Mannheim (Mannheim, Germany). All other chemicals were of the highest purity grade commercially available.

Statistical analysis

Analyses of phenotypic values, included descriptive statistics (s.e.mean by genotype) were performed by analysis of covariance, adjusting for sex, obesity status and age. In some cases chi-square and a Student's unpaired *t*-test were performed. A 5% significance level was used for hypothesis testing. Because of the bimodal distribution of BMI, due to the study design, the analysis of variance was done by stratifying for BMI status (obese/non-obese), instead of using BMI values.

Results

Allelic frequencies

We found 12 subjects who carried the 164Ile variant in heterozygous form. The remaining subjects were homozygous for the Thr allele. The observed frequency is about $\approx 5\%$. Furthermore, among the 164Ile carriers eight were men and four women. This gender distribution differed significantly from the distribution of 164Thr carriers between the sexes ($P<0.01$), which may reflect bad luck in the selection of our sample. Therefore, we also genotyped DNA from 266 non-obese subjects obtained from the population-based Swedish twin register (Boosma, 1998). The frequency of 164Ile subjects in the latter cohort was 2.5%.

The difference in gender distribution among the Ile carriers is most probably due to the fact that there were only 12 individuals in the cohort and is unlikely to represent a sexual dimorphism inherent in the polymorphism. This notion is further supported by the lack of finding of a significant sexual dimorphism in the twin material (2% of men and 3% of women had the 164Ile variant). Because of the difference in sex distribution, all the statistical analyses were corrected for gender.

Clinical findings

The clinical data for comparison between genotypes are shown in Table 1. No significant differences between Thr and Ile carriers were seen for either age or BMI.

Lipolysis

The lipolysis results for the whole cohort are shown in Table 2. The basal rate of lipolysis was similar in the two groups. However, pD_2 for terbutaline was significantly (about 0.8 log unit) lower in Ile carriers than in Thr carriers. When these values were translated into half-maximal effective concentrations, it was found that the Ile group had substantially larger EC_{50} values for terbutaline (300 nmol l^{-1}) than did the Thr group (50 nmol l^{-1}). This represents a 6 fold difference in β_2-adrenoceptor sensitivity. However, the mean pD_2 value for dobutamine was almost identical in the two groups indicating that β_1-adrenoceptor sensitivity was not influenced by the Thr 164Ile polymorphism. The EC_{50} for dobutamine was about 50 mmol l^{-1}. The mean concentration response curves for terbutaline and dobutamine are shown for illustrative purposes in Figure 1. The terbutaline curve for Ile carriers was shifted to the right by about 1.0 log unit compared to that for Thr carriers. The two dobutamine curves were almost superimposed. IA and responsiveness (i.e. lipolysis rates at maximum effective agonist concentration) for terbutaline, dobutamine and isoprenaline were similar when Ile and the Thr carriers were compared. Since two agonists (terbutaline and dobutamine) were investigated, we also adjusted the *P*-values for multiple comparison. The difference in pD_2 for terbutaline between the Ile and Arg carriers was still statistically significant after this adjustment ($P=0.04$).

The number of women and obese subjects was too few to allow for a separate analysis. However, the pD_2 values for terbutaline was significantly lower (1.2 log units) among male Ile than male Arg carriers (Table 3). This 20 fold difference in EC_{50} remained statistically significant even after adjustment for multiple comparison ($P=0.02$). For the lean subjects (Table 3) the mean pD_2 for Ile carriers was 0.8 log units lower than that for Arg carriers. This difference was only of border line statistical significance ($P=0.07$). No other influence of genotype was seen for lipolysis data in the lean or male subjects (values not shown).

It is possible that the pD_2 for terbutaline is influenced by the polymorphism in the β_2-adrenoceptors at codon 16 and 27. We therefore genotyped all subjects for these polymorphisms. Six Ile carriers also had the 16Gly and 27Glu alleles in homozygous or heterozygous form. Among the Arg carriers, 105 subjects had the 16Gly and 27Glu alleles in homozygous or heterozygous form. We made a separate analysis of 16Gly27Glu subjects (Table 3). The 164Ile carriers had a significantly lower pD_2 for terbutaline (1.0 log unit) than did the 164Thr carriers. This difference was also significant after adjustment for multiple comparison ($P=0.04$). No other lipolytic differences were observed between the two codon 164 genotypes in 16Gly27Glu carriers (values not shown).

