Receptor occupancy in lumbar CSF as a measure of the antagonist activity of atenolol, metoprolol and propranolol in the CNS

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- 1 The antagonist activity of atenolol, metoprolol and propranolol in the CNS was estimated by determining the extent to which the drugs occupy animal β_1 - and β_2 -receptors in CSF *ex vivo* at the time of lumbar puncture.
- 2 Five CSF and plasma samples were obtained 4 h after drug intake from subjects treated for hypertension with atenolol, 100 mg once daily and five from subjects treated with metoprolol, 50 mg three times daily. Twenty-four samples were obtained 1, 2, 4 or 12 h after drug intake from subjects receiving a single 40 mg dose of propranolol.
- 3 The receptor occupancy in the samples was determined by adding β_1 -receptors of rabbit lung and β_2 -receptors of rat reticulocytes into the samples and labeling the receptors with a nonselective β -adrenoceptor antagonist, (–)-[³H]-CGP-12177.
- 4 Atenolol and metoprolol occupied, as expected, larger fractions of β_1 - than β_2 -receptors in CSF and plasma samples. The receptor fraction occupied by atenolol in CSF was significantly ($P < 0.05$) lower than that occupied by metoprolol. The differences in occupancy between the drugs in plasma, however, were not statistically significant.
- 5 Propranolol occupied larger fractions of β_2 - than β_1 -receptors in the samples. Although propranolol concentrations in CSF were only 1/20–1/40 of those in plasma, the receptor occupancy of propranolol in CSF was similar to that in plasma. The differences in the extent of receptor occupancy at the various time points were not statistically significant (ANOVA for the differences, $P > 0.05$), because of the high variation in the occupancy at 1 and 12 h following the dose.
- 6 The 40 mg dose of propranolol occupied the largest, metoprolol therapy the intermediate and atenolol therapy the least fraction of β_2 -receptors in CSF at 4 h after drug intake. The order for receptor occupancy was thus identical to the reported order for the incidence of CNS side effects.
- 7 Thus, we can quantitate accurately the β_1 - and β_2 -antagonist activity of atenolol, metoprolol and propranolol in CSF. The data on how these drugs occupy animal β -receptors in CSF *ex vivo* may be of value in analyzing their CNS-related effects.

Keywords atenolol metoprolol propranolol lumbar CSF
 β_1 -receptor occupancy β_2 -receptor occupancy

Introduction

β -adrenoceptor blocking drugs produce subtle and vague symptoms considered to be caused by drug action on the CNS (Cruickshank & Prichard, 1988; Dimănes *et al.*, 1990; Patel & Turner, 1981). The main interest has focused on relations between these effects and the lipophilicity of various drugs (Conant *et al.*, 1989), but no clear correlation has emerged (Hoffman & Lefkowitz,

1990). The β -receptor functions in the CNS (Nielson & Vestal, 1987; Patel & Turner, 1981) and the vague symptoms are difficult to assess using the traditional methods of drug evaluation (Dimănes *et al.*, 1990). Therefore the mechanisms of the central side effects of β -adrenoceptor blocking drugs are poorly established.

In this study we evaluated the intensity and selectivity

of the antagonist activity of atenolol, metoprolol and propranolol in the CNS by determining the extent to which the drugs occupy animal β_1 - and β_2 -receptors (Wellstein *et al.*, 1987) in lumbar CSF and plasma following oral drug administration. The extent of receptor occupancy of the agents in CSF was compared with the literature concerning their effects on the CNS.

Methods

Subjects

Patients were admitted to the Department of Neurology at Turku University Hospital for diagnostic examinations including a lumbar puncture. The subjects participated in the study after informed consent. Details of the subjects receiving atenolol or metoprolol are presented in Table 1. Twenty-four normotensive subjects received orally a 40 mg dose of propranolol. Sixteen of the subjects were females with a mean $\pm$ s.d. age of 49 ± 9 years and weight of 55 ± 12 kg. The mean age of the eight males was 52 ± 11 years and their mean weight was 68 ± 16 kg. Subjects with a contraindication to β -adrenoceptor blockers, history of adverse drug reactions, diseases altering significantly pharmacokinetics or the blood-brain barrier were excluded. The protocol was approved by the Ethics Committee at Turku University Hospital.

