Pharmacokinetics of β-adrenoceptor blockers in obese and normal volunteers

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Aims Obesity can modify the pharmacokinetics of lipophilic drugs. As β-adrenoceptor blockers (BB) are often prescribed for obese patients suffering from hypertension or coronary heart disease, this study compares the pharmacokinetics of lipophilic β-adrenoceptor blockers in obese and control subjects.

Methods Nine obese (157 ± 24% of ideal body weight (IBW) mean ± s.d.) and nine non-obese healthy volunteers (98 ± 10% IBW), aged 32 ± 9 years, were included in the study. Subjects were randomly given a single i.v. infusion of one of the following racemic β-adrenoceptor blockers, whose doses (expressed as base per kg of IBW) were: propranolol (0.108 mg), labetalol (0.99 mg) and nebivolol (0.073 mg). The plasma concentrations of unchanged drugs were measured by h.p.l.c. The ionisation constants and lipophilicity parameters of β-adrenoceptor blockers were assessed.

Results The pharmacokinetic data for the three drugs were qualitatively similar. There was a trend towards a greater total distribution volume (V_{ss}) in obese patients than in controls. However, V_{ss} expressed per kg body weight was slightly smaller in obese patients. The relationship between V_{ss} and lipophilicity of five β-adrenoceptor was studied by combining the current results with those previously obtained with a moderately lipophilic drug (bisoprolol) and a hydrophilic one (sotalol). The V_{ss} of the five drugs was positively and well-correlated ($r^2 = 0.90$; $P < 0.01$) with their distribution coefficient at pH 7.4 ($\log D^{7.4}$), but not with their partition coefficients. The linear regression coefficients for lean and obese subjects were very similar.

Conclusions Lipophilic β-adrenoceptor blockers seem to diffuse less into adipose than into lean tissues. All electrical forms of the drugs (i.e. cations, neutral forms, or zwitterions) present at physiological pH contribute to their tissue distribution, in both obese and lean subjects.Their tissue distribution in obese patients could be restricted by the sum of hydrophobic forces and hydrogen bonds they elicit with macromolecules in lean tissues.

Keywords: β-adrenoceptor blockers, pharmacokinetics, distribution, obese subjects, lipophilicity

Introduction

Obesity is known to modify the distribution and elimination of a number of drugs [1]. The distribution volume of some highly lipophilic subtances such as tradozone, sufentanil and some benzodiazepines, is greater in obese subjects, while their elimination half-life is prolonged. Conversely, the pharmacokinetics of more hydrophilic drugs, such as antipyrine and digoxin is not significantly altered by obesity [2]. These modifications may require dosage adjustements.

β-adrenoceptor antagonists are used to treat systemic hypertension and coronary heart disease, for which obesity is a risk factor. However, there have been few studies on the kinetics of β-adrenoceptor blockers in obese patients. The pharmacokinetic parameters of sotalol, a markedly hydrophilic drug, were similar in obese and lean subjects [3]. The highly lipophilic drug propranolol had a smaller distribution volume in obese subjects than in lean subjects, while for the less lipophilic bisoprolol the distribution volume was similar in obese and lean patients [4, 5]. These results suggest that such drugs diffuse less extensively into adipose than lean tissues, and they seem to contradict findings with other lipid-soluble drugs [2]. Thus, factors other than lipophilicity may also influence the pharmacokinetics of β-adrenoceptor blockers in obese patients.

This study was therefore carried out to determine whether differences in distribution volume were specific to some β-adrenoceptor blockers, and to identify the factors responsible for adipose tissue affinity. The pharmacokinetics of propranolol and of labetalol and nebivolol, two other lipophilic β-adrenoceptor blockers whose pharmacodynamics differ from that of propranolol, were studied in lean and obese subjects. Labetalol is a non-selective β-adrenoceptor blocker, also acting as a moderate α-blocker [6]. Nebivolol is a potent selective β_1-adrenoceptor antagonist [7]. The data of the present study were compared with those of drugs with different lipophilicities, sotalol [3] and bisoprolol

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[5], previously studied under the same experimental conditions. The ionisation constants and lipophilicity parameters were also assessed for all five drugs, in an attempt to derive a physicochemical interpretation for the pharmacokinetic results. In addition, haemodynamic effects of the three drugs were monitored in order to verify that the administered doses were within the effective range.

