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Lipomobilizing effects of procaterol and yohimbine in the conscious dog: comparison of endocrinological, metabolic and cardiovascular effects

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1 Lipid mobilization during a hypocaloric diet may be enhanced by a pharmacological approach using β_2-adrenoceptor agonists or α_2-adrenoceptor antagonists. Studies were undertaken in the dog, an animal model presenting fat cell antilipolytic α_2- and lipolytic β-adrenoceptors, in order, firstly, to demonstrate the presence of β_2 subtype adrenoceptors on adipocytes and, secondly, to compare the effects of procaterol (β_2-adrenoceptor agonist) and of yohimbine (α_2-adrenoceptor antagonist) on metabolic, endocrinological and cardiovascular parameters.

2 Procaterol strongly stimulates lipolysis in dog adipocytes *in vitro*. The utilisation of selective β_1- and β_2-adrenoceptor antagonists (bisoprolol and ICI 118,551) in both lipolysis and binding studies (displacement of [^{3}H]-dihydroalprenolol binding) demonstrated the presence of the two β-adrenoceptor subtypes in dog fat cells.

3 Infusion of either yohimbine or procaterol (10 and 0.4 nmol min^{-1} kg^{-1}, respectively for 30 min). provoked an equivalent increase in plasma non-esterified fatty acids (+100%). Procaterol, but not yohimbine, induced hyperglycaemia (+120%). Plasma insulin was weakly enhanced by yohimbine (+120%) as compared to the increase given by procaterol (+500%).

4 Both drugs stimulated sympathetic nervous system activity, as indicated by the increased plasma noradrenaline concentration, but only yohimbine increased the plasma adrenaline level.

5 Cardiovascular measurements indicated that procaterol strongly enhances heart rate and transiently decreases mean blood pressure. Yohimbine exhibits a weaker effect on heart rate and slightly increases mean blood pressure.

6 The present work clearly indicates that lipid mobilization is enhanced during fasting in the dog by selective β_2-adrenoceptor stimulation or by α_2-adrenoceptor blockade. This enhanced lipolytic effect may result either from a direct action of the drugs on the adrenoceptors of fat cells or from an activation of the sympathetic nervous system. Procaterol suffers major limitations since it strongly increases heart rate, immunoreactive insulin and glycaemia. On the other hand, yohimbine induces only minor modifications both in cardiovascular and endocrinological parameters.

Introduction

Lipid mobilization is accelerated during the earlier part of a period of energy restriction or fasting in man and animals. It is likely that the decrease in plasma insulin levels is the major factor involved in the induction of lipid mobilization since insulin is a potent antilipolytic hormone (Cahill *et al.*, 1966; Lilavivathana *et al.*, 1978; Jensen *et al.*, 1987). Moreover, some endocrinological modifications, consisting of a reduction in plasma thyroid hormones (Suda *et al.*, 1978; Stockholm *et al.*, 1987) and a probable decrease in sympathetic activity (Landsberg & Young, 1978), could also be partly responsible for the weight-loss resistance usually observed during obesity therapy. However, data concerning the activity of the nervous system during caloric restriction are controversial. It seems probable that in man (James *et al.*, 1981; Scheen *et al.*, 1981) and in the rat (Young & Landsberg, 1977)

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starvation is accompanied by a lowering of the level of adrenergic neuronal activity. Finally, it has also been shown that both the number of β-adrenoceptors and the response to catecholamines are decreased in subcutaneous adipose tissues during fasting (Östman et al., 1984). This latter effect could enhance the antilipolytic action of catecholamines mediated by α_2-adrenoceptors (Berlan & Lafontan, 1985) and heighten the weight-loss resistance. On the whole, these observations support the idea that several adaptive mechanisms preserve energy mobilization by reducing the metabolic rate.

Pharmacological approaches aimed at the improvement of lipid mobilization could have potential interest in the treatment of obesity. Two major approaches may be proposed for enhancement of lipolysis. First, it should be possible to stimulate directly fat cell β-adrenoceptors with β-adrenoceptor agonists (Ricks et al., 1984). The use of a non-specific β-adrenoceptor agonist (e.g. isoprenaline) or physiological catecholamines should be avoided since these agents possess powerful cardiovascular effects (Stanton et al., 1969) and are rapidly eliminated after administration. A selective β_2-adrenoceptor agonist with a high degree of selectivity would theoretically be more suitable in minimising cardiac effects (O'Donnell & Wanstall, 1979). Moreover, β_2-adrenoceptor agonists should be efficient as lipolytic agents since the human fat cell possesses lipolytic mechanisms linked to β_2-adrenoceptors (Mauriège et al., 1988).

The use of α_2-adrenoceptor antagonists may also offer potential interest for lipid mobilizing strategies. These drugs could act by blockade of the antilipolytic α_2-adrenoceptor on fat cell membranes (Arner & Östman, 1976; Fain & Garcia-Sainz, 1983; Berlan & Lafontan, 1985; Mauriège et al., 1987). They could also activate sympathetic tone either through actions on the central nervous system (Papeschi & Theiss, 1975) or indirectly by blockade of the presynaptic α_2-adrenoceptors located on the sympathetic nerve endings (Starke et al., 1975; Langer, 1980) or by a mixture of both actions. These mechanisms could be additive in the improvement of lipid mobilization. This strategy for lipid mobilization has recently been applied by our group in man (Galizky et al., 1988).

