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BLOCKADE OF ISOPRENALINE-INDUCED CHANGES IN PLASMA FREE FATTY ACIDS, IMMUNOREACTIVE INSULIN LEVELS AND PLASMA RENIN ACTIVITY IN HEALTHY HUMAN SUBJECTS, BY PROPRANOLOL, PINDOLOL, PRACTOLOL, ATENOLOL, METOPROLOL AND ACEBUTOLOL

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1 The effects of intravenously administered propranolol 0.01 and 0.03, pindolol 0.001 and 0.003, practolol 0.12 and 0.36, atenolol 0.03 and 0.09, metoprolol 0.045 and 0.135 and acebutolol 0.12 and 0.36 mg/kg, on isoprenaline-induced changes in heart rate, blood pressure, plasma free fatty acids, immunoreactive insulin plasma levels and plasma renin activity were determined in six healthy human subjects.

2 Propranolol, atenolol and metoprolol had a stronger effect on resting heart rate than practolol, acebutolol and pindolol, probably reflecting differences in intrinsic β-sympathomimetic activity. Antagonist potencies against isoprenaline-induced changes in heart rate and blood pressure suggested cardioselectivity for practolol, atenolol, metoprolol and the lower dose of acebutolol and non-cardioselectivity for propranolol, pindolol and the higher dose of acebutolol.

3 All six β-adrenoceptor blocking agents were able, to a varying extent, to antagonize the isoprenaline-induced increases in plasma free fatty acids and plasma immunoreactive insulin levels. In general, the cardioselective agents were relatively less effective antagonists than the non-cardioselective agents.

4 Resting plasma renin activity was reduced by all six β-adrenoceptor blocking agents, suggestive of the presence of β_1-adrenoceptors mediating renin release, but the non-cardioselective agents propranolol and pindolol seemed relatively more effective in antagonizing isoprenaline-induced increases in plasma renin activity than the cardioselective agents, which indicates that β_2-adrenoceptors might also be involved.

5 The results are compatible with the hypothesis that both β_1- and β_2-adrenoceptors are involved in the regulation of lipolysis, insulin release and renin release.

Introduction

Since the withdrawal of practolol because of serious side-effects, there is a need for an alternative cardioselective β-adrenoceptor antagonist. Among agents more recently introduced, metoprolol, atenolol and acebutolol have been shown in animals to act selectively on cardiac β_1-adrenoceptors. In addition there is evidence for a certain degree of cardioselectivity on human β-adrenoceptors (Harms, 1976a). However, there are few studies to date on the metabolic effects of these newer β-adrenoceptor antagonists in man.

In view of the possible arrhythmogenic effects of elevated plasma free fatty acid levels in certain clinical situations (Rowe, Neilson & Oliver, 1975) we have been interested in the effects of cardioselective β-adrenoceptor blocking agents on lipolysis in human adipose tissue (Harms & Van der Meer, 1975; Harms, 1976b) and we wish to report here the results of a study in healthy subjects in which the antagonism of isoprenaline induced elevation of plasma free fatty acid levels by a number of cardioselective and non-cardioselective agents was determined.

There are practically no data available on the effects of the newer cardioselective β-adrenoceptor blocking agents on β-adrenergic stimulation of insulin release. Therefore, we also measured the effects of propranolol, pindolol, practolol, atenolol, metopolol and acebutolol on isoprenaline induced rises of plasma immunoreactive insulin levels.

Because there is some controversy on the classification of the β-adrenoceptors involved in the sympathetic regulation of plasma renin activity (for reviews, see Bühler, Burkart, Lütold, Küng, Marbet & Pfisterer, 1975; Guthrie, Dornier, Boucher, Kuchel, Rojo-Ortega, Nowaczynski & Genest, 1976) we also measured the effects of the antagonists on resting and isoprenaline stimulated plasma renin activity.

Methods

Six healthy male volunteers, aged 23–27 years, participated in this study. The aims and possible risks were explained fully to them and they all passed a thorough clinical and laboratory investigation. The protocol of the investigation was approved by the ethical committee of the Free University Academic Hospital.