Protocol

A 100 mg tablet of atenolol (Tenoblock® tbl., Leiras, Turku, Finland) or 50 mg tablet of metoprolol (Spesicor® tbl., Leiras) was administered 4 h before lumbar puncture,

Table 1 Details of subjects receiving atenolol 100 mg once daily (a) or metoprolol 50 mg three times daily (b) therapy for hypertension

Sex	Age (years)	Weight (kg)	WHO drug therapy
a			
1 F	43	63	WHO II t.d.s. 50 mg hydrochlorothiazide
2 M	60	82	WHO II o.d. 50 mg hydrochlorothiazide + amiloride
3 F	33	47	WHO I
4 M	60	72	WHO II
5 M	58	55	WHO II t.d.s. hydrochlorothiazide 50 mg + amiloride 5 mg
b			
1 M	38	99	WHO II t.d.s. hydrochlorothiazide + amiloride t.d.s.
2 F	52	85	WHO II o.d. hydrochlorothiazide 50 mg + amiloride 5 mg
3 M	41	77	WHO I
4 M	52	85	WHO I
5 F	45	71	WHO II o.d. hydrochlorothiazide 50 mg + amiloride 5 mg

The cause of admittance was cephalalgia. WHO I-II refers to the classification of the severity of the hypertensive disease.

which was performed at the level of IV lumbar vertebra. Twenty-four other subjects received orally a 40 mg tablet of propranolol (Reducor® tbl, Leiras, Turku, Finland). They were randomized to four groups ($n = 6$) with the time of drug administration 1, 2, 4 or 12 h before lumbar puncture.

Samples

CSF (4 ml) and venous blood (8 ml) samples were drawn into disposable polypropylene tubes (Sarsted, FRG) containing K_2 -EDTA as anticoagulant and sodium-bisulphite as antioxidant (Rochester *et al.*, 1980); immediately centrifuged for 10 min at 1,000 g; and cell-free CSF and plasma were stored at -60°C until analyzed. Drug-free pools of CSF and plasma for use in affinity determinations were obtained from other subjects at the Department using the same inclusion and exclusion criteria.

Chemicals

The following chemicals were gifts from the corresponding pharmaceutical companies: atenolol, propranolol and ICI 118,551: ICI, Macclesfield, UK. Metoprolol and 4-OH-metoprolol: Hässle, Södertälje, Sweden. (–)-timolol: MSD, Palo Alto, USA. (–)- $[^3\text{H}]$ -CGP-12177 (s.a. 54.6 Ci mmol^{-1}) was purchased from Amersham, Braunschweig, FRG.

β -receptors

A membrane of rabbit lung was used as a β_1 -adrenoceptor in the binding assays. The lung tissue was obtained from three male New Zealand White Rabbits of 3 months age (about 2.0 kg) killed by an overdose of barbiturate. The membrane was prepared as described previously (Brodde *et al.*, 1983; Pauwels *et al.*, 1988) and stored at -60°C at 1 mg ml^{-1} (Lowry *et al.*, 1951) for use in the binding experiment. A purified rat reticulocyte membrane was used as a source of β_2 -adrenoceptors in the binding assays (Wellstein *et al.*, 1984). The reticulocytes were obtained from 10 male Wistar rats of 3 months age (about 200 g) treated with acetylphenylhydrazide. The membrane was prepared as described previously (Kaila, 1991).

K_D values

K_D values (equilibrium binding constants) of (–)- $[^3\text{H}]$ -CGP-12177 (Staehelin *et al.*, 1983) were determined according to the method of Scatchard (1949). Buffer, drug-free lumbar CSF and drug-free plasma were used as assay fluids in order to study their effects on receptor binding. Briefly, the K_D -values for the β_1 -receptors were determined by equilibrating a constant 50 μl aliquot of rabbit lung membrane with 30 nM ICI 118,551 (O'Donnell & Wanstall, 1980) in buffer (Pauwels *et al.*, 1988), CSF and with 150 nM ICI 118,551 in plasma with 10 different concentrations of (–)- $[^3\text{H}]$ -CGP-12177 (10 pM–10 nM). The K_D -values for the β_2 -receptors, were determined by equilibrating a constant 50 μl aliquot of rat reticulocyte membrane without ICI 118,551 with 10 different concentrations of (–)- $[^3\text{H}]$ -CGP-12177. The