Methods

Subjects

The study was conducted on nine obese (157 ± 24% of ideal body weight (IBW)); body mass index (BMI) 34.6 ± 5.6) and nine non-obese healthy volunteers (98 ± 10% of IBW; BMI 21.4 ± 2.6). Each group contained four men and five women, including one poor debrisoquine hydroxylator. The normal subjects were aged 32 ± 9 years and the obese subjects 31 ± 9 years. (Table 1).

IBW was defined from life insurance tables as follows: IBW = 'X' kg + 2.3 kg/2.5 cm over 152 cm in height, where 'X' = 45.5 (female) or 50.0 (male) [8]. The percent IBW was calculated as the ratio of total body weight to IBW, multiplied by 100. Body mass index, defined as weight in kg/height^2 in metres, was also calculated. All subjects had normal cardiac, respiratory, hepatic and renal functions. The weight of all subjects had been stable for at least 2 months before the study and the subjects had taken no medication, other than oral contraceptives, for two weeks before entry.

The study was approved by the Ethics Committee of the Saint-Antoine Hospital and each subject gave written informed consent.

Study design

Each subject fasted overnight and remained supine. He/she was given, in random order, a single i.v. dose of β-adrenoceptor blocker: *rac*-propranolol (0.108 mg base kg^{-1} IBW), *rac*-labetabol (0.99 mg base kg^{-1} IBW), or *rac*-nebivolol (0.073 mg base kg^{-1} IBW). Both groups of subjects were given similar total doses (see Table 2). Drugs were infused at 1.81 ml min^{-1}, using an electric syringe, over a period of 5–10 min, depending on the amount given.

Table 1 Subject characteristics (n = 9/group).

	Control	*Obese*
Age (years)	32 ± 9	31 ± 9
Weight (kg)	60 ± 11	99 ± 23
% ideal body weight	98 ± 10	157 ± 24
BMI	21.4 ± 2.6	34 ± 5.6
Creatinine (mmol l^{-1})	80 ± 15	87 ± 12
Blood sugar (mmol l^{-1})	5.0 ± 0.5	5.1 ± 0.8
Triglycerides (mmol l^{-1})	0.8 ± 0.2	1.4 ± 0.9
Cholesterol (mmol l^{-1})	4.6 ± 1.0	4.9 ± 1.1
Phospholipids (mmol l^{-1})	2.8 ± 0.4	3.1 ± 0.6
Albumin (g l^{-1})	43.8 ± 4.5	39.0 ± 6.3
AAG (g l^{-1})	0.8 ± 0.2	0.8 ± 0.2

Data are means ± s.d.

AAG: α_1- acid glycoprotein.

Subjects continued to fast for 3 h after the infusions. Thereafter, a standard breakfast was served and they had a light lunch 5 h after drug administration. Smoking, coffee, tea and alcoholic beverages were forbidden for the day of the study. There was a 2–3 weeks wash-out period between study days. Venous blood samples were collected just before drug infusion and thereafter at 0, 5, 10, 15, 30, 45 min, hourly from 1 to 8 h and at 24, 48 h. Additional blood samples were taken from poor metabolisers at 72 and 96 h post-infusion. All plasma samples were stored at −20° C until assayed.

Heart rate (HR), systolic and diastolic blood pressure (SBP, DBP) were monitored (Dynamaps TM 1846) every 30 min for 8 h once drug administration was completed. Cardiac output (CO) was measured by echocardiography (Diasonics Vingmed CV 700) at rest before and at 0.5, 2, 4 h after drug administration [9].