The comparative evaluation of lipid mobilization together with endocrinological and cardiovascular responses induced by α_2-adrenoceptor antagonist and β_2-adrenoceptor agonist compounds is of importance for the therapeutic utilization of these drugs. Such a comparative approach is presented in this paper, using the dog as a model. The properties of dog fat cell α- and β-adrenoceptors have been defined in previous studies. In dog adipocytes the pharmacological properties of the β- and α_2-adrenoceptor binding sites were found to be very similar to those defined in human adipocytes (Milavec-Krisman & Wagner, 1978; Berlan et al., 1982; Taouis et al., 1987), although the presence of β_2-adrenoceptors still has not been clearly demonstrated.

The following studies were undertaken. Firstly, to explore the presence of β_2-adrenoceptor subtypes in dog fat cells and to establish the validity of the model. Secondly, to compare *in vivo* the relative potencies of yohimbine, an α_2-antagonist and procaterol, a highly selective β_2-agonist (Yabuuchi, 1977; O'Donnell & Wanstall, 1985) with special attention being paid to their side-effects on the cardiovascular system and to various endocrinological and metabolic indices such as plasma glucose, insulin and catecholamines.

Methods

Adipose tissue was obtained from female mongrel dogs, weighing 15 to 20 kg which had been fasted overnight before omentum biopsy taken immediately after the induction of general anaesthesia by 30 mg kg^{-1} pentobarbitone (i.v.).

Preparation of dog adipocyte membranes

The isolated adipocytes, obtained as previously described (Mauriège et al., 1987), were washed four times in a hypotonic lysing medium to elicit total cell breakage and recover fat cell ghosts. The lysing medium was composed of $MgCl_2$ 3.5 mM, $KHCO_3$ 1 mM, Tris HCl 2 mM, pH 7.5 and the following protease inhibitors: leupeptin (5 μg ml^{-1}), EGTA (3 mM), benzamidine (10 μM) and phenylmethylsulphonyl fluoride (100 μM). Homogenization had to be performed at 20–22°C to minimize trapping of plasma membranes in the coalescing fat cake. Crude adipocyte ghosts were pelleted by centrifugation (40,000 *g*, 10 min) at 4°C, washed twice in the same buffer and pelleted in similar conditions. At the end of the washing procedure, they were suspended in the lysing buffer at a final concentration of 2–2.5 mg protein ml^{-1} and immediately frozen. The membrane preparation was stored at −80°C and generally used within 1 week for binding analysis. Protein was assayed by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Radioligand binding studies

[^{3}H]-dihydroalprenolol was used, as previously described (Mauriège et al., 1987), for the identification of β-adrenoceptor binding sites. Thawed frozen membranes were rehomogenized with four pestle strokes in a Potter homogenizer and washed once

with the buffer used in the binding studies. Binding assays were performed in a final volume of 400 μl as previously described (Lafontan *et al.*, 1983; Berlan & Lafontan, 1985) with only minor modifications. Incubations were carried out at 25°C in a water bath for 20 min under constant shaking at 120 cycles min^{-1}. At the end of the incubation, duplicate 400 μl aliquots of medium were diluted in 4 ml of ice-cold incubation buffer (50 mM Tris-HCl, 0.5 mM $MgCl_2$, pH 7.5) and vacuum filtered immediately through Whatman GF/C glass fibre filters placed on a Millipore manifold. The filters were washed twice with 10 ml portions of ice-cold incubation buffer, placed in minivials containing 4 ml of liquid scintillation medium (Ready-Solv MP, Beckman Instruments Ltd) and counted in a Packard scintillation spectrometer at an efficiency of 40%. Specific binding was taken as the amount of radioactivity bound to the membranes and defined as the difference between the total binding and binding in the presence of 10 μM unlabelled ligand (propranolol). Specific binding ranged between 70–80% of the total binding for [^{3}H]-dihydroalprenolol at a final concentration of 5 nM. For competition experiments, various adrenoceptor antagonists were dissolved in the binding buffer, diluted, and added to the assay (100 μl) just before experimentation.

Lipolysis measurements

The lipolytic activity was analysed on isolated adipocytes prepared according to the technique of Rodbell (1964) with minor modifications. Krebs-Ringer bicarbonate buffer containing bovine serum albumin (3.5%) and glucose (6 mM), adjusted to pH 7.4 with 1N NaOH just before use, was utilized as previously described (Berlan & Lafontan, 1985). After collagenase action (1 mg ml^{-1} in Krebs-Ringer bicarbonate buffer), isolated fat cells were washed three times and the packed cells were brought to a suitable dilution in Krebs-Ringer bicarbonate buffer. The cells were incubated in plastic vials (1 ml incubation medium containing 10,000–15,000 cells) with gentle shaking in a water bath under an air phase at 37°C. After 90 min, the incubation tubes were placed in an ice bath, the adipocytes were separated from the buffer and 200 μl of the underlying fluid were removed for the enzymatic determination of glycerol which was taken as an index of the lipolytic rate. Total lipids were evaluated gravimetrically after extraction. Pharmacological agents were added just before the beginning of the incubation in 10 μl volume of vehicle to obtain the final concentration. The lipolytic activity of the isolated fat cells was tested using isoprenaline (a non-selective β-adrenoceptor agonist) and procaterol (a new highly selective β_2-adrenoceptor agonist) and various selective β_2- and β_1-adrenoceptor antagonists (respectively ICI 118,551, O'Donnell & Wanstall, 1980; and bisoprolol, Kaumann & Lemoine, 1985).