binding maximum ($B_{\max}$) of the membrane aliquots were adjusted to be about $7,000 \text{ d min}^{-1}$. Nonspecific binding was estimated by measuring binding in the presence of $1,000 \text{ nM}$ ($-$)-timolol. The assay buffer consisted of 0.100 mM NaCl , 5 mM MgCl and 10 mM Tris-HCl buffer, pH 7.4 at room temperature.

The volume of buffer, CSF and plasma was $200 \mu\text{l}$ and the total assay volume as $300 \mu\text{l}$. The bound and free radioactivity were separated by filtering the samples through Gelman A/E glass fibre filters (Gelman Sciences, Ann Arbor, Michigan, USA) using Brandel M-12 Cell Harvester (Brandel Biomedical Research Instruments, Gaithersburg, Maryland, USA). The radioactivity retained in the filters was counted using Lipoluma and Lumasolve scintillation liquids (Lumac AG, Landgraaf, Netherlands) and LKB/Wallac Betarack 1214 beta-scintillation counter (Wallac Oy, Turku, Finland). The experiments were carried out in triplicate. The best fit for the values was obtained by analyzing the saturation isotherms using a curve-fitting program for binding data, LIGAND (McPherson, 1985; Munson & Rodbard, 1980).

The K_D -values of the radioligand in the rabbit lung and rat reticulocyte membrane in buffer, CSF and plasma are presented at Table 2. The values are in agreement with earlier published data (Pauwels *et al.*, 1988; Staehelin *et al.*, 1983; Wellstein *et al.*, 1984).

K_i values

Apparent affinity constants (K_i values) were determined by displacing the radioligand from receptor sites by

Table 2 The K_D and K_i -values of the compounds for the rabbit lung β_1 -adrenoceptor and rat reticulocyte β_2 -adrenoceptor

	β_1	β_2
K_D -values of ($-$)-[^3H]-CGP-12177		
buffer	0.12 ± 0.01	0.25 ± 0.02
CSF	0.13 ± 0.02	0.26 ± 0.03
plasma	0.14 ± 0.02	0.28 ± 0.03
K_i -values		
Atenolol		
buffer	251 ± 12	$7,620 \pm 370$
CSF	255 ± 19	$7,650 \pm 480$
plasma	274 ± 30	$7,660 \pm 520$
Metoprolol		
buffer	63 ± 4.9	$1,390 \pm 90$
CSF	67 ± 5.4	$1,440 \pm 130$
plasma	72 ± 11.0	$1,520 \pm 180$
4-OH-metoprolol		
buffer	910 ± 61	$39,200 \pm 3,300$
CSF	923 ± 75	$40,100 \pm 3,500$
plasma	948 ± 82	$41,500 \pm 4,200$
Propranolol		
buffer	2.4 ± 0.2	0.72 ± 0.04
CSF	2.8 ± 0.3	0.85 ± 0.06
plasma	35.3 ± 4.7	11.7 ± 1.2

The K_D -values were determined using Scatchard analysis and the K_i -values by displacing 2.1 nM ($-$)-[^3H]-CGP-12177 from the receptor sites in the membranes. The best fit for the values were calculated using the LIGAND program. The values are nM, means $\pm$ s.d., $n = 3$.