Drug assays

The plasma concentrations of unchanged *rac*-propranolol, *rac*-nebivolol and *rac*-labetalol were determined by h.p.l.c. with fluorescence detection [10, 11, 12]. The limits of accurate determination were 1 ng ml^{-1} for propranolol, 0.1 ng ml^{-1} for nebivolol and 5 ng ml^{-1} for labetalol. The intra and inter-day coefficients of variation were ranging 8.0–4.3% and 10.2–6.5% respectively for propranolol (plasma concentrations of 1–64 ng ml^{-1}), 8.7–1.0% and 9. 6–3.7% for labetalol (5–400 ng ml^{-1}), 10. 2–5.7% and 8.7–7.0% for nebivolol (1–200 ng ml^{-1}). All plasma concentrations are expressed as drug base.

Nebivolol metabolism is subject to hydroxylation genetic polymorphism, with reduced clearance in poor hydroxylators [13]. Phenotyping was therefore done before inclusion in the study, by giving 40 mg dextromethorphan (DEM) orally and collecting urine for 10h. The urinary DEM and its *O*-demethylated metabolite (DOR) were assayed by h.p.l.c. and the ratio DOR/DEM calculated. Poor metabolizers gave a value < 10 [14]. To avoid a bias, the data from poor hydroxylators were excluded from calculations of nebivolol cardiovascular effects and pharmacokinetics. Parameters were calculated with n = 8/group.

Pharmacokinetic and statistical analysis

The plasma concentrations of the β-adrenoceptor blockers were analyzed by the nonlinear least-squares fitting program SIPHAR® [15]. The following pharmacokinetic parameters were determined: elimination half-life ($t_{1/2,z}$), area under the concentration-time curve from zero to infinity (AUC) by the linear trapezoidal method; total body clearance (CL = dose/AUC); and total distribution volume (V_{ss} = dose. AUMC/AUC^2), in which AUMC is the area under the first-moment *vs* time curve; $V_z = CL/\lambda_z$, in which λ_z is the terminal slope. V was also corrected per kg actual body weight (V kg^{-1}). The pharmacokinetic parameters for the two groups of subjects were compared by Student's t-test and analysis of variance, with a significance limit of $P \leq 0.05$. The same statistical procedures were used to compare baseline values and maximum variations of haemodynamics effects of drugs. A correlation was calculated with the 18

subjects in the study, between distribution volume for of each β-adrenoceptor blocker studied and % IBW.

Ionisation constants and lipophilicity

The same methodology was used for the three β-adrenoceptor blockers of the current study, plus two other (bisoprolol and sotalol) whose pharmacokinetics were previously studied [3, 5]. The ionization constants and lipophilicity parameters of β-adrenoceptor blockers in *n*-octanol/water and in *n*-dodecane/water systems were reexamined at 25 °C with potentiometric techniques (PCA 101, Sirius Analytical Instruments [16, 17]. Some measurements were also performed using centrifugal partition chromatography (CPC) [18]. Details of these techniques can be found elsewhere [19]. The pK_a for sotalol and labetalol were attributed and the pK_a of nebivolol (which is not soluble in water) was determinated by the Yasuda-Shedlowsky method [20] with methanol as co-solvent. The lipophilicity parameters determined or estimated were:

a) The distribution coefficient in the system octanol/water at pH 7.4 (log $D^{7.4}$). The values for sotalol and labetalol were taken from Barbato *et al*. [21]. The values for bisoprolol, propranolol and nebivolol were derived from distribution curves measured with the two-phase titrator, and calculated with a mathematical model taking into account the partition coefficients of both the neutral and cationic forms.

b) The distribution coefficient of the two zwitterionic β-adrenoceptor blockers, sotalol and labetalol, near their isoelectric pH (log P^I).

c) The partition coefficient of the cationic form (log P^+) in the octanol/water system, calculated from distribution curves.

d) The partition coefficient of the neutral forms (log P). Log P in octanol/water was obtained by correcting log D for ionization. It is an expression of hydrophobic interactions and hydrogen bonds between solutes and solvent. The value for labetalol was estimated from the log P^I measured by the Sirius titrator, the equilibrium constant between the neutral and zwitterionic forms measured experimentally ($K_z = 26$), and the difference (2.6) between the partition coefficients of the neutral and zwitterionic forms. The partition coefficient of the neutral form of sotalol cannot be calculated from log P^I because K_z (the equilibrium constant between the zwitterion and the neutral form) is not known.