Table 1 Clinical data and the Thr164Ile polymorphism

	Thr	*Ile*	P
n	224	12	
Age, years	39±1	34±3	NS
Obese/lean	110/114	2/10	NS
Male/female	53/171	8/4	<0.001
BMI kg m^{-2}			
Obese	39.9±0.6	36.9±3.0	NS
Lean	23.9±0.2	23.9±0.7	NS

Values are s.e.mean. They were compared by Student's *t*-test or chi-square analysis. *n*=number of subjects. NS=not significant

Table 2 Lipolysis data and the Thr16Ile polymorphism in the whole cohort

Measure	*Thr*	*Ile*	P
n	224	12	
pD_2			
Terbutaline	7.6±0.1	6.8±0.2	0.02
Dobutamine	7.5±0.1	7.4±0.3	NS
IA			
Terbutaline	0.89±0.02	0.92±0.05	NS
Dobutamine	0.85±0.02	0.90±0.07	NS
Glycerol release (μmol 2 h^{-1} 10^7 cells)			
Basal	8.0±0.6	6.7±0.9	NS
Terbutaline	29.7±1.1	29.0±3.1	NS
Dobutamine	28.7±1.0	29.0±3.2	NS
Isoprenaline	32.8±1.1	31.9±3.0	NS

pD_2 is the negative logarithm of half-maximal effective concentration. IA is intrinsic activity in relation to maximum effect of isoprenaline. Values for glycerol release are in the absence (basal) or presence of a maximum effective concentration of the agonist. Values are s.e.mean. They were compared by analysis of covariance, with age, obesity status and gender as covariants. NS=not significant. *n*=number of subjects

Discussion

This study demonstrates significant effects of the Thr164Ile polymorphism in the β_2-adrenoceptor gene on native receptor

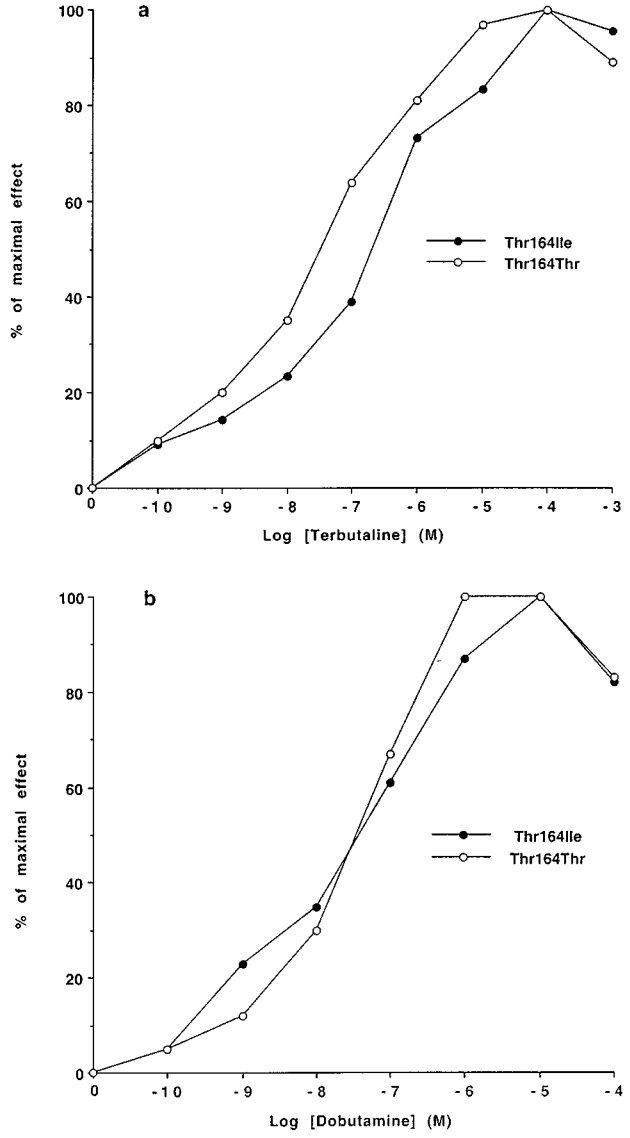

Figure 1 Mean concentration response curves for lipolysis induced by: (a) terbutaline; (b) dobutamine. Values are expressed as percentage of glycerol release at maximum effective agonist concentration. The curves were constructed from data of all 12Ile carriers and 12 randomly selected Thr carriers.