Figure 1 The inhibition of 2.1 nM ($-$)-[^3H]-CGP-12177 binding by atenolol, metoprolol, 4-OH-metoprolol and propranolol in lumbar CSF and plasma from the β_1 -adrenoceptors of the rabbit lung membrane. The triangles refer to propranolol, squares to metoprolol, circles to atenolol and diamonds to 4-OH-metoprolol. The closed symbols refer to the inhibition in CSF and the open symbols to the inhibition in plasma. The bars represent the standard deviation of the curve ($n = 3$) calculated using the LIGAND program.

competing drugs. Experiments were performed using buffer, lumbar CSF and plasma as assay fluids to study their effects on the binding. Briefly, we equilibrated atenolol, metoprolol, 4-OH-metoprolol and propranolol at 10 – 12 different concentrations with 2.1 nM ($-$)-[^3H]-CGP-12177 and with a $50 \mu\text{l}$ membrane aliquot of rabbit lung in the presence of 30 nM (in buffer and in CSF) or 150 nM ICI 188,551 (in plasma) for determining the K_i values for β_1 -receptors and, respectively, with a $50 \mu\text{l}$ membrane aliquot of rat reticulocyte membrane for determining the K_i values for β_2 -receptors. The $B_{\max}$ of membrane aliquots was adjusted to about $7,000 \text{ d min}^{-1}$. The total assay volume was $300 \mu\text{l}$ and the volume of drug standards was $220 \mu\text{l}$. The incubation time, separation of bound and free radioactivity and counting of radioactivity were performed as described above in determining the K_D -values. The best fit for the K_i -values was obtained by analyzing the inhibition isotherms using the LIGAND program. Whether the one site model exists in competition between the β -receptor, ($-$)-[^3H]-CGP-12177 and displacing agents was analyzed by studying the linearity of inhibition of binding and the Hofstee transformation of the data. The inhibition curves of atenolol, metoprolol and 4-OH-metoprolol were found to be almost identical in buffer and lumbar CSF. Figures 1 and 2 present the inhibition curves of the competing drugs in CSF and plasma. The respective K_i -values are presented in Table 2. The values are in agreement with values for the compounds in the literature (Brodde *et al.*, 1983; Pauwels *et al.*, 1988; Wellstein *et al.*, 1984, 1985b).

Receptor occupancy

Receptor occupancy was measured by modifying the assay described by Wellstein *et al.* (1985b). In our modification, CSF and plasma samples ($220 \mu\text{l}$) were equilibrated with 0.2 or 1.0 nM ($-$)-[^3H]-CGP-12177

Figure 2 The inhibition of 2.1 nM (-)-[³H]-CGP-12177 binding by atenolol, metoprolol, 4-OH-metoprolol and propranolol in lumbar CSF and plasma from the β_2 -adrenoceptors of the rat reticulocyte membrane. The explanations for the symbols, see the explanations for the Figure 1.

(30 μ l) and with an aliquot of rabbit lung or rat reticulocyte receptor preparation with a $B_{\max}$ about 7,000 d min⁻¹ (50 μ l). The incubation time, separation of bound and free radioactivity and counting of radioactivity were performed as described above in determining the K_D -values. Receptor occupancy as percent was calculated using equations of Williams & Lefkowitz (1978) for receptor binding assays. The ratio of concentration (i) and affinity (K_i) of the competing agent (i/K_i) is obtained by relating the amount of radioligand bound to receptor sites (B_o) and the total amount of receptors in the assay ($B_{\max}$) to the concentration (L) and affinity (K_D) of the radioligand as follows:

$$i/K_i = \left[\frac{L}{K_D} \times \left(\frac{B_{\max} - B_o}{B_o} \right) - 1 \right] \times f$$

The parameter f (= 1,36) in the equation corrects the effect of plasma dilution caused by assay performance. In our experiments L, the concentration of (-)-[³H]-CGP-12177, was 0.2 or 1.1 nM, $B_{\max}$ was about 7,000 d min⁻¹. The K_D -values in plasma are presented at Table 2. The receptor occupancy as percent was thereafter calculated using the ratio i/K_i and the equation (2):

$$2) \text{ Receptor occupancy \%} = 100 \times i/K_i / (i/K_i + 1)$$

The detection limit was defined by the concentration of the displacing agent, which decreased by 10% the specific radioligand binding. Using 0.2 nM (-)-[³H]-CGP-12177 the detection limit was thus about 35% for β_1 - and 23% for β_2 -receptor occupancy of the drugs in plasma. The intra- and inter-assay variation ($n = 10$) in occupancy was in analyzing standards with an occupancy of 35% ($n = 10$) $\pm$ 3% and in analyzing standards with an occupancy of 95% $\pm$ 0.3% ($n = 10$).