Log P in dodecane/water was obtained by correcting log D in this system for ionization. It expresses hydrophobic interactions of solutes and solvent. The log D(dod) of sotalol was too low to be measurable by the potentiometric method or by CPC. This value was estimated for labetalol from the distribution coefficient measured by CPC near the isoelectric point (−2.0 at pH = 8.75), the equilibrium constant between the neutral and zwitterionic forms measured experimentally ($K_z = 26$), and an estimated difference (2.6) between the partition coefficients of the neutral and zwitterionic forms. This value was estimated for nebivolol from the partition coefficient of the cationic form measured in dodecane by CPC (−1.87 at pH = 4.0), assuming the same difference between the partition coefficients of the cationic and neutral forms in the octanol/water and dodecane/water systems. The log P of the neutral form cannot be measured directly as it precipitates in the dodecane/water system.

e) The difference, log P(oct) minus log P(dod), which is a measure of the hydrogen-bonding capacity of solutes.

Results

Biochemical data (Table 1)

All subjects had blood parameters for renal and liver functions within the reference values. Mean concentrations were similar in both groups.

The serum triglycerides, cholesterol and phospholipids of obese subjects were slightly higher than in controls, but their serum albumin was lower. The median (range) of the DOR/DEM ratio for extensive hydroxylators (8/9 in each group) was 466 (22–1082) in the control group and 177 (16–3270) in the obese group. The ratios for poor hydroxylators (1/9 in each group) were 0.23 (control) and 0.70 (obese subject).

Pharmacokinetics (Table 2)

Propranolol There was no statistically significant difference between the groups of subjects for V, CL and $t_{1/2,z}$. The correlation, calculated with the 18 subjects, between total V_{ss} and % IBW was positive, but not significant ($r = 0.43$).

Nebivolol The total V_{ss} in obese subjects was significantly higher than in controls ($P < 0.05$), but the V_{ss} corrected for kg actual body weight was similar. The CL in obese subjects was significantly higher than in controls ($P < 0.05$). The $t_{1/2,z}$ of the groups did not differ.

The CL was 14.9 l h^{-1} and the $t_{1/2,z}$ was 34 h in the obese poor metabolizer, while the CL was 17.7 l h^{-1} and $t_{1/2,z}$ 31.9 h in the control poor metabolizer.

There was a positive but barely significant correlation, for the whole subjects, between total V_{ss} and % IBW ($r = 0.462$; $P = 0.05$).

Labetalol The total V_{ss} in the obese group was significantly greater than in the control group ($P < 0.05$). In contrast, the V_{ss} corrected for body weight was not-significantly different in obese patients. The CL and $t_{1/2,z}$ of the two groups were similar. The correlation between V_{ss} and % IBW was significantly positive ($r = 0.643$; $P < 0.01$).

Cardiovascular effects (Table 3)

The basal values of the cardiovascular parameters were within the normal range. The four recorded parameters decreased in response to each β-adrenoceptor blocker with a maximal effect within 1–2 h for BP and HR, and within 0.5–2 h for CO. All effects had ended by 4–5 h. The maximum changes in all parameters were statistically significant for both groups of subjects ($P < 0.001$–0.05). Drugs effects on SBP, DBP and HR were of similar magnitude in both groups of subjects (NS). The decrease in CO was significantly less in obese patients than in control subjects ($P < 0.05$).

Table 2 Pharmacokinetic parameters of the β-adrenoceptor blockers studied.