In vivo *studies*

Twelve normal female mongrel dogs (15–20 kg) were used for the *in vivo* studies. Several days before each experiment, the dogs were trained to stand still for 3 to 4 h on a Pavlov table and accustomed to intravenous infusion and blood sampling. Systolic and diastolic blood pressure were recorded by means of a catheter introduced into the abdominal aorta via the left femoral artery under local anaesthesia (xylocaine 5%) and connected to a Gould P23ID transducer on one channel of a Honeywell recorder. Mean blood pressure was measured by automatic electrical integration of systolic and diastolic blood pressure. Heart rate was obtained using a heart period meter triggered by blood pressure. At 09 h 00 min, after 24–36 h fasting, the dogs were placed on the Pavlov table. Another catheter was placed in the antecubital vein for drug perfusion. After that, the dog remained quiet until the beginning of the experiment. Two blood samples were taken before drug infusion (times −15 min and 0 min). Then yohimbine or procaterol were infused for 30 min. Other blood samples were taken 5 min, 15 min and 30 min after the beginning of infusion and every 15 min after the end of infusion.

Blood samples were collected in heparinized tubes and immediately centrifuged; plasma was collected and frozen at −20°C until use. Blood glucose was determined with a glucose-oxidase technique (Biotrol kit, Paris, France) and insulin by radioimmunoassay (commercial radioimmunoassay kit, Institut Pasteur, Paris). Glycerol was determined by an enzymatic procedure and non-esterified fatty acids (NEFA) by the Wako enzymatic method (commercial radioimmunoassay kit, Biolyon, Lyon).

For catecholamine determinations, blood was collected on lithium heparin with 10 mM sodium metabisulphite and centrifuged at 5000 *g* at 0°C; the plasma was stored at −80°C. Catecholamines were selectively isolated from the plasma by adsorption on activated alumina, then eluted with 0.1 M perchloric acid. Dihydroxybenzylamine was used as internal standard to monitor recovery from the extraction step. Catecholamines were assayed by high performance liquid chromatography using electrochemical (amperometric) detection (Waters h.p.l.c. system) (Valet *et al.*, 1988).

Data analysis

All experiments were performed in duplicate. Values are means ± s.e.mean. Student's paired *t* test was

used for comparisons between matched pairs, differences were considered significant when P was less than 0.05. The Mann Whitney U test was used for analysis of unpaired data. The determination of the relative affinity of adrenoceptor compounds for β-adrenoceptor binding sites was carried out by computer-assisted calculations (Barlow, 1983).

Drugs and chemicals

[^{3}H]-dihydroalprenolol (specific activity 75 Ci mmol^{-1}) was obtained from Amersham (Amersham, U.K.). Yohimbine hydrochloride, isoprenaline and propranolol were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.). The following drugs were obtained from the companies mentioned: procaterol hydrochloride (5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril), Warner-Lambert; ICI 118,551 (erythro-DL-(7 methylindan-4-yloxy)-3-isopropylaminobutan-2-ol); (−)-bisoprolol hemifumarate, Merck (Darmstadt, F.R.G.). Collagenase, bovine serum albumin and enzymes for glycerol assays came from Boehringer Mannheim (F.R.G.). All other chemicals and organic solvents were of reagent grade.

Results

In vitro *studies*

Lipolytic studies on dog fat cells The first aim of the study was to determine the properties of the β-adrenoceptor controlling the lipolytic processes in dog fat cells. Comparative lipolytic responses to isoprenaline and procaterol in the same batch of dog fat cells are shown in Figure 1. An increase in glycerol production was observed in the range 10^{-9}–10^{-5} M for the two β-adrenoceptor agonists. The maximal lipolytic action of procaterol was 80–85% of the maximal effect of isoprenaline. Figure 1b shows the dose–response curves with the responses expressed as a percentage of the maximal effect given by each agent at 10^{-5} M. The mean EC_{50} value (agonist concentration required for half maximal stimulation of lipolysis) for procaterol was not significantly different from that of isoprenaline (62.2 ± 21.3 nM and 27.2 ± 13.5 nM, respectively, $n = 5$).

Figure 2 shows the antagonism of isoprenaline with the highly selective β_2- (ICI 118,551) and β_1-adrenoceptor (bisoprolol) antagonists on dog fat cell lipolysis. There is no significant difference in the