The volunteers were asked not to eat and drink only water for at least 4 h prior to the experiments. In each experiment, the subject rested on a comfortable couch. Blood pressure was recorded with an Arteriosonde 1216; electrocardiogram and heart rate were registered on a Mingograph 804 recorder. An infusion catheter with 0.9% saline drip was inserted in an antecubital vein. After 20 min rest, a blood sample was drawn and heart rate and blood pressure measurements were taken. Then the test agent or saline was injected intravenously, slowly over 10 min, and 10 min after the end of the injection, a second blood sample was obtained and cardiovascular parameters recorded, immediately followed by the infusion of isoprenaline sulphate (0.025 $\mu g\ kg^{-1}\ min^{-1}$) for 20 min. At the end of the infusion period, cardiovascular measurements were repeated and a third blood sample was taken. Blood for free fatty acid, glucose and insulin determinations was collected in heparinized tubes and for plasma renin activity determination in sodium EDTA tubes, and centrifuged immediately at 0°C.

Plasma was frozen immediately and stored at −30°C. Free fatty acids were determined according to Ko & Royer (1967). Immunoreactive insulin was determined according to Yalow & Berson (1960). Glucose was measured by the glucose oxidase peroxidase method. Plasma renin activity was assayed by the method of Lehfeldt & Hutchens (1971) with slight modifications: angiotensin I samples were taken at 0, 30 and 60 min instead of 1, 2 and 3 h. In each subject two experiments with saline placebo and 12 experiments with the six test agents were performed.

Two doses of each agent were tested in separate experiments. In the first set of experiments the following doses were chosen, on the basis of literature data (Aström & Vallin, 1974; Johnsson, Nyberg & Sölvell, 1975; Lang & Heck, 1972; Basil, Jordan, Loveless & Maxwell, 1973; Wilson, Brooks, Lloyd & Robinson, 1969) to cause a similar degree of blockade of cardiac β-adrenoceptors: propranolol, 0.03 mg/kg; pindolol 0.003 mg/kg; practolol 0.12 mg/kg; atenolol 0.03 mg/kg; metoprolol 0.045 mg/kg, acebutolol 0.12 mg/kg.

In the first series of experiments propranolol and pindolol were found to block the effects of isoprenaline almost completely and a lower dose (0.01 mg/kg and 0.001 mg/kg respectively) was selected for later experiments. In contrast, because the other agents proved less effective than propranolol, the following doses were used in the second set of experiments: practolol, 0.36 mg/kg; atenolol, 0.09 mg/kg; metoprolol, 0.135 mg/kg; acebutolol, 0.36 mg/kg.

A period of at least 3 days between consecutive experiments was maintained, which was sufficient, in view of the low doses used and the half-lives of elimination of the various test agents, to exclude interference of one experiment with the next.

Mean ± s.e. mean were calculated for all parameters measured. However the s.e. mean values should be considered only as a rough estimate of variability of the data, because the condition of a normal distribution of the data was not fulfilled completely for all sets of values. Differences between data obtained were tested for significance using the rank sign test.

Results

Heart rate and blood pressure

Propranolol, atenolol and metoprolol, three agents lacking intrinsic β-sympathomimetic activity (ISA), exerted the largest reductions in resting heart rate, while practolol, acebutolol and pindolol, three agents that possess ISA, had less or no effect on resting heart rate. None of the six agents had an important effect on resting blood pressure. The changes in isoprenaline induced rise in systolic and fall in diastolic blood pressure, caused by the various β-adrenoceptor blocking agents, are shown in Table 1. Propranolol, 0.03 mg/kg, was more potent than atenolol, 0.03 mg/kg and metoprolol, 0.045 mg/kg, as an antagonist of isoprenaline induced tachycardia and drop of diastolic blood pressure ($P < 0.05$).