Table 3 pD_2 and the Thr164Ile polymorphism in subgroups

Subgroup	*Thr*	*Ile*	P
Male subjects			
n	53	8	
Terbutaline	7.8±0.2	6.6±0.2	<0.01
Dobutamine	7.5±0.1	7.4±0.2	NS
Lean subjects			
n	114	10	
Terbutaline	7.9±0.1	7.1±0.2	0.07
Dobutamine	7.6±0.1	7.6±0.2	NS
Subjects carrying 16Gly and 27Glu			
n	105	6	
Terbutaline	7.6±0.1	6.6±0.2	0.02
Dobutamine	7.5±0.1	7.5±0.5	NS

Values are s.e.mean. pD_2 is the negative logarithm of half-maximal effective concentration. Values were compared by analysis of covariance with age and, when appropriate, gender and obesity status as covariant. NS = not significant.

function in apparently healthy subjects, as assessed by lipolysis in freshly isolated subcutaneous adipocytes. It was previously found using transfected cell lines that the Ile substitution decreases receptor function due to altered affinity, coupling and internalization (Green *et al*., 1993). We have demonstrated here that native 164Ile receptor function is also decreased. We observed between groups no differences in basal lipolysis. However, a marked, between 6 fold and 20 fold (dependent on which group that was analysed) decrease in adipocyte β_2-adrenoceptor sensitivity (terbutaline pD_2) but no change in IA or maximum agonist effect (responsiveness) of terbutaline was observed among Ile carriers. Furthermore, the effect of an Ile substitution was selective for the β_2-adrenoceptor function as judged by findings with the β_1-adrenoceptor selective agonist dobutamine. A change in pD_2 may reflect different alterations in receptor signal transduction (Kenakin, 1990). It is quite possible that some of the defects observed in the recombinant experiments, such as decreased receptor affinity and impaired coupling to adenylyl cyclase (Green *et al*., 1993), explain the impaired native receptor function. Unfortunately, the small amount of adipose tissue available (barely sufficient for lipolysis experiments in lean subjects) made it impossible for us to perform more detailed investigations of the native human adipocyte β_2-adrenoceptor.

The Thr164Ile polymorphism is rare and according to our data occurs in 2.5% of the Swedish population-based sample. Although as many as 236 subjects underwent a fat biopsy, only 12 carried the Ile variant. Thus, due to paucity of subjects it is possible that type I errors may have influenced the statistical analysis. The difference in gender distribution most likely represents such an error because it was not reproduced in the population-based sample. However, for several reasons it is unlikely that the findings with pD_2 are influenced to an important extent by a type I statistical error. In the analysis of the whole material we made statistical corrections for heterogeneity with respect to gender, obesity status and age. The difference between the Ile and Thr carriers remained statistically significant after correction for multiple comparison. Furthermore, in the subgroup analysis (Table 3) the genotype effect on terbutaline pD_2 was statistically significant in two out of the three subgroups. In the third subgroup it reached borderline significance. It remains to be established if the polymorphism is also functional in fat cells of obese women.

As mentioned earlier, several polymorphisms in the β_2-adrenergic receptor are functional. However, it is unlikely that background variation in the β_2-adrenoceptor gene influenced the present results. We were able to identify six Ile carriers and 105 carriers of Arg who also had the codon 16Gly allele and the codon 27Glu allele in the β_2-adrenoceptor gene in homozygous or heterozygous form. This subgroup also showed a significant effect of the Thr164Ile polymorphism on the terbutaline pD_2.

The absence of a decrease in terbutaline responsiveness is perhaps surprising given a 6 to 20 fold change in EC_{50}. This can be explained due to spare receptors. When β_2- and β_1-adrenoceptors in isolated human subcutaneous fat cells were subjected to various degrees of irreversible non-selective blockade, the EC_{50} had to be shifted more than 100 fold before the maximum agonist effect on lipolysis was reduced (Arner *et al*., 1988).

At present, we do not know if the Thr164Ile polymorphism is also functional in other human tissues. There is indirect evidence for a functional effect in the heart, since patients with end-stage cardiac failure who carry the Ile variant have a poor prognosis (Liggett *et al*., 1998). Furthermore, myocardial signalling defects and impaired cardiac function of the human Thr164Ile β_2-adrenergic receptor polymorphism have been demonstrated in transgenic mice (Turki *et al*., 1996).

