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β-adrenoceptors and human skeletal muscle characterisation of receptor subtype and effect of age

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1 Rectus abdominis muscle biopsies were obtained from 28 patients undergoing abdominal surgery. In membranes prepared from these biopsies β-adrenoceptor binding was examined. The apparent affinity (K_D) and the density (B_{max}) of the receptors for the radioligand (−)-[^{125}I] cyanopindolol were 28.5 ± 2.7 (pM) and 25.9 ± 2.1 (fmol mg^{-1} protein) (mean ± s.e. mean) respectively. In forceps biopsies from vastus lateralis muscle from four healthy volunteers the values for K_D and B_{max} were 22.5 ± 4.4 (pM) and 16.4 ± 2.2 (fmol mg^{-1} protein). The binding characteristics for the radioligand were similar in the biopsies from the two muscle sites.

2 Inhibition of the radioligand binding by the selective β_2-adrenoceptor antagonist ICI 118551 (K_I = 117 ± 45 nM) and selective β_1-adrenoceptor antagonist metoprolol (K_I = 15229 ± 5046 nM) suggests the dominance of β_2-adrenoceptor subtype in human skeletal muscle.

3 There were no significant differences in the skeletal muscle β-adrenoceptor densities or affinities between the young and older patients.

Keywords β-adrenoceptor skeletal muscle receptor subtype

Introduction

β-adrenoceptors in skeletal muscle contribute to a variety of muscle activities such as contraction (Bowman & Zaimis, 1958; Bowman & Nott, 1969) and metabolism (Meyer & Stull, 1971). Furthermore β-adrenoceptors in skeletal muscle have been suggested to maintain physiological concentrations of potassium in plasma especially during exercise and stress and also to mediate the hypokalaemic response to β_2-adrenoceptor agonists in animals and man (Lockwood & Lum, 1974; Brown *et al.*, 1983; Struthers *et al.*, 1983; Brown, 1985; Williams *et al.*, 1985; Haalboom *et al.*, 1985; Elfellah & Reid, 1987a). Recent studies have shown that in various animal species the skeletal muscle β-adrenoceptors are of the β_2-subtype (Minneman *et al.*, 1979; Ijzerman *et al.*, 1984; Elfellah & Reid, 1987b). However, in man, β-adrenoceptors in skeletal muscle have not been fully characterised.

There is a general agreement that the function of β-adrenoceptors in various systems decreases with advancing age in both man and animals (Fleisch & Hooker, 1976; Vestal *et al.*, 1979; Lakata, 1980; Bertel *et al.*, 1980; Krall *et al.*, 1981; Scarpace & Abrass, 1983; Rodeheffer *et al.*, 1984). Based on radioligand studies in lymphocytes, the age-related, reduced function of β-adrenoceptors in man has been attributed to the reduction of the density of the receptors (Schocken & Roth, 1977). On the other hand, others have shown that the density of the receptor was unaltered in aged man (Feldman *et al.*, 1984). Most of these human studies were carried out on lymphocytes; few radioligand studies have been carried out in other human tissues.

Our aims are therefore to characterise β-adrenoceptors and to investigate the effect of ageing on β-adrenoceptors in skeletal muscle using a radioligand technique.

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Methods

Rectus abdominis muscle

Biopsies, approximately 0.25–0.5 g each, from rectus abdominis muscle were obtained from 28 subjects, 14 males and 14 females with age range of 18–77 years and an average of 48 years. The biopsies were obtained during abdominal operations for various gastrointestinal diseases including cancer, duodenal ulcer and cholecystitis. Four of the patients had been treated with β-adrenoceptor blockers and one patient received salbutamol pre-operatively. Nine of the patients were being treated with drugs that are known not to alter β-adrenoceptors, such as antacids, H_2-receptor blockers and diuretics. Fourteen of the patients received no drugs in the 2 week period before surgery.

Vastus lateralis muscle

Vastus lateralis muscle biopsies were used to compare β-adrenoceptors of rectus abdominis muscle with other muscles. Forceps biopsies, approximately 0.25 g each, were obtained by the method of Henriksson (1979) from four healthy volunteers, three males and one female with age range of 27–41 years.