In several samples receptor occupancy was below the detection limit of the method. In such cases we approximated the receptor occupancy by dividing the drug

concentration obtained from the RRA (i) by its affinity constant in plasma or CSF (K_i , expressed as ng ml⁻¹) using the values presented at Table 2. Receptor occupancy was then calculated using the equation (2).

Selectivity

Selectivity of the compounds *in vitro* was estimated by dividing the K_i -value (Table 2) for the β_2 -receptor by the K_i -value for the β_1 -receptor. The selectivity of atenolol, metoprolol and propranolol was estimated by dividing the percentage of their β_1 -receptor occupancy by the respective percentage of their β_2 -receptor occupancy.

Drug concentrations

Propranolol concentrations were determined using a radioreceptor assay (RRA) method (Kaila, 1991). Concentrations of atenolol and metoprolol were determined with the same RRA using the rabbit lung membrane with 30 nM ICI 118,551 as β_1 -receptor (Pauwels *et al.*, 1988). The sensitivity of the method for atenolol was 13.2 ng ml⁻¹, for metoprolol 3.8 ng ml⁻¹ and for propranolol 0.08 ng ml⁻¹. Because we did not separate metabolites from their parent compounds before the binding step in the analysis, active metabolites are included in the results, expressed as ng ml⁻¹ of their parent compounds (Kaila, 1991).

Statistical analysis

Drug concentrations are ng ml⁻¹, means $\pm$ s.d. The receptor occupancies are percents, means $\pm$ s.d. Differences in the receptor occupancy between atenolol and metoprolol were compared using paired *t*-test. Differences in the receptor occupancy of propranolol at various time points were analyzed by ANOVA using the time points as categorical variables.

The calculations were performed using SYSTAT programs (Systat Inc, Evanston, Ill., USA).

P values less than 0.05 were considered significant.

Results

Atenolol occupied 4 h after the dose 35–52% of rabbit lung β_1 -receptors in CSF and 80–90% in plasma of subjects receiving continuous treatment. The extent of rat reticulocyte β_2 -receptor occupancy varied between 2–4% in CSF and 11–25% in plasma. Thus, the receptor occupancy of atenolol in CSF was significantly lower than that in plasma ($P < 0.001$). The receptor occupancy of atenolol in the samples is presented in Table 3.

Metoprolol occupied 4 h after the dose 73–89% of rabbit lung β_1 -receptors in plasma and 60–85% in CSF. The extent of rat reticulocyte β_2 -receptor occupancy varied between 12–30% in plasma and between 11–27% in CSF. Thus, the β_1 - and β_2 -receptor occupancies of metoprolol in CSF were significantly higher than those of atenolol ($P < 0.01$), but differences in occupancy between the drugs in plasma were not significant. The receptor occupancy of metoprolol is shown in Table 4.

Table 3 Receptor occupancy and concentrations of atenolol in CSF and plasma 4 h after drug intake

	β_1 -RO (%)	CSF β_2 -RO (%)	ng ml ⁻¹	β_1 -RO (%)	Plasma β_2 -RO (%)	ng ml ⁻¹
1	36	2	40	88	25	453
2	49	3	55	86	17	351
3	52	4	70	80	11	257
4	35	2	36	90	25	492
5	35	2	53	85	13	305
Mean	45	3	53	88	18	372
s.d.	9	1	15	7	7	99

The receptor occupancy was measured using β_1 -receptors of rabbit lung and β_2 -receptors of rat reticulocytes. Atenolol concentrations were determined with a radioreceptor assay (Kaila, 1991), however, using the rabbit lung β_1 -adrenoceptor as the binding site. The abbreviations β_1 -RO and β_2 -RO refer to the β_1 - and β_2 -receptor occupancy of atenolol.