The effects of atenolol, metoprolol and practolol on isoprenaline induced changes in heart rate and diastolic blood pressure were not clearly dose-dependent in the dose range studied, indicating some degree of cardioselectivity.

Even the lower doses of propranolol and pindolol caused more than 50% blockade of isoprenaline induced tachycardia and lowering of diastolic blood

Table 1 The changes (mean ± s.e. mean) in isoprenaline-induced response of heart rate, blood pressure, plasma renin activity (PRA), plasma free fatty acid levels (FFA) and insulin levels by various β-adrenoceptor blocking agents.

	Dose[a]	*Heart rate*		*Blood pressure*[b]		*PRA*[c]				
Test agent	*mg/kg*	*Δ1*	*Δ2*	*Δsystolic*	*Δdiastolic*	*I*	*II*	*III*	*ΔFFA*[d]	*ΔInsulin*[e]
Saline		0 ± 1	34 ± 3	33 ± 1	−20 ± 2	3.3 ± 0.4	3.3 ± 0.4	5.7 ± 0.5	0.83 ± 0.08	17.5 ± 3.4
Propranolol	0.01	−4 ± 1	11 ± 2	22 ± 3	− 8 ± 3	4.0 ± 0.7	3.6 ± 0.7	4.2 ± 0.6	0.54 ± 0.10	6.3 ± 1.4
Propranolol	0.03	−6 ± 1	7 ± 1	13 ± 2	− 4 ± 2	3.8 ± 0.4	3.2 ± 0.4	3.4 ± 0.3	0.32 ± 0.06	1.3 ± 2.0
Practolol	0.12	−3 ± 1	17 ± 3	22 ± 3	−15 ± 3	4.1 ± 0.4	3.7 ± 0.5	4.8 ± 0.6	0.69 ± 0.13	10.2 ± 1.9
Practolol	0.36	−2 ± 1	16 ± 3	21 ± 1	−14 ± 4	3.6 ± 0.5	3.0 ± 0.4	4.0 ± 0.7	0.58 ± 0.12	10.3 ± 2.1
Atenolol	0.03	−7 ± 1	17 ± 2	26 ± 3	−15 ± 2	4.2 ± 0.7	3.3 ± 0.2	4.8 ± 0.5	0.58 ± 0.09	13.2 ± 3.4
Atenolol	0.09	−8 ± 1	14 ± 2	20 ± 2	−14 ± 2	4.4 ± 0.4	3.3 ± 0.4	3.7 ± 0.4	0.53 ± 0.15	5.8 ± 1.4
Metoprolol	0.045	−5 ± 1	19 ± 2	25 ± 4	−12 ± 2	3.2 ± 0.4	3.0 ± 0.4	3.7 ± 0.3	0.43 ± 0.06	12.6 ± 2.9
Metoprolol	0.135	−7 ± 1	15 ± 2	19 ± 3	−11 ± 3	2.9 ± 0.3	2.4 ± 0.3	3.0 ± 0.3	0.53 ± 0.14	11.4 ± 2.6
Acebutolol	0.12	−3 ± 1	15 ± 2	21 ± 3	−17 ± 3	4.0 ± 0.3	3.0 ± 0.2	3.0 ± 0.4	0.56 ± 0.06	5.8 ± 1.2
Acebutolol	0.36	−1 ± 1	11 ± 2	13 ± 1	−10 ± 2	4.0 ± 0.7	3.3 ± 0.6	3.8 ± 0.5	0.28 ± 0.07	5.5 ± 1.4
Pindolol	0.001	−0 ± 1	14 ± 4	24 ± 2	− 9 ± 2	3.6 ± 0.4	3.4 ± 0.4	4.4 ± 0.5	0.51 ± 0.10	2.6 ± 2.2
Pindolol	0.003	−1 ± 1	9 ± 3	14 ± 2	− 9 ± 2	3.9 ± 0.7	3.4 ± 0.6	3.8 ± 0.6	0.18 ± 0.02	1.2 ± 1.6

[a] Average heart rate at the start of the experiment 59 beats min^{-1}. Δ_1, heart rate after test agent minus heart rate before test agent; Δ_2, heart rate at the end of the isoprenaline infusion minus heart rate after test agent.