Skeletal muscle membrane preparation

Muscle biopsies were homogenised in Tris-HCl buffer (50 mM) at pH 7.4 using five successive bursts of the Polytron (Kinetica) each for 20 s at setting 5. The homogenates were centrifuged at 20,000 *g* for 15 min and the tissue membranes were stored at −60° C till assayed within 1 week. The tissue membranes could not be routinely purified further because of the small size of the muscle biopsy. However, in one single experiment the tissue was processed by a previously described method (Elfellah & Reid, 1987b). This experiment was carried out in order to be able to compare the K_I for ICI 118551 in the human tissue with that of the guinea pig skeletal muscle membranes. In this experiment a 0.9 g muscle biopsy was obtained from the rectus abdominis muscle of a 30 year old male patient undergoing cholecystectomy operation. The muscle was homogenised in sucrose (0.32 M) and centrifuged at 500 *g* for 15 min. The supernatant was separated over four layers of cheese-cloth and diluted with Tris HCl (50 mM) pH 7.4 and centrifuged at 40,000 *g* for 30 min. The pellet was then washed and re-centrifuged. Incubation with ICYP in the presence of various concentrations of ICI 118551 was carried out as described below.

β-adrenoceptor binding assay

The tissue membranes were thawed, suspended in Tris HCl (50 mM) and centrifuged at 20,000 *g*. The pellets were finally suspended in Tris HCl and homogenised for 10 s to give a protein concentration of approximately 0.25 mg ml^{-1}. All the above procedures were carried out at 4° C. Aliquots of membrane suspension each of 200 μl were incubated with the radioligand (−)-[^{125}I] cyanopindolol (ICYP) in a total volume of 400 μl Tris buffer at pH 7.4 at 25° C for 2 h. Incubations were terminated by addition of 10 ml cold Tris HCl followed by vacuum filtration over glass microfibre filters (Whatman GF/B). The filters were then washed twice with 5 ml buffer and estimated for radioactivity in a gamma counter (Berthold). Assays were carried out in duplicate and the non-specific binding was estimated as the difference between total binding and binding in the presence of propranolol (1 μM). Protein concentration was measured in aliquots of tissue membrane samples (Lowry *et al.*, 1951). The maximum number of binding sites, B_{max}, and the apparent dissociation constant, K_D, for ICYP binding were calculated by Scatchard analysis.

For determination of potencies of β-adrenoceptor drugs in inhibiting ICYP (25 pM) binding to muscle membrane (200 μl), the radioligand was incubated with the competing drug in a final volume of 1.2 ml Tris HCl buffer and the apparent dissociation constants K_I for the competing drugs were calculated according to the method of Cheng & Prusoff (1973). In the competition experiments none of the tissues obtained from any individual who was taking any drugs was used.

Statistics

Results are expressed as mean ± s.e. mean. Student's *t*-test was used to test the difference between two means. Iterative non-linear regression to fit the Hill equation was used to test whether the displacement of the radioligand by ICI 118551 or metoprolol fitted one or two binding site models.

Results

Figure 1 shows the specific, non-specific and total binding of ICYP from a representative experiment in a biopsy from rectus abdominis muscle. Saturation was reached at approximately 55 pM of ICYP added.