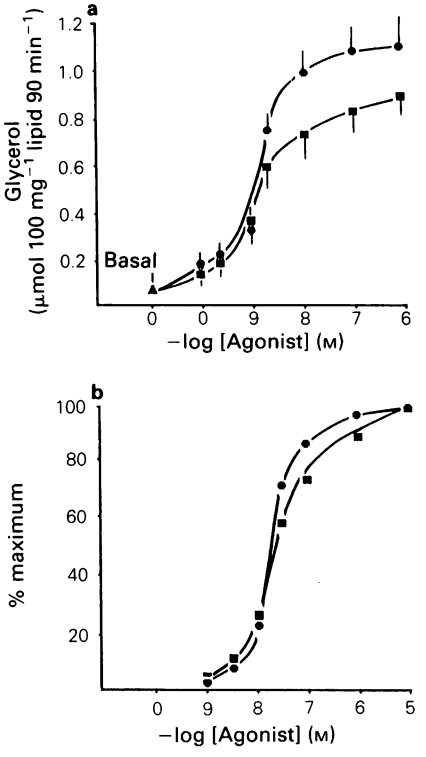

Figure 1 Effect of isoprenaline (●) and procaterol (■) on glycerol release by dog omental fat cells. Fat cells (15–20 mg total lipid) were incubated in 1 ml of Krebs-Ringer bicarbonate solution containing 3.5% bovine albumin and 6 mM glucose as described in Methods. (a) Lipolysis is expressed as μmol of glycerol released by the cells. Values are the mean, with vertical lines indicating s.e.mean, from 5 animals. (b). Lipolysis is expressed as a percentage of the maximal lipolytic response initiated by each agent (10^{-5} M).

potency of the two selective β-adrenoceptor antagonists. The IC_{50}s (concentrations required to suppress 50% of the lipolytic effect of isoprenaline) were both in the micromolar range. Then, to confirm the presence of β_2-adrenoceptors in dog fat cells, we compared the antagonistic effect of these selective β-adrenoceptor antagonists on procaterol-induced lipolysis (Figure 2b). The selective β_2-adrenoceptor antagonist ICI 118,551 was a more potent inhibitor of lipolysis than bisoprolol in such conditions, the IC_{50}s were 86.3 ± 29.6 nM and 36,900 ± 10,600 nM, respectively. This result strongly supports the presence of a β_2-adrenoceptor in dog fat cells. However, it should be noticed that the incubation medium used for lipolysis contains albumin (3.5 g% buffer)

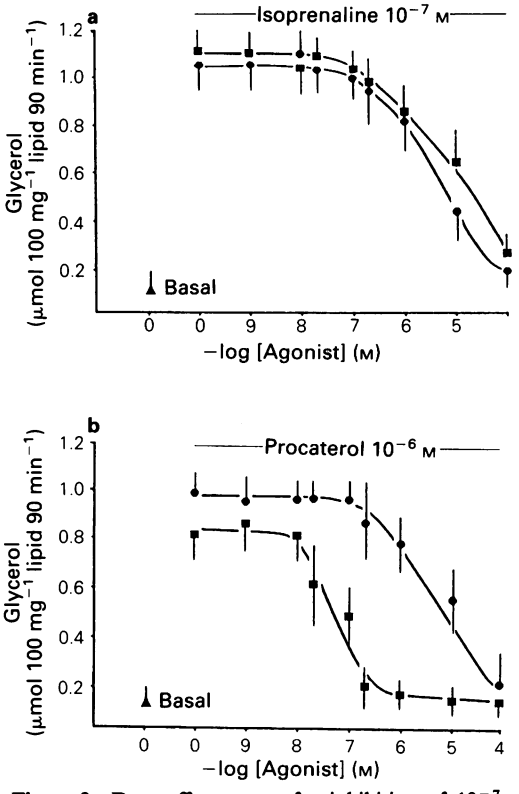

Figure 2 Dose–effect curves for inhibition of 10^{-7} M isoprenaline (a)- or 10^{-6} M procaterol (b)-stimulated lipolysis in the presence of the β_1-adrenoceptor selective antagonist bisoprolol (●) or the β_2-adrenoceptor selective antagonist ICI 118,551 (■). Lipolysis is expressed in μmol of glycerol released by the cells. Values are the mean, with vertical lines indicating s.e.mean, from 5 animals.

and it is not possible to exclude the possibility that adrenoceptor antagonists binding to albumin (or their relative lipid solubility) could introduce some discrepancies in the measurement of their relative affinities. So, in order to improve the characterization of β-adrenoceptor subtypes in dog adipocytes, binding studies were carried out on fat cell membranes.

Comparative binding of specific β_1- or β_2- adrenoceptor compounds on β-adrenoceptors of dog fat cell membranes In order to characterize the mixed population of β_1- and β_2-adrenoceptors in dog fat cell membranes, [^{3}H]-dihydroalprenolol binding competition studies were performed with the selective β_1- and β_2-adrenoceptors antagonists previously used in the lipolysis experiments.

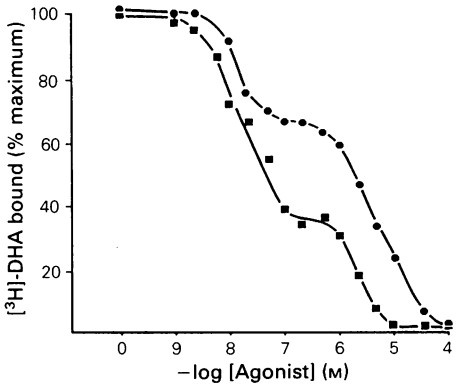

Figure 3 A typical example of the inhibition by either bisoprolol (●) (β_1-adrenoceptor selective antagonist) or ICI 118,551 (■) (β_2-adrenoceptor selective antagonist) of specific [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding to dog fat cell membranes.