[b] Average blood pressure at the start of the experiment 112/79 mmHg. Δsystolic, systolic blood pressure at the end of the isoprenaline infusion minus systolic blood pressure after test agent; Δdiastolic, idem for diastolic blood pressure.

[c] Plasma renin activity, I, at the start of the experiment, II, after injection of test agent, III, after isoprenaline infusion (ng ml^{-1} h^{-1}).

[d] Free fatty acid plasma level increase, induced by isoprenaline (μeq/ml).

[e] Immunoreactive insulin plasma level increase, induced by isoprenaline (μU/ml).

All values are given as mean ± s.e. mean; the s.e. mean values should be considered only as rough estimate of variability because the condition of a normal distribution of the data was not fulfilled completely for all sets of numbers.

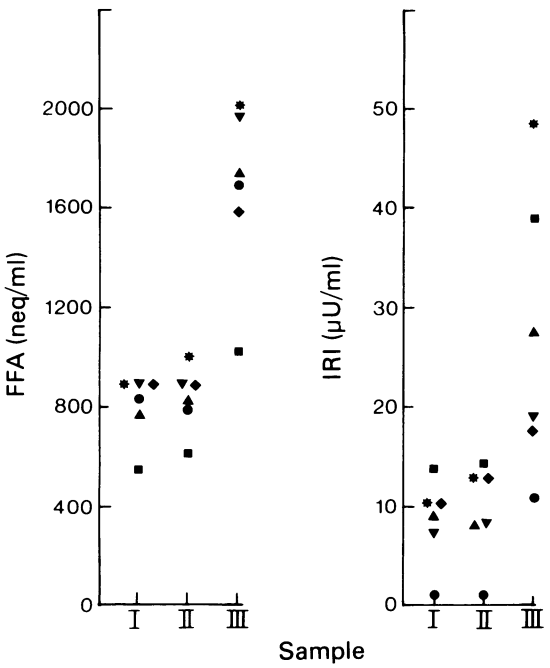

Figure 1 Resting (I), post-saline injection (II) and isoprenaline stimulated (III) plasma free fatty acid (FFA) and immunoreactive insulin levels (IRI). Each point is the average of data from two experiments, for six subjects. Each subject is represented by a different symbol.

pressure. Therefore, a clear dose-dependency could not be demonstrated, but the relatively strong antagonism of isoprenaline induced lowering of diastolic blood pressure suggests a lack of cardioselectivity. The lower dose of acebutolol had approximately the same effects as practolol, 0.12 mg/kg, but at 0.36 mg/kg, the effects on isoprenaline induced changes in heart rate and diastolic blood pressure were similar to those of propranolol, 0.01 mg/kg.

Plasma free fatty acid levels

None of the six agents had an important effect on resting free fatty acid plasma levels. The effects of isoprenaline on plasma free fatty acid levels are shown in Figure 1.

Figure 2 gives the individual values of the rises in plasma free fatty acid levels, induced by isoprenaline in the absence and presence of the various β-adrenoceptor blocking agents. Average values $\pm$ s.e. mean are given in Table 1. The effects of propranolol and pindolol were similar. The cardioselective agents seemed to have a relatively weak effect, compared to the effect of propranolol, at doses that are approximately equipotent with respect to blockade of sympathetic stimulation of the heart.

