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ISOPRENALINE ANTAGONISM OF CARDIOSELECTIVE β-ADRENERGIC RECEPTOR BLOCKING AGENTS ON HUMAN AND RAT ADIPOCYTES

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1 The β-adrenergic blocking potencies of practolol, ICI 66082, tolamolol, acebutolol, H 93/26, H 87/07, pindolol and Ro 3-4787 were compared with that of propranolol, on human and rat adipocytes.

2 A good correlation was found between the potencies on adipocytes of the two species but not between our results and literature data on antagonism of isoprenaline tachycardia in the anaesthetized cat.

3 The results indicate that differences between adrenergic β-receptors in heart and adipose tissue may be detected using cardioselective β-adrenergic receptor blocking agents.

Introduction

The classification of the β-adrenergic receptor mediating free fatty acid mobilization is a matter of some controversy. Lands, Arnold, McAuliff, Luduena & Brown (1967) and Arnold (1972), in studies in which they used only catecholamine data, found similarities between lipolytic and cardiac adrenergic β-receptors. They proposed classification of both receptors as β_1. Others, however, found indications for differences between these receptors by using non-catecholamine β-adrenergic receptor stimulating and blocking agents (Grana, Cattarini Mastelli, Zonta, Lucchelli & Santagostino, 1972; Stanton, 1972; Harms, Zaagsma & van der Wal, 1974).

Most of the work on lipolytic β-adrenergic receptor classification has been done on rat adipose tissue and cells, but the possibility of species differences has to be considered. Kelly & Shanks (1971) found that practolol (3 mg/kg) antagonized isoprenaline-induced free fatty acid mobilization in the greyhound, but Stanton (1972) found less than 20% inhibition of isoprenaline-induced lipolysis in the rat, with doses up to 136 mg/kg. Species differences in β-adrenergic receptors were also reported by Davey, Malta & Raper (1974).

Although there is evidence that non-cardioselective β-adrenergic receptor blocking agents are capable of reducing free fatty acid levels during exercise or emotional stress (Björntorp, Ek, Olsson & Schröder, 1967; Carruthers, Taggart & Sommerville, 1973; Sommerville, Taggart & Carruthers, 1973; Taggart, Carruthers & Sommerville, 1973), few data on effects of cardioselective β-adrenergic receptor blocking agents on human lipid mobilization have been published.

Whether or not the effects of β-adrenergic receptor blocking drugs on free fatty acid mobilization are clinically important is not well known (Taylor, 1973), but there are indications that reduction of plasma free fatty acid levels can be beneficial in acute heart infarction (Rowe & Oliver, 1974) and a special type of diabetic ketoacidosis (Baker, Minuchin & Rosman, 1975). Therefore it seemed of interest to determine the β-adrenoceptor blocking potencies of a number of new experimental β-adrenergic receptor blocking drugs, most of which are thought to be cardioselective (Åblad, Carlsson & Ek, 1973; Basil, Jordan, Loveless & Maxwell, 1973; Barrett, Carter, Fitzgerald, Hull & Le Count, 1973; Adam, Baird, Burges & Linell, 1974) on human and rat adipocytes (1) to study possible species differences between human and rat lipolytic β-adrenoceptors and (2) as a base for future *in vivo* studies of effects of cardioselective β-adrenergic blocking agents on adrenergic lipid mobilization.

Methods

Rat adipocyte suspension

Eight male Wistar rats, body weight 160-180 g, fed *ad libitum* were used per experiment. After decapitation the epididymal fat pads were

dissected out and a cell suspension was prepared according to Rodbell (1964). The suspension medium contained (g/l) NaCl 6.97, KCl 0.36, $CaCl_2.2H_2O$ 0.38, $MgSO_4.7H_2O$ 0.30, $NaHCO_3$ 2.10, KH_2PO_4 0.17, bovine serum albumin 40. No glucose was added to prevent reesterfication of the free fatty acids (Rodbell, 1965).

The cell suspension was distributed over siliconized 8 ml Kimax tubes (2.7 ml cell suspension/tube); 0.15 ml of the test compound or solvent (suspension medium without albumin) was added immediately. Concentrations of the test compounds formed a geometric series with factor 2. Propranolol was used as the reference compound in each experiment. After 10 min 0.15 ml of isoprenaline (0.15 ml, 2×10^{-6} M) or solvent was added. After 2 h of incubation in a Dubnoff metabolic shaker at 37°C in an atmosphere of 95% O_2/5% CO_2, the amount of released free fatty acids was determined according to Ko & Royer (1967). Each experiment was carried out in triplicate and the titration values of the triplicates were averaged.