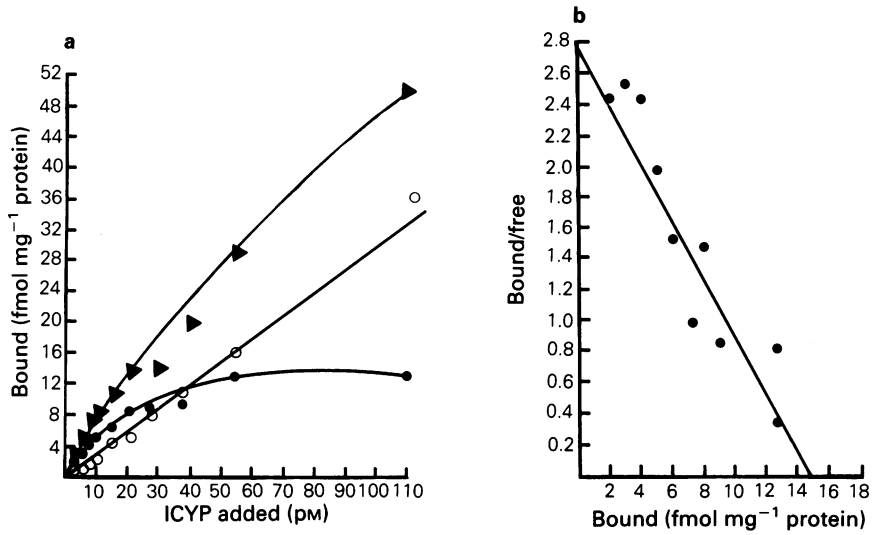

Figure 1 a) A representative saturation experiment of ICYP binding in a biopsy from human rectus abdominis muscle.
b) Scatchard plot obtained from (a). ▲ total, ○ non-specific, ● specific binding.

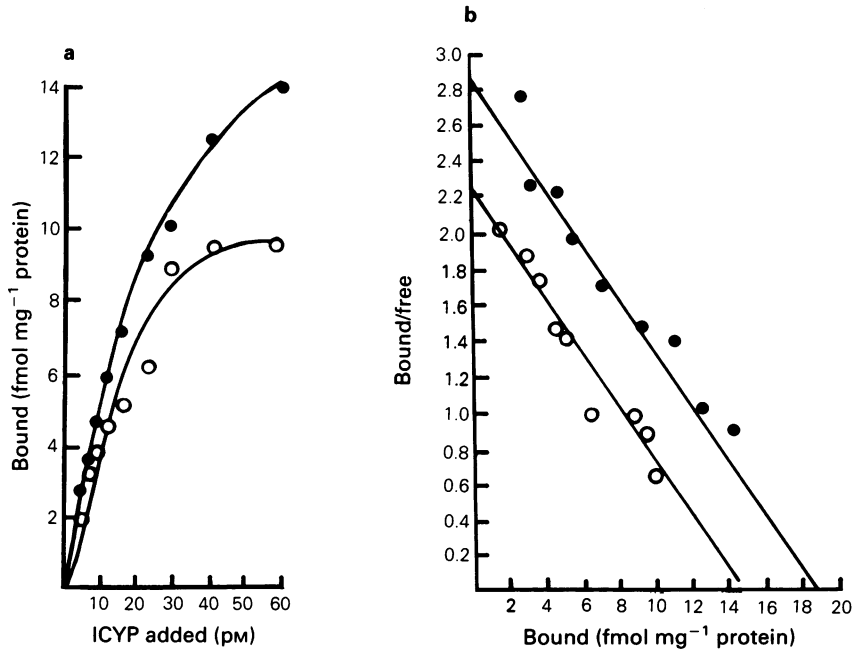

Figure 2 a) Saturation plots of ICYP binding in human rectus abdominis ● (n = 28 subjects) and vastus lateralis ○ (n = 4) muscle biopsies.
b) Scatchard plots obtained from (a).

Table 1 Binding characteristics for ICYP in human rectus abdominis and vastus lateralis muscle biopsies. Results are means ± s.e. mean

	Rectus abdominis muscle (n = 28)	*Vastus lateralis muscle (n = 4)*
B_{max} (fmol mg^{-1} protein)	25.9 ± 2.1	16.4 ± 2.2
K_D (pM)	28.5 ± 2.7	22.5 ± 4.4

No significant differences between the corresponding means ($P = 0.10$ for B_{max} and $P = 0.42$ for K_D means).

Figure 2 shows the saturation and Scatchard plots for ICYP specific binding in rectus abdominis and vastus lateralis muscles from all subjects in the study. The specific binding was saturable and the Scatchard plot was linear suggesting one class of binding sites. There were no significant differences in the values of the B_{max} or K_D between the rectus abdominis and vastus lateralis muscles (Table 1).