Plasma immunoreactive insulin levels

None of the six agents had an important effect on resting insulin plasma levels. The effects of isoprenaline on plasma insulin levels are shown in Figure 1. Figure 3 depicts the individual values of isoprenaline induced rises in plasma immunoreactive insulin levels after

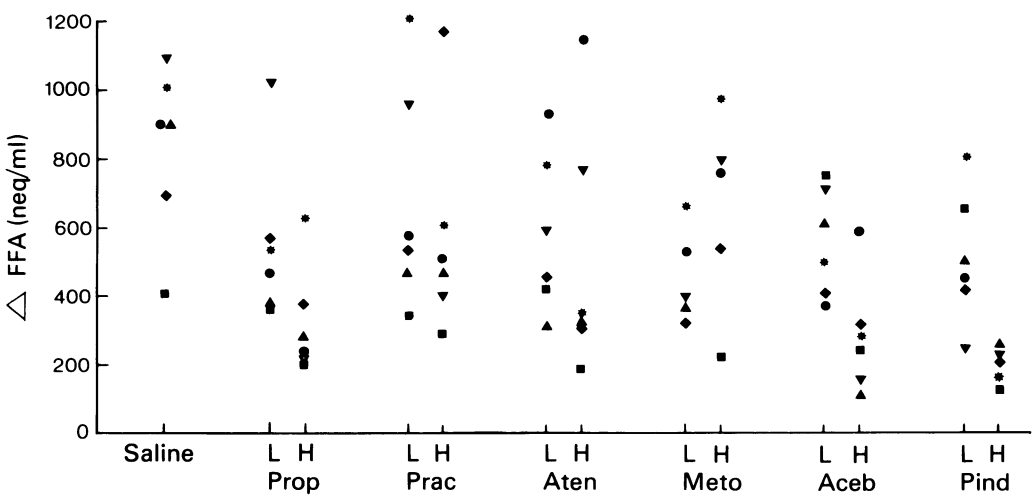

Figure 2 Isoprenaline-induced increases in plasma free fatty acid levels (FFA) after saline (average of two experiments) or β-adrenoceptor blocking agent in six subjects. L, low dose, H, high dose (see Methods section). Prop. propranolol; prac. practolol; aten. atenolol; meto. metprolol; aceb. acebutolol; pind. pindolol.

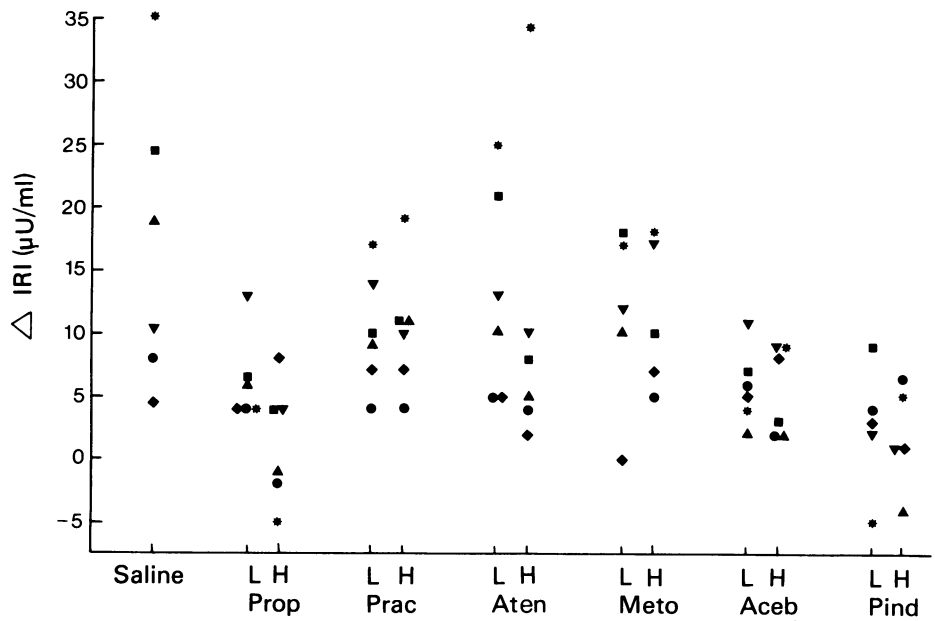

Figure 3 Isoprenaline-induced increases in plasma immunoreactive insulin levels (IRI) after saline (average of two experiments) or β-adrenoceptor blocking agent in six subjects. L, low dose, H, high dose (see Methods section). Prop. propranolol; prac. practolol; aten. atenolol; meto. metoprolol; aceb. acebutolol; pind. pindolol.

saline or β-adrenoceptor blocking agents. Average values ± s.e. mean are given in Table 1.