	Dose (*mg base*)	*AUC* ($\mu g\ l^{-1}\ h$)	*CL* ($l\ h^{-1}$)	V_{ss}		V_z		$t_{1/2,z}$ (*h*)
				(*l*)	($l\ kg^{-1}$)	(*l*)	($l\ kg^{-1}$)	
Propranolol								
Control group	6.7±1.0	161.7±16.6	41.6±6.8	180.0±28.4	3.1±0.7	199.0±34.2	3.4±0.8	3.4±1.0
Obese group	6.7±1.0	154.0±37.4	46.2±10.5	226.8±71.9	2.4±0.9	260.1±94.2	2.7±1.2	3.9±1.2
95% CIs		−20.9∼36.4	−13.3∼4.2	−7.3∼100.9	−0.02∼1.5	−9.08∼131.3	−0.3∼1.7	−0.6∼1.6
Nebivolol								
Control group	4.6±0.7	92.2±22.4	51.6±11.6	673±202	11.2±3.4	761±249	12.7±4.0	10.3±2.4
Obese group	4.6±0.6	69.2±23.4	71.6±17.4★	898±198★	9.4±2.8	994±187	10.4±2.8	10.0±2.6
95% CIs		−1.1∼47.1	4.5∼35.5	14.9∼435	−1.5∼5.1	1.7∼464	−1.3∼5.9	−2.3∼2.9
Labetalol								
Control group	60.7±8.9	777.0±88.4	81.5±14.7	278.6±69.3	4.8±1.7	515.3±138.9	8.8±2.9	4.4±1.2
Obese group	61.9±8.3	731.2±172.5	89.9±10.6	367.9±84.1★	3.8±1.0	590.5±104.1	6.2±1.7★	4.5±0.4
95% CIs		−89.9∼181.5	−4.3∼21.1	12.9∼166	−0.4∼2.4	−46∼197	0.2∼4.9	−0.8∼1.0

Number of subjects/group: $n=9$ for propranolol and labetatol; $n=8$ for nebivolol.
Data are means±s.d.
Difference obese *vs* control subjects. ★: $P<0.05$.
95% CIs: 95% confidence intervals on differences in mean values.

Physicochemical results

Table 4 summarizes the ionisation constants and partition coefficients. The zwitterionic (±) character of labetalol and sotalol was demonstrated unambiguously by the changes in their pKa in the presence of the organic solvent, leading to a different lipophilicity profile. The two compounds exist at pH=7.4 as mixtures of several electrical forms (cation, anion, zwitterion and neutral) because of the proximity of the two pKa.

The results of partition coefficient of cationic forms (log P^+) are comparable to those obtained by Recanatini [22] for other β-adrenoceptor blockers, and confirm the relatively high log P^+ values of these drugs. This implies that the partition of cationic species into the organic phase cannot be neglected when calculating distribution coefficients of drugs at pH=7.4 (i.e., log $D^{7.4}$) from log P and pKa values.

The results of the present study were combined with the data previously obtained for sotalol and bisoprolol [3, 5], to look for correlations between distribution volume and physicochemical parameters. The V_{ss} values for sotalol were 81.0±12.3 in obese subjects and 70.8±11.6 l in controls; those for bisoprolol were 173.0±35.2 (obese) and 146.0±22.5 l (controls).The physicochemical parametres in Table 4 describe a variety of related properties: log P (partition coefficient) for the compounds in a single electrical state, and log D (distribution coefficient) for a mixture of electrical states existing at a given pH (here 7.4). The best relationship found was between the total distribution volume V_{ss} (in l) and the distribution coefficient at pH 7.4 in octanol/water (log $D^{7.4}$) (Figure 1). The correlation, for non-obese subjects (Figure 1a) was:

$$\log V_{ss} = 0.23\ (\pm 0.04)\ \log D^{7.4} + 2.1\ (\pm 0.07)$$

$$n=5;\ r^2=0.89;\ P=0.01$$

The correlation for obese subjects (Figure 1b) was:

$$\log V_{ss} = 0.25\ (\pm 0.05)\ \log D^{7.4} + 2.2\ (\pm 0.07)$$

$$n=5;\ r^2=0.90;\ P=0.01$$

These two equations are statistically identical.

Discussion

Haemodynamic monitoring was carried out in this study for safety reasons and in order to verify that the administered doses were effective. The cardiovascular effects, with significant decreases in BP, HR, and CO are clearly the result of β-adrenoceptor blockade. Their intensity and duration were similar to those previously reported after acute i.v. perfusion of chlorhydrate of propranolol (2–15 mg) [23], or labetalol (1–1.5 mg kg^{-1}) [24], or oral administration of nebivolol (5 mg) [25].