Figure 3 shows the inhibition of ICYP specific binding by various β-adrenoceptor agonists and antagonists and Table 2 shows the K_I values for these antagonists. The potency order for the agonists in displacing the radioligand was isoprenaline > adrenaline > noradrenaline. The (−)-isomer of propranolol is approximately 80 times the potency of the (+)-isomer, which indicates the stereoselectivity of the binding site. The β_2-adrenoceptor selective antagonist ICI 118551 is much more potent than the selective β_1-adrenoceptor antagonist metoprolol. Furthermore, when non-linear regression to fit the Hill equation was applied we found that the displacement plots for ICI 118551 or metoprolol in Figures 3 and 4 are best fitted for one site model. Furthermore, the slope of the Hill plot for ICI 118551 (Figure 4) was 0.9. This indicates that most β-adrenoceptors in the rectus abdominis muscle are mainly of the β_2-adrenoceptor subtype.

Table 2 K_I values for β-adrenoceptor antagonists in human rectus abdominis muscle biopsies (n = 3–8). Results are mean ± s.e. mean obtained from the inhibition of ICYP plots by the method of Cheng & Prusoff (1973)

	K_I *value (nM)*
(−)-propranolol	19.3 ± 5.5
(+)-propranolol	1544± 772
ICI 118551	117 ± 45
Metoprolol	15229 ± 5046

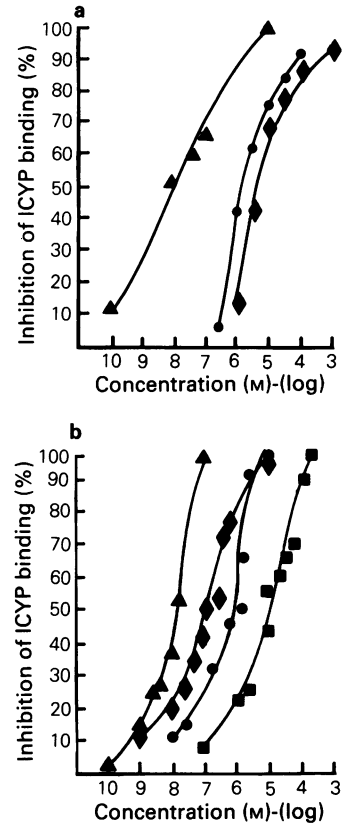

Figure 3 Inhibition of specific ICYP binding in human rectus abdominis muscle biopsies (n = 1–8 subjects) by various concentrations of (a) adrenoceptor agonists (◆ noradrenaline n = 1, ● adrenaline n = 1 and ▲ isoprenaline n = 1) and (b) antagonists (▲ (−)-propranolol n = 3, ● (+)-propranolol n = 3, ◆ ICI 118551 n = 4, ■ metoprolol n = 8).

The K_I values for the antagonists including ICI 118551 and metoprolol were higher than previously reported in the guinea pig skeletal muscle (Elfellah & Reid, 1987b). This is perhaps due to the use of rather crude membrane preparation and the higher non-specific binding in the present study. Thus in one experiment when a purified membrane preparation was used, the K_I for ICI 118551 was reduced to 1.1 nM which was comparable with that of the guinea pig skeletal muscle (0.39 nM) where β-adrenoceptors are all of β_2-subtype.

As treatment with β-adrenoceptor blockers (Hedberg *et al.*, 1986) and agonists (Martinsson *et al.*, 1987) may alter β-adrenoceptors, none of the patients under these drugs was included in the analysis of the effect of sex or age on binding site number.

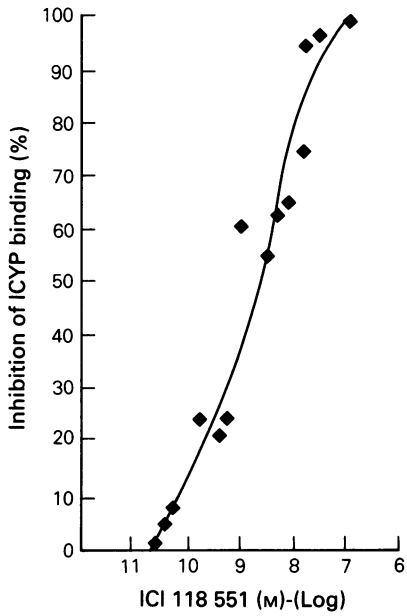

Figure 4 One experiment showing the inhibition of ICYP binding in purified rectus abdominis muscle biopsy. (See method for details.) The K_I of ICI 118551 was 1.1 nM.