The dissociation of [^{3}H]-dihydroalprenolol specific binding (75 to 85% of the total binding) in the presence of 10^{-4} M ICI 118,551 or bisoprolol was rapid in both cases ($t_{1/2}$ for dissociation was 10.7 min and 7.6 min, respectively) and represented a first order process. The calculated first order rate constants for the displacement of 5 nM [^{3}H]-dihydroalprenolol (slope of the semilogarithmic plot calculated by regression analysis) by ICI 118,551 (0.0021 min^{-1}) and bisoprolol (0.0019 min^{-1}) were not significantly different. The displacement studies showed that both ICI 118,551 and bisoprolol suppressed 80–90 of the specific [^{3}H]-dihydroalprenolol binding on dog fat cell membranes.

Typical competition curves for two compounds are depicted in Figure 3. Bisoprolol and ICI 118,551 completely displaced the binding of [^{3}H]-dihydroalprenolol in dog fat cell membranes. The competition curves were shallow and a shoulder was observed between 10^{-8} M and 10^{-6} M. The results clearly showed two competition phases for each compound indicating the presence of two β-adrenoceptor subtypes. The relative proportions of β_1- and β_2-adrenoceptor subtypes were evaluated by a computed-aided curve fitting technique. The results, depicted in Table 1 indicate that the β_2-subtype represents about 60–70% of the total number of [^{3}H]-dihydroalprenolol binding sites whatever the specificity of the β-adrenoceptor antagonist used for subtype definition. In contrast, the non-selective β-adrenoceptor antagonist, propranolol, gave steep displacement curves with a mean K_i of 4.0 ± 0.1 nM and a Hill coefficient of 1; the competition curves could be modelled by a single homogeneous class of binding site.

Table 1 Relative percentages of β_1- and β_2-adrenoceptor binding sites in dog fat cell membranes: characteristics of competition between [^{3}H]-dihydroalprenolol binding and ICI 118,551 (β_2-adrenoceptor antagonist) or bisoprolol (β_1-adrenoceptor antagonist)

	(n)	%	β_1 K_D (nM)	%	β_2 K_D (nM)	*Selectivity ratio*
ICI 118,551	(5)	34 ± 5	3795 ± 640	66 ± 5	44 ± 12	104 ± 25
Bisoprolol	(5)	32 ± 3	74 ± 36	68 ± 3	7724 ± 2490	252 ± 85

%: percentages of β_1- or β_2-sites evaluated from [^{3}H]-dihydroalprenolol competition curves (as indicated in Figure 3). The Selectivity ratio is calculated from the formula: $K_D\ \beta_1/K_D\ \beta_2$ for ICI 118,551 and $K_D\ \beta_2/K_D\ \beta_1$ for bisoprolol. Non-linear analysis of the competition curves by the method of Barlow (1983) yielded the two dissociation constants and the percentages of the receptor subtypes. Each evaluation is the mean ± s.e.mean of *n* experiments.

In vivo *studies*

As expected, a 24–36 h fasting period induced an increase in plasma NEFA concentrations in the dog (283 ± 35 nM and 602 ± 52 nM before and after fasting).

Metabolic and endocrinological effects of yohimbine or procaterol infusion In a preliminary study, we compared the effects of the infusion of various increasing doses of yohimbine and procaterol on plasma NEFA levels in normal fasted dogs. Yohimbine provoked an increase in plasma NEFA concentrations, the maximal effect was obtained with the dose rate of 10 nmol min^{-1} kg^{-1} and persisted after the end of the infusion (Figure 4). The lowest procaterol dosing rate tested (0.08 nmol min^{-1} kg^{-1}) slightly increased plasma NEFA levels only at 5 min after the beginning of the infusion (Table 2) while a higher dose of procaterol (4 nmol min^{-1} kg^{-1}) promoted an increase in plasma NEFA concentrations which was equivalent to that induced by the dose of yohimbine selected for this study (Figure 4).

The time-course of plasma NEFA concentration increments induced by these two agents was different (Figure 4). A significant NEFA mobilizing effect with yohimbine only appeared 15 min after the beginning of the infusion and the maximal effect was reached within 30 min (a longer infusion period was without any additional effect). Moreover, the increment in plasma NEFA levels persisted until the end of the experimental period. However, the lipomobilizing action of procaterol was rapid, the increase in plasma NEFA levels was significant 5 min after the beginning of the infusion and the maximal effect was reached after 15 min. A progressive decrease was observed after cessation of the infusion.

Modifications in various metabolic and endocrinological parameters during and after the infusion periods of the drugs are presented in Figure 4 and Table 2. At the dose used, yohimbine did not significantly modify plasma glucose levels. Plasma immunoreactive insulin concentrations were transiently increased 15 to 30 min after the beginning of yohimbine infusion while post-infusion plasma immunoreactive insulin levels quickly returned towards the

Table 2 Effect of an infusion of procaterol (0.08 nmol min^{-1} kg^{-1}) on plasma non-esterified fatty acids (NEFA) and glucose concentrations, immunoreactive insulin (IRI) and heart rate in the conscious dog

Time (min)	*−15*	*0*	*5*	*15*	*30*	*45*	*60*
NEFA (μM)	802 ± 129	833 ± 136	*938 ± 152	1004 ± 179	780 ± 160	517 ± 131	*507 ± 119
Glucose (mM)	5.5 ± 0.3	5.6 ± 0.3	*6.3 ± 0.4	*7.6 ± 0.5	*8.5 ± 0.8	*7.3 ± 0.6	*6.1 ± 0.6
IRI (μu ml^{-1})	24 ± 6	21 ± 7	*89 ± 46	*155 ± 50	*126 ± 47	41 ± 14	25 ± 8
Heart rate (beats min^{-1})	94 ± 4	95 ± 4	*138 ± 3	*163 ± 11	*175 ± 16	*144 ± 7	*130 ± 8

Values are the mean ± s.e.mean from 5 animals.
* Significantly different ($P < 0.05$) compared to the values measured at time 0.