The main objectives of this study were pharmacokinetic, and the results for normal and obese subjects will be discussed separately. The results for the normal volunteers were similar to those reported for propranolol by Castleden *et al.* [26] and Bowman *et al.* [27], and for labetalol by Elliot *et al.* [28] and Abernethy *et al.* [29)]. Some pharmacokinetic parameters were obtained for nebivolol by Van Peer *et al.* [13], but the 10-fold smaller dose used made it difficult to compare their data with the present findings. In the present study, obese patients showed differences in drug distribution compared with non-obese subjects. Total V_{ss} was higher for nebivolol and labetalol, and was positively and significantly correlated with the % IBW. The tendancy was similar for propranolol, but the correlation was not significant. In contrast, V_{ss} expressed per kg body weight was slightly smaller in obese patients. There were similar observations for bisoprolol [5]. When the five β-adrenoceptor blockers, sotalol, bisoprolol, labetalol, nebivolol and propranolol are considered together, general trends become even more

Table 3 Effects of propranolol (PP), nebivolol (NEB) and labetalol (LAB) on systolic (SBP), diastolic (DBP) blood pressure, heart rate (HR) and cardiac output (CO). Basal values (T0) and maximum changes (Δ) are shown for each drug.

	Control subjects						*Obese subjects*					
	PP		*NEB*		*LAB*		*PP*		*NEB*		*LAB*	
	t0	Δ	t0	Δ	t0	Δ	t0	Δ	t0	Δ	t0	Δ
SBP	106.9	−18.7***	110.7	−22.8***	108.8	−22.7***	113.1	−18.4***	113.2	−23.2***	110.9	−18.5***
(mmHg)	(±13.7)	(±5.3)	(±8.0)	(±9.8)	(±11.6)	(±9.9)	(±7.9)	(±9.8)	(±7.9)	(±7.4)	(±7.6)	(±9.5)
DBP	61.5	−12.8***	64.7	−16.7***	64.4	−19.5***	64.1	−11.7***	63.9	−15.6***	63.0	−14.7***
(mmHg)	(±7.2)	(±5.3)	(±7.9)	(±6.8)	(±8.6)	(±7.4)	(±5.0)	(±3.3)	(±9.4)	(±8.6)	(±7.5)	(±7.8)
HR	66.1	−12.7***	63.4	−10.8***	64.4	−8.8**	69.7	−10.9***	71.9	−14.0***	70.7	−6.0**
(beats min^{-1})	(±6.6)	(±5.4)	(±7.3)	(±3.3)	(±7.0)	(±6.6)	(±8.4)	(±6.5)	(±8.8)	(±7.1)	(±8.8)	(±4.5)
CO	4.9	−1.3***	4.9	−1.3***	4.8	−0.6*	5.3	−0.67**	5.2	−0.6***	5.1	−0.2*
(l min^{-1})	(±1.0)	(±0.7)	(±1.1)	(±0.8)	(±1.2)	(±0.7)	(±1.0)	(±0.4)	(±0.6)	(±0.2)	(±0.7)	(±0.2)

Number of subjects/group: $n=9$ for PP and LAB; $n=8$ for NEB.

Data are means ± s.d.

Significance of Δ: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 4 Physicochemical parameters of five β-adrenoceptor blockers. The drugs are arranged in increasing order of log $D^{7.4}$ values.

	Sotalol	*Bisoprolol*	*Labetalol*	*Propranolol*	*Nebivolol*
pKa (base)	9.72	9.57	9.38	9.50	8.22
pKa (acid)	8.28	—	7.44	—	—
log $D^{7.4}$(oct)	−1.50	−0.02	1.09	1.29	2.39
log P^+(oct)	−0.85	−1.22	−0.03	0.38	1.36
log P^I(oct)	−0.44	—	1.14	—	—
log P(oct)	—	2.15	2.6	3.34	3.23
log P(dod)	< −3	−0.24	−0.6	1.34	0.0
Δlog P	>3.5	2.4	3.2	2.0	3.2

●log $D^{7.4}$: distribution coefficient at pH = 7.4.
●log P^+(oct): partition coefficient of the cationic form in octanol/water system.
●log P^I(oct): distribution coefficient near isoelectric pH.
●log P: partition coefficients of the neutral forms in octanol/water (oct) or dodecane/water (dod) systems.
●Δlog P: log P(oct) minus log P(dod).