Human adipocyte suspension

Subcutaneous adipose tissue samples were obtained from patients undergoing different surgical procedures, mainly breast or gall bladder resections. The adipose tissue was transported from the operation theatre to the laboratory in 0.9% w/v saline, minced with scissors and a cell suspension was prepared in the same way as with rat epididymal fat pads, with one modification: during the collagenase treatment, glucose (5.5 mM) was added to the medium; washings and final incubation were done without glucose. The cell suspension was distributed over siliconized 8 ml Kimax tubes. The procedure for testing β-adrenergic receptor blocking agents was similar to that used in the rat adipocyte assay, except that duplicates were used instead of triplicates and that the concentrations of the test compounds formed a geometric series with factor 3, because of the limited supply of human adipose tissue.

Evaluation of the results

The effect of isoprenaline (10^{-7}M) was put at 100%. Other values were calculated as percentages of this maximum and potency ratios of each of the β-adrenergic receptor blocking agents to propranolol were calculated for each dose. Only points on the quasi-linear part of the log dose-response curves were used.

Drugs and chemicals

Propranolol, practolol, ICI 66082 (ICI); tolamolol (Pfizer); pindolol (Sandoz); Ro 3-4787 (Hoffman-La Roche); H 87/07, H 93/26 (Hässle); acebutolol (May & Baker); isoprenaline sulphate (ACF, Holland); collagenase CLS (Worthington); demineralized bovine serum albumin (Povite, Holland). All other chemicals were of pure analytical grade and obtained from Merck. All drugs were dissolved in distilled water and diluted in suspension medium without albumin; for dissolution of the compounds that were available as free bases the appropriate amount of HCl 0.1 N was added.

Results

Table 1 gives the structural formulae of the compounds tested and the potencies on human and rat adipocytes in blocking isoprenaline (10^{-7}M), compared to propranolol, which was assigned an arbitrary potency of 1000.

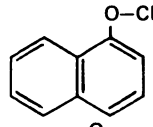
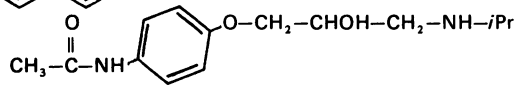
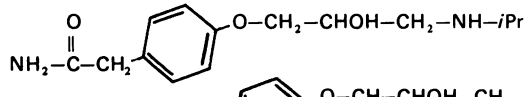
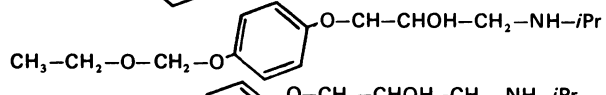
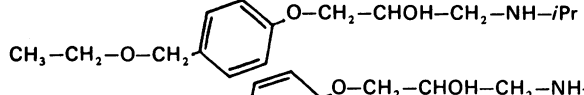
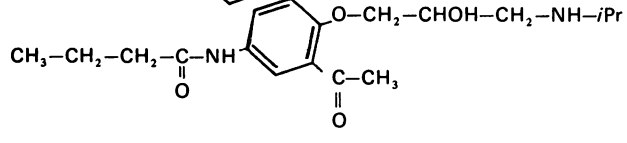
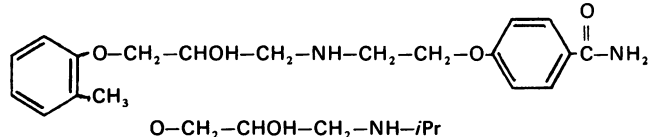
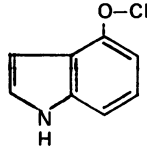
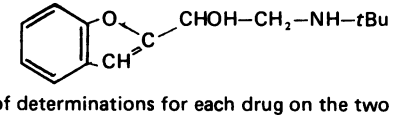
Discussion

In 1974, Harms *et al.*, presented evidence against the classification of Lands *et al.* (1967) that the β-adrenergic receptor in rat adipose cells is β_1. The present results seem to support the hypothesis that compounds that owe their cardioselectivity to (para-)substitution in the phenyl ring have lipolytic β-adrenoceptor blocking potencies on rat adipocytes that do not correlate well with potencies on cardiac receptors.