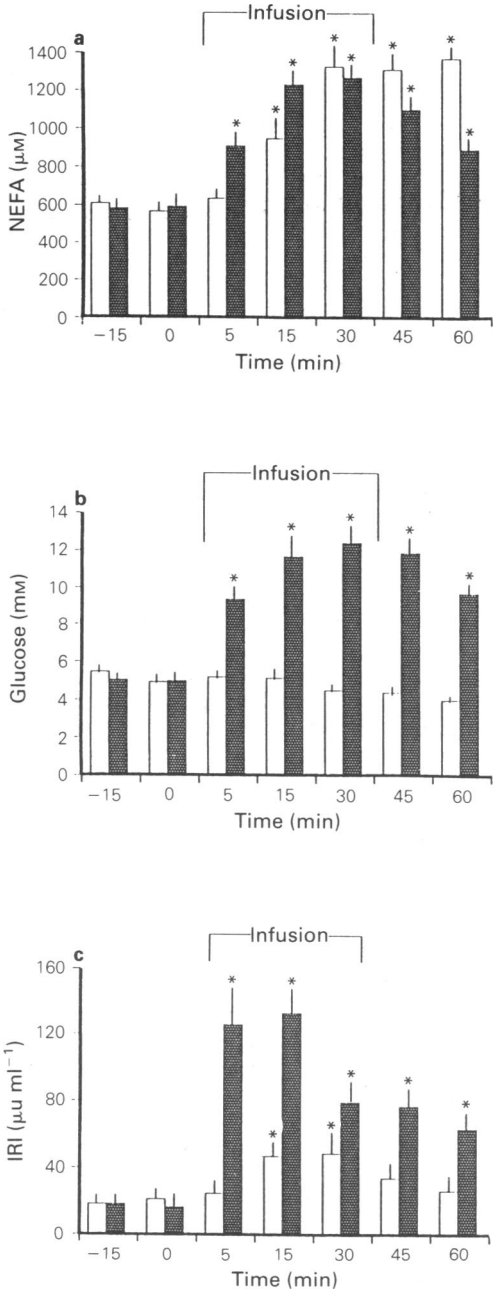

Figure 4 Comparative effects of i.v. infusion of yohimbine (10 nmol min^{-1} kg^{-1}, open columns) and procaterol (0.4 nmol min^{-1} kg^{-1}, stippled columns) on dog plasma levels of (a) non-esterified fatty acids (NEFA), (b) glucose (c) immunoreactive insulin (IRI). Values are the mean, with vertical lines indicating s.e.mean, from 5 animals. * Significantly different ($P < 0.05$) as compared to the values measured before drug infusion.

Table 3 Effect of 30 min i.v. infusions of procaterol or yohimbine on plasma noradrenaline and adrenaline levels in dog

	Noradrenaline (pg ml^{-1})	*Adrenaline* (pg ml^{-1})
Resting values	341 ± 63	188 ± 47
	Procaterol (0.4 nmol min^{-1} kg^{-1})	
A	1065 ± 203*	149 ± 30
B	904 ± 119*	229 ± 26
	Yohimbine (10 nmol min^{-1} kg^{-1})	
A	1349 ± 194*	578 ± 97*
B	1517 ± 258*	755 ± 170*

Resting values: before drug infusion.
A: at the end of drug infusion
B: 30 min after the end of drug infusion.
Values are the mean ± s.e.mean from 6 animals.
* Significantly different ($P < 0.05$) as compared to the values measured before drug infusion (resting values).

pre-infusion values. Plasma noradrenaline and adrenaline levels (basal values: 341 ± 63 pg ml^{-1} and 188 ± 47 pg ml^{-1}, respectively) increased during the infusion period and remained elevated after the infusion was stopped (Table 3).

With regard to the effects of procaterol, an early increase in plasma glucose levels was observed; the hyperglycaemia persisted during the infusion and the effect was dose-dependent (Table 2 and Figure 4). It was noticeable that the lower dose of procaterol increased plasma glucose levels but was ineffective on NEFA mobilization. Procaterol strongly stimulated insulin secretion with the two doses used in this study (500% whatever the dose). Plasma noradrenaline levels increased during the infusion and remained elevated after the infusion. Plasma adrenaline levels, however, were unchanged during the whole experimental period (Table 3).

Cardiovascular effects of yohimbine and procaterol Yohimbine infusion induced a progressive and significant rise in blood pressure until 15 min (Figure 5). The maximal effect occurred at the end of infusion and persisted until the end of the experimental period. The heart rate also increased, maximal values were measured at the end of the infusion.

Procaterol induced a significant and transient decrease in mean blood pressure. The maximal effect appeared at 5 min from the start of the infusion (Figure 5). After that, a return to normal pressure was observed during the rest of the infusion period.