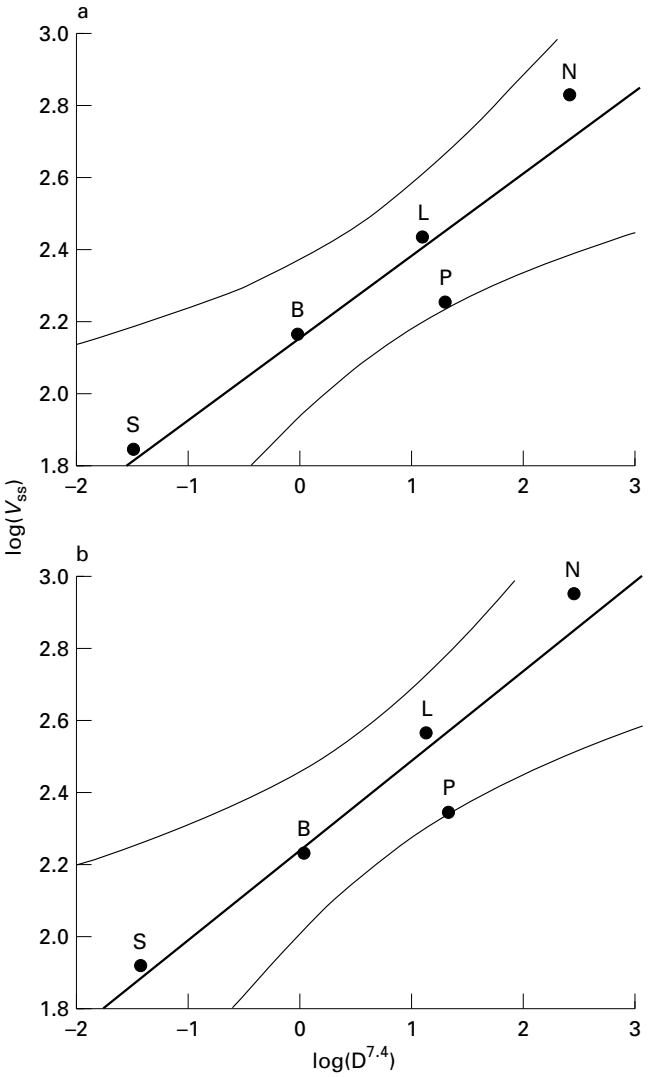

Figure 1 Relationship between the total distribution volume V_{ss} (in l) and the distribution coefficient at pH 7.4 (log$D^{7.4}$) for controls (Figure 1a and Equation 1a) and obese subjects (Figure 1b and Equation 1b). The drugs are sotalol (S), bisoprolol (B), labetalol (L), propranolol (P), and nebivolol (N).

apparent. Indeed, the ratios of distribution volume in obese versus control subjects concerning total V_{ss} (l) or V_{ss}/kg^{-1} body weight ($l\,kg^{-1}$), range from 1.18 to 1.33 and from 0.65 to 0.84, respectively. Thus obese patients appear to have an approximately 24% higher total V and 23% lower $V\,kg^{-1}$ for β-adrenoceptor blockers, than control subjects.

When discussing these data, it must be remembered that obese individuals have a larger absolute amount of lean body mass as well as fat than lean subjects of the same age, height and sex. Forbes *et al.* [30] reported that the lean component of the body in obese patients accounts for 20–40% of the excess weight. Womersley *et al.* [31] showed in a group of young female obese patients, compared with non-obese subjects, that lean weight was higher in absolute terms (51 *vs* 42 kg) but reduced in percentage terms (56 *vs* 75%). In addition, there was almost a two-fold increase in the percentage of fat (44 *vs* 25%). The increase in the total body weight and in the percentage of fat tissue, can explain the significant increase in total V and $V\,kg^{-1}$ in obese patients, observed for lipophilic drugs such as trazodone, sulfentamil and some benzodiazepines, which apparently diffuse mostly in the excess fat. Nevertheless, tissue distribution of other highly lipophilic drugs (e.g., prednisolone and cyclosporine) does not follows this pattern. Their total V is higher in obese patients than in controls, but the $V\,kg^{-1}$ is lower [1]. Similar findings were made in the present study, as in previous publications for propranolol and bisoprolol [4, 5].These results suggest that the lipophilic β-adrenoceptor blockers diffuse less into adipose tissue than into lean tissue. Thus lipophilicity alone cannot account for the pharmacokinetic differences between normal and obese subjects. A combination of biological and physicochemical factors must be involved.