Propranolol and pindolol had a considerable blocking effect, already at the lower doses. The cardioselective agents atenolol and metoprolol had significantly less effect than propranolol ($P < 0.05$) at doses considered to be equipotent with respect to blockade of sympathetic stimulation of the heart. Plasma glucose levels were not influenced by isoprenaline, in accordance with previous reports (Gibbons, Lant, Ashford, Collins & Pinder, 1976).

Plasma renin activity

All six agents caused a reduction of resting plasma renin activity and an inhibition of the effect of isoprenaline infusion. Only atenolol, 0.03 mg/kg did not antagonize isoprenaline to a significant degree, although the effect on resting plasma renin activity was significant ($P < 0.05$). Average values ± s.e. mean are given in Table 1. Individual values are shown in Figure 4.

Discussion

Blockade of cardiac and vascular β-adrenoceptors

The relatively weak antagonism of the increase in heart rate, caused by isoprenaline, by the cardioselective agents, practolol, atenolol and metoprolol, compared to propranolol, at doses which are roughly equipotent against sympathetic stimulation of the heart, is in accordance with literature data (Johnsson *et al.*, 1975; De Plaen, Amery & Reybrouck, 1976) and might be explained by interference of vagal withdrawal and/or cardiac β_2-adrenoceptor stimulation by isoprenaline.

Surprisingly, the lower dose of acebutolol showed a considerable blockade of isoprenaline tachycardia but only very weak antagonism of the drop in diastolic blood pressure, whereas the higher dose produced approximately the same effects as propranolol, 0.01 mg/kg, on both heart rate and diastolic blood pressure. This apparent non-cardioselectivity of the higher dose of acebutolol is in accordance with the report of Briant, Dollery & George (1974) who also found no cardioselectivity of acebutolol, comparing its effects against isoprenaline tachycardia and vasodilation. On the other hand, acebutolol has been shown to possess cardioselectivity *in vitro* on human cardiac vs. bronchial β-adrenoceptors (Harms, 1976a). No satisfactory explanation for these contrasting results seems available.

Blockade of adipose tissue β-adrenoceptors mediating lipolysis

In 1967, Lands, Arnold, McAuliff, Luduena & Brown proposed a subclassification of the β-adrenoceptors in