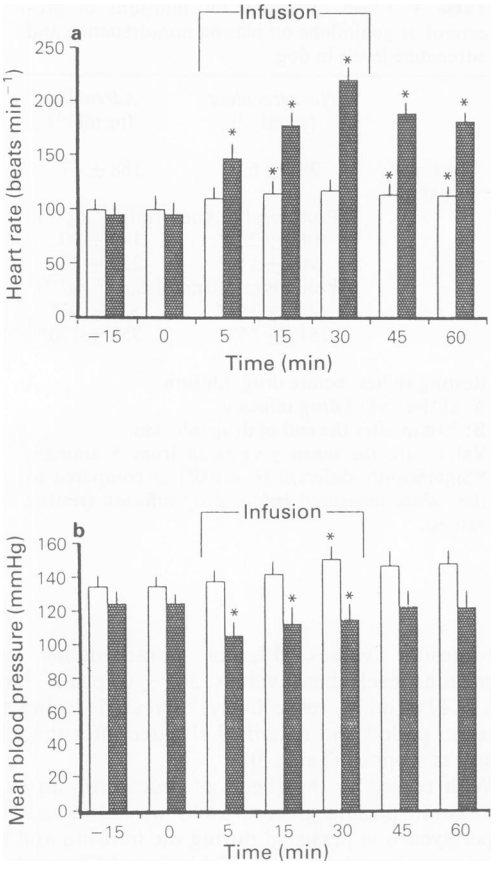

Figure 5 Comparative effects of i.v. infusion of yohimbine (10 nmol min^{-1} kg^{-1}, open columns) and procaterol (0.4 nmol min^{-1} kg^{-1}, stippled columns) on heart rate (a) and mean blood pressure (b). Values are mean, with vertical lines indicating s.e.mean, from 5 animals. *Significantly different ($P < 0.05$) compared to the values measured before drug infusion.

Procaterol induced a strong increase in heart rate which was dose-dependent (Table 2 and Figure 5), the lower dose used in this study provoked an 84% increase while the higher dose induced a 133% increase in heart rate.

Discussion

The present results demonstrate firstly that the dog is a suitable model for the exploration of the lipid-mobilizing effects of various compounds active at adrenoceptors, since the properties of dog fat cell β- and α_2-adrenoceptors (present study and Taouis *et al.*, 1987) are similar to those of man (Lafontan & Berlan, 1980; Mauriège *et al.*, 1988). Secondly, β_2-adrenoceptor agonists and α_2-adrenoceptor antagonists are both useful tools for induction of lipid mobilization. Thirdly, in the dog, the α_2-adrenoceptor antagonist yohimbine has fewer side-effects (endocrino-metabolic, cardiovascular) than the β_2-adrenoceptor agonist, procaterol.

Previous studies on dog fat cell membranes showed that the specific binding of [^{3}H]-dihydroalprenolol, a non-selective β-adrenoceptor antagonist, was saturable and of high affinity. Scatchard analysis defined a population of binding sites which was characterized by an equilibrium dissociation constant (K_D) of 2.2 ± 0.6 nM and a maximum binding of 378 ± 76 fmol mg^{-1} protein (Taouis *et al.*, 1987). The first part of the present work was devoted to the demonstration that dog fat cells possess a typical β_2-adrenoceptor. Lipolytic measurements and binding studies using either a highly specific β_2-agonist procaterol (O'Donnell & Wanstall, 1985; Brodde *et al.*, 1988) or antagonist ICI 118,551 (O'Donnell & Wanstall, 1980) clearly demonstrate the existence of β_2 adrenoceptors in dog adipocytes. The stimulation with the β_2-adrenoceptor agonist enhanced lipolysis with the same efficiency as the non-selective β adrenoceptor agonist isoprenaline. It can be concluded that stimulation of the β_2-adrenoceptor (which constitutes 60–70% of the total population of the β-adrenoceptors) is able to provoke the maximal lipolytic rate in fat cells. The presence of a mixed population of β-adrenoceptors is also supported by the analysis of the dose-response relations of ICI 118,551 and bisoprolol on isoprenaline-induced lipolysis. The antagonistic effect of these drugs only appeared at concentrations around 10^{-6} M when the selectivity of the compounds is lost. The weaker concentrations of each selective compound were without any effect on isoprenaline-enhanced lipolysis. Moreover, in binding studies, bisoprolol and ICI 118,551 displaced 60–70% and 40–30% of [^{3}H]-dihydroalprenolol specific binding, respectively, at concentrations lower than 10^{-7} M. So this last result and previous findings on α_2-adrenoceptors in dog adipocytes (Taouis *et al.*, 1987) led us to conclude that the dog is a valuable model for performing *in vivo* pharmacological studies in this field. The β-adrenoceptors of fat cells of the most commonly used species, the rat, are atypical β-adrenoceptors (Harms *et al.*, 1982; Bojanic & Nahorski, 1983). The adipocytes of other species have generally been less extensively studied (Harms *et al.*, 1982; Mersmann, 1984).

Another important point is that the dog shows early and late endocrino-metabolic adaptations to fasting that are quite similar to those observed in

man. De Bruijne (1979) obtained an increase in NEFA and a decrease in insulin levels in the dog. A concomitant reduction of thyroid function, as reflected by a progressive reduction of plasma triiodothyronine and thyroxine levels during fasting, was also observed (De Bruijne *et al.*, 1981).