Previous studies have shown that some common polymorphisms in the β_2-adrenoceptor gene associate with obesity. The present lack of association between the Thr164Ile variant and BMI might be more apparent than real bearing in mind that only 12Ile carriers were found. It is apparent that a much larger and maybe population-based cohort needs to be investigated in order to elucidate the possible role of the polymorphism for obesity.

In summary, this study demonstrates that the Thr164Ile polymorphism in the β_2-adrenoceptor gene is associated with variations in native receptor function, as evidenced by a low pD_2 for terbutaline stimulated lipolysis in human fat cells of Ile carriers. This in turn suggests that genetic variations in the β_2-adrenoceptor gene can be important for inter-individual variations in receptor sensitivity and therapeutic effects of β_2-adrenergic agents.

The excellent technical assistance of Eva Sjölin, Kerstin Wåhlen, Britt-Marie Leijonhufvud and Catharina Hertel is greatly appreciated. This study was supported by grants from the Swedish Medical Research Council, Swedish Diabetes Association, Swedish Heart and Lung Foundation and the Foundations of Novo Nordisk and Söderberg.

References

ARNER, P., HELLMÉR, J., WENNLUND, A., ÖSTMAN, J. & ENGFELDT, P. (1988). Adrenoceptor occupancy in isolated human fat cells and its relationship with lipolysis rate. *Eur. J. Pharmacol.,* **146,** 45 – 56.

BOOSMA, D.I. (1998). Twin registers in Europe: an overview. *Twin Res.,* **1,** 34 – 51.

CARPENE, C., BOUSQUET-MELOU, A., GALITZKY, J., BERLAN, M. & LAFONTAN, M. (1998). Lipolytic effects of β_1- β_2- and β_3-adrenergic agonists in white adipose tissue of mammals. *Ann. N.Y. Acad. Sci.,* **839,** 186 – 189.

GREEN, S.A., COLE, C., JACINTO, M., INNIS, M. & LIGGETT, S.B. (1993). A polymorphism of the human β_2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J. Biol. Chem.,* **268,** 23116 – 231121.

GREEN, S.A., TURKI, J., HALL, I.P. & LIGGETT, S.B. (1995). Implications of genetic variability of human β_2-adrenergic receptor structure. *Pulm. Pharmacol.,* **8,** 1 – 10.

KENAKIN, T. (1990). Drugs and receptors. An overview of the current state of knowledge. *Drugs,* **40,** 666 – 687.

KOLACZYNSKI, J.W., MORALES, L.M., MOORE, Jr. J.H., CONSIDINE, R.V., PIETRZKOWSKI, Z., NOTO, P.F., COLBERG, J. & CARO, J.F. (1994). A new technique for biopsy of human abdominal fat under local anaesthesia with Lidocaine. *Int. J. Obes. Relat. Metab. Disord.,* **18,** 161 – 166.

LARGE, V., HELLSTRÖM, L., REYNISDOTTIR, S., LÖNNQVIST, F., ERIKSSON, P., LANNFELT, L. & ARNER, P. (1997). Human β_2-adrenergic gene polymorphisms are highly frequent in obesity and associate with altered adipocyte β_2-adrenoceptor function. *J. Clin. Invest.,* **100,** 3005 – 3013.

LIGGETT, S.B., WAGONER, L.E., CRAFT, L.L., HORNUNG, R.W., HOIT, B.D., MCINTOSH, T.C. & WALSH, R.A. (1998). The Ile164 β_2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. *J. Clin. Invest.,* **102,** 1534 – 1539.

LÖNNQVIST, F., KRIEF, S., STROSBERG, D., NYBERG, B., EMORINE, L.J. & ARNER, P. (1993). Evidence for a functional β_3-adrenoceptor in man. *Br. J. Pharmacol.,* **110,** 929 – 936.

TURKI, J., LORENZ, J.N., GREEN, S.A., DONNELLY, E.T., JACINTO, M. & LIGGETT, S.B. (1996). Myocardial signalling defects and impaired cardiac function of a human β_2-adrenergic receptor polymorphism expressed in transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 10483 – 104888.

(Received October 17, 2000
Revised March 19, 2001
Accepted April 19, 2001)