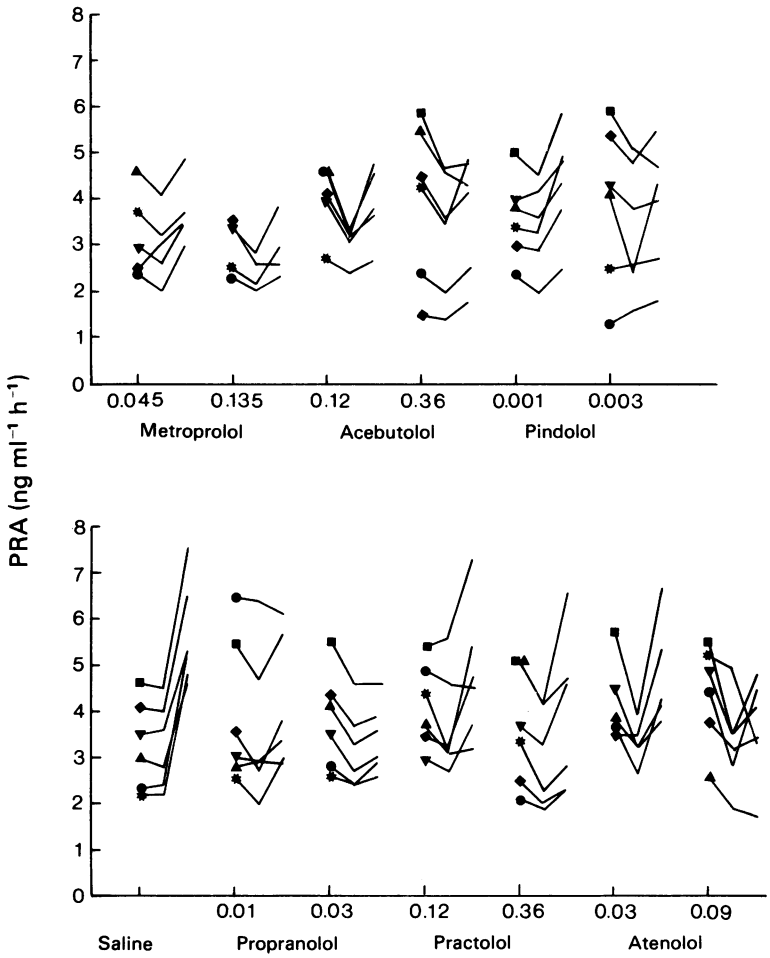

Figure 4 Plasma renin activity (PRA): each line connects, from left to right, resting level, level after β-adrenoceptor blocking agent and level after isoprenaline infusion in six subjects.

heart and adipose tissue as β_1. However, a considerable number of reports indicate that there are differences between the pharmacological characteristics of cardiac and adipose tissue β-adrenoceptors, both in animals and in man (Harms, Zaagsma & van der Wal, 1974; Goldberg, Joffe, Bersohn, Van As, Krut & Seftel, 1975; Gibbons *et al.*, 1975; Harms, 1976b). Comparison of the antagonist potencies of several cardioselective β-adrenoceptor blocking agents on human cardiac, bronchial and adipocyte β-adrenoceptors against isoprenaline induced effects (Harms & van der Meer, 1975; Harms, 1976a) also suggests that these agents are somewhat less effective in blocking adipocyte β-adrenoceptors than cardiac β-adrenoceptors.

Alternatively, the human adipocyte may have significant numbers of both β_1 and β_2-adrenoceptors (Åblad, Borg, Carlsson, Ek, Johnsson, Malmfors & Regårdh, 1975).

Our data, showing relatively weak antagonism of isoprenaline induced increase in plasma free fatty acids by the cardioselective agents at doses that are approximately equipotent to those of non-cardioselective compounds with respect to antagonism of sympathetic stimulation of the heart, are consistent with such a hypothesis.

Blockade of pancreatic β-adrenoceptors mediating insulin release

Loubatieres, Mariani, Sorel & Savi (1971) suggested that the β-adrenoceptors in the pancreas mediating insulin release are of the β_2-type.

Our data suggest that indeed the noncardioselective

agent propranolol is more effective than the cardioselective compounds atenolol and metoprolol in suppressing the rise in plasma immunoreactive insulin, induced by isoprenaline, at doses that are equipotent with respect to blockade of sympathetic stimulation of the heart.

In some experiments, however, the cardioselective agents caused considerable partial blockade of the isoprenaline effect, suggesting that also in the pancreas a mixed population of β_1- and β_2-adrenoceptors might exist.

Blockade of renal β-adrenoceptors mediating renin release

The significant drop in unstimulated plasma renin activity after the lower dose of the cardioselective agents, including the lower dose of acebutolol, which had hardly any effect on vascular β_2-adrenoceptors, suggests that β_1-adrenoceptors are involved in plasma renin activity regulation, in accordance with Bühler *et al.* (1975).

On the other hand, the fact that atenolol, 0.03 mg/kg, had significantly less blocking effect against isoprenaline than propranolol, 0.01 mg/kg ($P < 0.05$), although both agents are approximately equipotent against exercise induced tachycardia (Aström & Vallin, 1974) suggests that β_2-adrenoceptors might also be involved in plasma renin activity regulation. Thus, also in plasma renin activity regulation, both β_1 and β_2-adrenoceptors may be involved, in line with the hypothesis of Carlsson *et al.* (1972).

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