Two possible strategies can be proposed with the aim of improving lipid mobilization during fasting or hypocaloric diet. Arner *et al.* (1981) showed that the increased NEFA levels during fasting could be enhanced by an infusion of noradrenaline. However, this strategy cannot be currently used in obesity therapy since noradrenaline induces large increments in systolic and diastolic blood pressures. The use of β-adrenoceptor agonists has also been proposed in order to stimulate fat cell lipolysis and thermogenesis (Henny *et al.*, 1987). An approach based on the use of a selective β_2-agonist is of putative interest in order to minimize side-effects on the cardiovascular system. The recent characterization of β_2-adrenoceptors on human fat cells by our group (Mauriège *et al.*, 1988) supports this idea. The second approach involves the use of α_2-adrenoceptor antagonists which can act directly on fat cell antilipolytic α_2-adrenoceptors and indirectly stimulate lipolysis by sympathetic nervous system activation. This strategy has recently been tested in obese patients by Berlin *et al.* (1986) and Zahorska-Markiewicz *et al.* (1986) but their results exhibit some discrepancies.

The present data demonstrate that yohimbine and procaterol infusions promote an increment of plasma NEFA levels. In the dog, as in the rat (Pfister & Keeton, 1988) and in man (Galitzky *et al.*, 1988), yohimbine enhances sympathetic activity as indicated by the increased plasma catecholamine levels. This effect can be attributed to a peripheral action either on sympathetic nerve endings (blockade of presynaptic α_2-adrenoceptors) or to a central action (Wemer *et al.*, 1979; Hedler *et al.*, 1981), or both. Procaterol increases plasma noradrenaline levels while leaving adrenaline concentration unchanged. This differential action suggests a direct effect of the drug on the presynaptic β-adrenoceptors located on sympathetic nerve endings (Majewski, 1983; Rump & Majewski, 1987); these presynaptic β-adrenoceptors seem to belong to the β_2-subtype (Brodde *et al.*, 1988). The lipid mobilizing effect of yohimbine may be explained through sympathetic activation, α_2-adrenoceptor blockade on fat cells, or both. The effect of procaterol may consist of both direct stimulation of the β_2-adrenoceptor of adipocytes and noradrenaline release.

Thus, the present data indicate both pharmacological approaches are satisfactory with regard to induction of lipid mobilisation. However, the two drugs provoked various endocrino-metabolic side-effects and also altered cardiovascular functions. For a comparison of the undesirable effects of the drugs, we selected yohimbine and procaterol doses inducing equivalent lipomobilization.

Procaterol administration increased plasma insulin levels by acting on the β_2-adrenoceptors located on the pancreatic β cells (Loubatières *et al.*, 1971) and also raised plasma glucose levels. In addition it provoked strong tachycardia. This chronotropic effect of procaterol on the heart could be attributed to a direct effect of the drug on cardiac β_2-adrenoceptors (Hedberg *et al.*, 1980; Johansson & Persson, 1983; O'Donnell & Wanstall, 1985; Arnold *et al.*, 1985). Moreover, it is noticeable that a five fold lower dose of procaterol increases plasma insulin and glucose levels and the heart rate without any major effect on NEFA levels. The drug also induced a weak and transient hypotensive action which was probably counteracted during the infusion by baroreflex tachycardia, by the increase in noradrenaline levels or both. Similar effects have been described in man by Henny *et al.* (1987) with a new type of β-adrenoceptor agonist (RO 16-8714), the selectivity of which is still undefined. Even with the highly selective β_2-adrenoceptor agonist used in the present experiments there is no reduction of side-effects. The strong stimulation of heart rate, the hyperglycaemia and hyperinsulinaemia consequent upon procaterol infusion represent limitations for the use of β_2-adrenoceptor agonists as lipomobilizing agents.

As compared to procaterol, yohimbine had a weaker effect on some of these parameters (its effect on heart rate and plasma insulin levels represent only 14% and 26% of the increase induced by procaterol). Plasma glucose concentration was not modified by yohimbine administration. As opposed to procaterol, yohimbine weakly increased the mean blood pressure in spite of an increment of both catecholamines in the plasma. This lack of major hypertensive effect is not surprising since the blockade of vascular α_2- and also probably of α_1-adrenoceptors by yohimbine (a weakly selective adrenoceptor antagonist) could minimize the vascular responses consecutive to catecholamine release. However, the hypertensive effect could represent a potential limitation for the use of this compound in man. Nevertheless, in a previous study (Galitzky *et al.*, 1988), we showed that acute oral administration of yohimbine ($0.2\,mg\,kg^{-1}$) in normal fasting volunteers increased NEFA levels without any major impact on heart rate or blood pressure.

In summary, these results demonstrate firstly, that the dog fat cell β-adrenoceptors are composed of two subtypes that can be interpreted in terms of classical β_1- and β_2-adrenoceptors, as demonstrated by competition studies conducted with highly selective β_1- and β_2-adrenoceptor antagonists. Secondly, that

both yohimbine and procaterol induce a lipomobilizing effect in the dog. Procaterol stimulates β_2-, while yohimbine blocks α_2-adrenoceptors in fat cells. These effects are enhanced by the activation of the sympathetic nervous system (as indicated by the increase in plasma catecholamine levels). Owing to their side-effects, selective β_2-adrenoceptor agonists (like procaterol) may have major limitations in their use as lipomobilizing agents since they strongly enhance heart rate, insulin secretion and glucose production. In contrast, α_2-adrenoceptor antagonist compounds (like yohimbine) could offer interesting possibilities for the improvement of lipid mobilization since they have limited side-effects.

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