Table 4 Receptor occupancy and concentrations of metoprolol in CSF and plasma 4 h after drug intake

	β_1 -RO (%)	CSF β_2 -RO (%)	ng ml ⁻¹	β_1 -RO (%)	Plasma β_2 -RO (%)	ng ml ⁻¹
1	85	27	106	89	30	141
2	71	18	69	87	27	122
3	75	13	47	73	12	45
4	60	11	39	79	18	64
5	84	26	93	85	25	106
Mean	75	19	69	83	22	91
s.d.	10	7	32	7	7	40

The receptor occupancy was measured using β_1 -receptors of rabbit lung and β_2 -receptors of rat reticulocytes. Metoprolol concentrations were determined with a radioreceptor assay (Kaila, 1991), however, using the rabbit lung β_1 -adrenoceptor as the binding site. The abbreviations β_1 -RO and β_2 -RO refer to the β_1 - and β_2 -receptor occupancy of metoprolol.

The receptor binding of propranolol was similar in plasma and CSF samples. The rabbit lung β_1 -receptor occupancy of propranolol, however, was 1–20% lower than the respective rat reticulocyte β_2 -receptor occupancy. At 4 h after the dose the mean β_1 - and β_2 -receptor occupancies of propranolol in plasma were $81 \pm 6\%$ and $88 \pm 6\%$ ($n = 6$). The respective values in CSF were $67 \pm 5\%$ and $72 \pm 6\%$. Differences between the time points 1, 2, 4 and 12 h following the dose were not statistically significant because variation in the extent of receptor occupancy at 1 and 12 h was high. The time-course of receptor binding of propranolol in plasma and CSF is presented in Figures 3 and 4.

In the preliminary binding experiments atenolol,

metoprolol and 4-OH-metoprolol showed marked β_1 -selectivity. The most selective compound was 4-OH-metoprolol. Atenolol was a degree more selective than metoprolol. Propranolol, in contrast to the other compounds, showed a degree of β_2 -selectivity. Selectivity

Table 5 The concentrations of propranolol in lumbar CSF and plasma following its oral 40 mg dose

Time following the dose (h)	CSF (ng ml ⁻¹)	Plasma (ng ml ⁻¹)
1	0.64 ± 0.49	25.1 ± 30.2
2	3.42 ± 2.83	73.2 ± 43.8
4	1.62 ± 0.84	48.4 ± 32.0
12	0.75 ± 0.52	14.8 ± 15.3

Propranolol concentrations were determined using radioreceptor assay (Kaila, 1991). The values are means $\pm$ s.d., $n = 6$.

Figure 3 The mean β_1 -receptor occupancy of propranolol in 24 subjects following its oral 40 mg dose. The closed triangles refer to the mean receptor occupancy in CSF and the open triangles to the mean receptor occupancy in plasma. The bars represent the s.d. of the parameters ($n = 6$) at each time point.

Figure 4 The mean β_2 -receptor occupancy of an oral 40 mg dose of propranolol. The explanations for the symbols, see the explanations for Figure 3.

Table 6 Selectivity *in vitro*

K_i -ratio, β_2/β_1 :	CSF	Plasma
Atenolol	30	28
Metoprolol	22	22
4-OH-metoprolol	44	44
propranolol	0.3	0.3

The selectivity refers to the difference in the binding affinity between receptor-subtypes.

The differences was defined by dividing the K_i -value for the rat reticulocyte β_2 -receptor by the K_i -value for the rabbit lung β_1 -receptor.

Table 7 Selectivity in subjects

	CSF		Plasma	
	Atenolol	Metoprolol	Atenolol	Metoprolol
1	18.0	3.1	3.5	3.0
2	14.0	3.9	5.1	3.2
3	13.3	5.8	7.2	6.1
4	17.5	5.5	3.6	4.4
5	17.5	3.2	6.1	3.4
Mean	16.5	4.3	5.2	4.0
s.d.	2.0	1.3	1.7	1.2

The selectivity in subjects (1–5) refers to the ratio of the percents between the rabbit lung β_1 -receptor and rat reticulocyte β_2 -receptor occupancy in the samples.

was similar in assay buffer, CSF and plasma. The selectivity of the compounds *in vitro* is presented in Table 6. The selectivity was determined by dividing percentage rabbit lung β_1 -receptor occupancy by percentage rat reticulocyte β_2 -receptor occupancy. Atenolol showed high β_1 -selectivity in the CSF samples. In plasma samples, however, the selectivity of atenolol was markedly less than in the CSF. The difference between CSF and plasma was significant ($P = 0.025$). Metoprolol showed similar selectivity in CSF and plasma samples. The selectivity of atenolol and metoprolol was similar in plasma, whereas in CSF atenolol was more selective than metoprolol ($P = 0.01$). The selectivity of atenolol and metoprolol in CSF and plasma is presented in Table 7.