Table 3 Effect of ageing on binding characteristics of ICYP in rectus abdominis muscle biopsies. Results are mean ± s.e. mean. Five patients on β-adrenoceptor medication were excluded from this analysis

Age (years)	n	B_{max} *(fmol mg^{-1} protein)*	K_D *(pM)*
18–50	15	22.9 ± 2.8	25.9 ± 3.5
54–77	8	28.6 ± 3.85	25.6 ± 5.3

No significant difference between the corresponding means ($P = 0.24$ for B_{max} and $P = 0.96$ for K_D means).

There were no significant differences in the binding characteristics between males and females. B_{max} values were 27.8 ± 3.8 (n = 11) and 22.3 ± 2.6 fmol mg^{-1} protein (n = 12) and K_D values were 31.2 ± 5.0 and 23.8 ± 3.8 pM in males and females respectively.

There were no significant differences in the binding characteristics for ICYP between the young and elderly groups of patients (Table 3). The absence of any correlation between the density of β-adrenoceptors and age in the individual patients (r = 0.02, P = 0.57) is shown in Figure 5.

The binding characteristics for ICYP in patients receiving treatment with β-adrenoceptor drugs

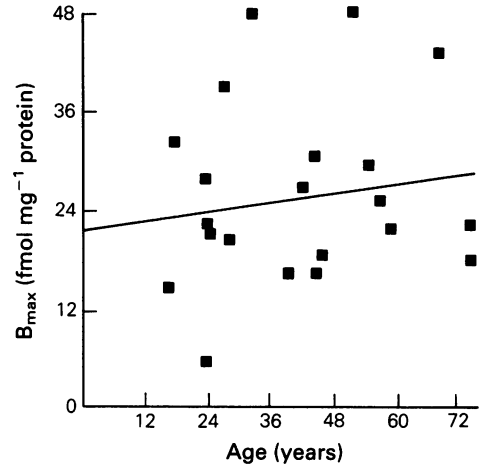

Figure 5 Correlation between age and density of β-adrenoceptors (B_{max}) in human rectus abdominis muscle biopsies (r = 0.02, P = 0.57). Each point represents result from one subject.

Table 4 Binding characteristics for ICYP in rectus abdominis muscle biopsies from patients receiving drug treatment

Treatment	n	B_{max} *(fmol mg^{-1} protein)*	K_D *(pM)*
None	14	26.6 ± 7.3	27.3 ± 3.9
Propranolol	1	45.6	46.8
Oxprenolol	1	26.4	30.8
Atenolol	2	33.3	33.3
Salbutamol	1	13.4	25.7

are shown in Table 4. The B_{max} for the patient treated with propranolol was much higher than the B_{max} for the patients receiving no drug treatment. Treatment with oxprenolol or atenolol did not appear to alter the B_{max}. In one patient receiving salbutamol the B_{max} was approximately half of the average B_{max} for the non-treated patients. With the exception of propranolol, drug treatment did not alter the K_D. The high value of the K_D in the case of the propranolol may be an artefact caused by the blocker being retained in the tissue membranes.

Discussion

In the present study the specific binding of ICYP was saturable and the Scatchard plot was linear, indicating that it is likely that the binding of the radioligand was to one class of receptors in the skeletal muscle. The order of potency of β-adrenoceptor agonists in displacing the radioligand was isoprenaline > adrenaline > noradrenaline. In addition the stereoselectivity of the

binding to the (−)-isomer of propranolol in the rectus abdominus muscle indicates that the binding of ICYP is to β-adrenoceptors. The most potent antagonist in inhibiting the radioligand binding was (−)-propranolol followed by the selective β_2-adrenoceptor antagonist ICI 118551, while the selective β_1-adrenoceptor antagonist metoprolol was much weaker. Furthermore, by using non-linear regression to fit the Hill equation it was found that the displacement curve for either ICI 118551 or metoprolol was best fitted for a one site model. In addition the slope of the Hill plot for ICI 118551 in the purified membrane preparation (Figure 4) was approximately 1. This suggests the dominance of β_2-adrenoceptor subtype in the human skeletal muscle. In the skeletal muscle of other animal species such as cats (Minneman *et al.*, 1979), cattle (Ijzerman *et al.*, 1984), guinea pigs (Elfellah & Reid, 1987b) and rabbits (Deighton *et al.*, 1987) the β-adrenoceptors are exclusively of the β_2-adrenoceptor subtype. The presence of β_2-adrenoceptor binding sites on membranes together with the large pool of potassium which is within skeletal muscle is consistent with the involvement of these receptors in short term regulation of plasma potassium (Lockwood & Lum, 1974; Clausen & Flatman, 1980; Struthers *et al.*, 1983; Brown *et al.*, 1983).