The biological factors which may affect tissue distribution are the binding to plasma protein and the haemodynamic effects of drugs. Propranolol is 90% bound to α_1-acid glycoprotein, and there is no difference in the binding between obese and healthy volunteers [3, 4]. The protein binding of labetalol (50%), bisoprolol (30%) and sotalol (<5%) are too small to affect distribution. Nebivolol is 98% bound to serum albumin in healthy subjects, but no information is available for obese patients. The cardiovascular effects of propranolol, labetalol and nebivolol, in controls compared with obese patients, are similar (BP, HR) or higher (CO). Thus, neither the plasma protein binding nor the haemodynamic effects can explain the limited diffusion of the drugs tested into adipose tissues in obese subjects.

Several relevant ionisation constants and parameters of lipophilicity were measured in order to assess the physicochemical factors influencing distribution. All five drugs examined are strong bases (pKa around 9.5), but labetalol and sotalol are also weak acids (pKa around 8). Thus these drugs exist as a mixture of protonated (mainly) and zwitterionic forms at physiological pH, while bisoprolol, nebivolol and propranolol are mostly (*circa* 99%) cationic. These ionized forms each have distinct partition coefficients (e.g. log P for the neutral forms, log P^+ for the cations), which often proves useful in investigating structure-permeability relationships.

The solvent systems octanol/water and dodecane/water produce different mixtures of intermolecular forces between

solutes and solvents (hydrophobic forces, van der Waals interactions and hydrogen bonds) [32–34]. The difference between log P(octanol) and log P(dodecane) (i.e.Δlog P) is mainly an expression of the hydrogen-bonding capacity of solutes, another physicochemical property known to influence blood-brain barrier or skin drug permeation [35]. Neither log P, Δlog P, nor log P^{+}, was related in a statistically meaningful manner to the distribution volume of the five β-adrenoceptor blockers studied. This indicates that our pharmacokinetic observations cannot be explained by the partition and/or intermolecular interactions of a single electrical form of the drugs.

In contrast, the distribution coefficients at pH 7.4 (log $D^{7.4}$) express the sum of the proportional contributions of the various electrical forms present at physiological pH (i.e. cations and neutral forms, plus zwitterions for labetalol and sotalol). This parameter was well correlated with the distribution volume of the five β-adrenoceptor blockers in both obese and lean subjects. This implies that all electrical forms present at physiological pH contribute to the distribution of these drugs, for which the octanol/water system provides a fair physicochemical model of *in vivo* distribution.

Furthermore the linear regressions between log V_{ss} and log D (Equations 1a and 1b) are identical in control and obese subjects, suggesting that the tissue affinity of β-adrenoceptor blockers is similar in both groups of subjects.

Studies carried out by Bickel [36] shed a new light on these pharmacokinetic data, since his investigations demonstrate that many highly lipophilic drugs having a basic group are not stored *in vivo* in adipose tissue. He concluded that the key factor for storage in adipose tissue is a so-called 'binding competition' between lean and adipose tissue. Hence, storage in adipose tissue is low when binding to lean tissues is high. Consequently, the diffusion of the lipophilic β-adrenoceptor blockers studied could concern both adipose and lean tissues. Their distribution could be restricted and controlled by the sum of the hydrophobic forces and hydrogen bonds they make with lean tissues.

To conclude, the results of this study suggest that lipophilic β-adrenoceptor blockers diffuse less into adipose tissues than into lean tissues. It appears that all electrical forms of the drugs contribute to their tissue distribution, and that their tissue affinity is similar in control and obese subjects.

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(*Received 30 September 1996, accepted 14 February 1997*)