In Table 2, literature data on the potencies of some of the tested compounds against isoprenaline-induced tachycardia in the anaesthetized cat, compared to propranolol (= 1000) are summarized. Evidently, there is a poor correlation with our results on lipolysis. However, because a comparison between *in vitro* data on the one hand and *in vivo* data, from different laboratories, on the other is not ideal, *in vitro* studies with guinea pig atria are now in progress.

The high potency of tolamolol against isoprenaline-induced lipolysis is also consistent with our hypothesis, because this compound owes its selectivity to the N-substituent; compounds of this type were predicted to have lipolytic β-adrenoceptor blocking potencies that correlate well with cardiac β-adrenoceptor blocking potencies. Indeed our results and those of Adam *et al.* (1974) both show similar potencies for tolamolol and propranolol on respectively rat adipocytes and guinea pig right atrium.

Table 1 Lipolytic β-adrenergic receptor blocking potencies, compared to propranolol (= 1000) on human and rat adipocytes

Compound	*Structure*	*Human adipocytes (log_{10} β-adrenoceptor blocking potency ± s.e. mean)*	*Rat adipocytes (log_{10} β-adrenoceptor blocking potency ± s.e. mean)*
Propranolol		3	3
Practolol		0.600 ± 0.052 (14)	0.863 ± 0.051 (19)
ICI 66082		1.612 ± 0.043 (11)	1.683 ± 0.026 (14)
H 87/07		0.936 ± 0.084 (14)	1.100 ± 0.029 (12)
H 93/26		2.087 ± 0.057 (14)	2.104 ± 0.022 (13)
Acebutolol		0.821 ± 0.067 (12)	0.598 ± 0.039 (12)
Tolamolol		2.872 ± 0.055 (16)	2.871 ± 0.032 (41)
Pindolol		3.620 ± 0.038 (10)	3.497 ± 0.034 (23)
Ro 3-4787		2.540 ± 0.054 (10)	2.589 ± 0.038 (19)

The number of determinations for each drug on the two parameters measured is given in brackets.
In the structural formulae, *i*Pr isopropyl, *t*Bu tertiary butyl.

Table 2 Comparative β-adrenergic receptor blocking potencies on lipolytic (this paper) and cardiac β-adrenoceptors (literature data on isoprenaline induced tachycardia in anaesthetized cats), relative to propranolol (= 1000)

Compound	*Rat adipocytes (log_{10} β-adrenoceptor blocking potency)*	*Anaesthetized cat tachycardia (log_{10} β-adrenoceptor blocking potency)*	*Reference*
Propranolol	3	3	
Practolol	0.863	2.204	Åblad *et al.* (1973)
ICI 66082	1.683	3	Barrett *et al.* (1973)
H 87/07	1.100	2.204	Åblad *et al.* (1973)
H93/26	2.104	2.322	Åblad *et al.* (1973)
Acebutolol	0.598	2.463	Basil *et al.* (1973)

Table 1 also shows that the potencies of the tested drugs on rat and human adipocytes, compared to propranolol, are similar although there are some differences. For instance, on rat adipocytes, practolol is more active than acebutolol, while on human adipocytes acebutolol is the more active drug. Despite the small differences found, the interactions of the tested compounds with human and rat lipolytic β-adrenergic receptors appear to be rather similar. Regression analysis of the two sets of β-adrenoceptor blocking potencies gives a high correlation coefficient, $r = 0.991$, $n = 8$, $P \ll 0.001$.

Although many authors have presented evidence for the supposition that the lipolytic β-adrenergic receptor should be classified as β_1, (Lands *et al.*, 1967; Arnold, 1972; Grana, Lucchelli & Zonta, 1974; Lefkovitz & Coverstone, 1974) our results seem to indicate that this classification does not provide satisfactory explanation for the potencies of cardioselective β-adrenergic blocking agents against isoprenaline-induced lipolysis in rat and human adipocytes.

Based on our results, differences might be expected in effects on adrenergic lipid mobilization, in rat as well as in humans, of doses of cardioselective β-adrenergic receptor blocking agents that are equipotent as far as the β-adrenergic blockade on the heart is concerned.

Investigations to test this hypothesis are now in progress.

The gifts of propranolol, practolol, ICI 66082 (I.C.I. Ltd.), tolamolol (Pfizer), acebutolol (May & Baker), Ro 3-4787 (Roche), H 87/07, H 93/26 (Hässle) and pindolol (Sandoz) are gratefully acknowledged. We are very grateful to surgeons and staff of the departments of surgery and plastic surgery for the supply of adipose tissue samples and to George Wardeh for excellent technical assistance.

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