The density and affinity of the receptors for ICYP in both rectus abdominis and vastus lateralis muscles were similar, suggesting the former was representative of skeletal muscle in man.

In man abrupt withdrawal of propranolol after prolonged treatment could lead to serious reactions as a result of exaggerated responsiveness of β-adrenoceptors (Alderman *et al.*, 1974). Increased density, i.e. up-regulation of β-adrenoceptors has been suggested as the cause of the rebound reaction to propranolol (Hedberg *et al.*, 1985; Brodde *et al.*, 1986). In the present study the density of β_2-adrenoceptors in the skeletal muscle from one patient treated with propranolol was almost double the density of the receptors for the patients who received no drug treatment (Table 4). In a patient receiving oxprenolol the density of the receptors was not altered. Although oxprenolol is a non-selective β-adrenoceptor blocker it does possess some partial agonist activity. As expected, treatment with atenolol (a β_1-adrenoceptor selective antagonist) did not seem to alter skeletal muscle β_2-adrenoceptors in two patients. On the other hand in the skeletal muscle from one patient who was receiving salbutamol, β_2-adrenoceptor density was reduced by almost half. In asthmatic patients prolonged treatment with β_2-adrenoceptor agonists causes desensitisation to the bronchodilatation response (Svedmyr *et al.*, 1976; Jenne *et al.*, 1977). Based on studies in lymphocytes from such patients, the desensitisation was thought to result from down-regulation of β_2-adrenoceptors of the bronchial smooth muscle (Martinsson *et al.*, 1977). Thus our data on β-adrenoceptor blockers and agonists although based on very few subjects is consistent with reports of studies on lymphocytes (Whyte *et al.*, 1987).

Studies to elucidate the mechanism of the reduced function of β-adrenoceptors reported in the elderly have been carried out mainly on lymphocytes presumably because they represent an accessible model for studying alterations of β-adrenoceptors in man. Schocken & Roth (1977) demonstrated a reduced density of β-adrenoceptors in lymphocytes in elderly subjects and suggest that loss of β-adrenoceptors explains the reduced function of β-adrenoceptors. It remains to be seen whether ageing affects the function of β-adrenoceptors on skeletal muscle, nevertheless the present results in skeletal muscle clearly show that ageing does not affect either density or affinity of β-adrenoceptors for the radioligand ICYP. In our study a wide age range was examined and patients on drug treatment were excluded. Others have previously reported that although ageing reduced the function of β-adrenoceptors in lymphocytes it did not affect the density of the receptors (Abrass & Scarpace, 1981). Furthermore studies in animals showed that while ageing reduced the function of β-adrenoceptors it did not influence the density of β-adrenoceptors in solid tissues such as lung and heart (Guarnieri *et al.*, 1980; Scarpace & Abrass, 1983). Thus alteration of β-adrenoceptors with age in man seems to be due to a decrease in either adenylate cyclase activity (Abrass & Scarpace, 1982) or in a decreased coupling efficiency of the receptors to adenylate cyclase (Feldman *et al.*, 1984). In the present study the high and low affinity states of the receptors to the agonists, which occur in the absence or presence of guanosine nucleotide, were not measured. It is therefore possible that reduction in the high affinity state of the receptors such as occurs with ageing in human lymphocytes (Vestal *et al.*, 1979) and rat lung (Scarpace & Abrass, 1983) may have been overlooked.

In conclusion, β-adrenoceptors in human skeletal muscle are predominantly of the β_2-subtype and the density of these receptors did not change with advancing age.

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