Table 8 Factors affecting receptor binding of drugs and hormones in clinical samples

- 1 The receptor binding affinity and selectivity of the parent compound
  - 2 Drug concentration
  - 3 Drug binding to plasma proteins or other transport proteins
  - 4 Drug metabolites with affinity to the either of the receptor-subtypes
  - 5 Endogenous factors interfering in receptor-binding, such as autoantibodies against the receptor
  - 6 Stereoselective factors
- Differences in the receptor binding affinity, selectivity, metabolism or disposition between drug enantiomers, diastereomers, or their active metabolites

The list on the factors is adapted from the articles by Wellstein *et al.* (1985a,b) and Gorden & Weintraub (1985).

Propranolol did not show any selectivity in most samples (Figures 3 and 4). The drug showed, however, a degree of β_2 -selectivity at concentrations less than 1 ng ml^{-1} in CSF or 10 ng ml^{-1} in plasma.

The ratios of K_i -values (Table 6) and of β_1/β_2 -receptor occupancy (Table 7) were not comparable parameters, but they highlighted various aspects of selectivity. In analyzing the selectivity of drugs *in vivo* the major factors affecting their selectivity in clinical samples should be remembered (Table 8).

Concentrations of atenolol and metoprolol in CSF and plasma are presented in Tables 2 and 4 and those of propranolol in Table 5.

Discussion

Our study shows that atenolol, metoprolol and propranolol occupy large fractions of rabbit lung β_1 -receptors in plasma and CSF at 4 h after administration to man (Tables 3 and 4, and Figures 3 and 4). The finding is of pharmacodynamic importance, because receptor occupancy is the molecular basis of competitive receptor antagonism (Brown *et al.*, 1976; Williams & Lefkowitz, 1978). The antagonist activity of drugs, based on their K_i values (Table 2; from *in vitro* experiments (Figures 1 and 2) or pA_2 values from isolated organ preparations, reflects poorly antagonist drug activity *in vivo*, because active metabolites, drug binding to nonreceptor sites and enantioselective drug kinetics contribute frequently to the equilibrium between drug and receptor (Wellstein *et al.*, 1985a,b). Therefore, it is necessary to analyze clinical samples to estimate receptor occupancy of various drugs *in vivo*. The estimation of receptor occupancy of drugs *in vivo* by analysing their binding to receptors from animal sources in CSF or plasma samples, however, contains several limitations: Firstly, although β -adrenoceptor blocking drugs ordinarily bind to mammalian β_1 - and β_2 -receptors, receptors with a very similar affinity (Brodde *et al.*, 1983; Pauwels *et al.*, 1988; Wellstein *et al.*, 1985a), differences between species exist. Secondly, although the assay conditions mimicked those existing *in vivo*, they are not identical. Thus, unidentified factors can affect receptor binding of drugs *in vivo*, but not in

samples *ex vivo*. Thirdly, the receptor binding of drugs in samples may reflect poorly their binding in the effect compartment: the site of effect can be separated from the CSF or plasma compartment by diffusional and metabolic barriers, which can decrease or increase drug concentrations at the site of effect (Wellstein *et al.*, 1985a). The role of these factors in each individual case is difficult to evaluate, because the literature on this topic is limited. The liposolubility, pKa and the extent of protein binding govern the rate and extent of penetration of drugs into CSF (Conant *et al.*, 1989; Cruickshank & Prichard, 1988; Patel & Turner, 1981). Studies of lipophilicity and CNS-related effects of various β -adrenoceptor blocking agents, however, have not revealed any clear correlation (Hoffman & Lefkowitz, 1990). Lipophilicity, receptor binding affinity and receptor occupancy of β -adrenoceptor blocking drugs are almost entirely independent from each other (Williams & Lefkowitz, 1978). Therefore, the amount of drug in CSF or other areas of brain predicts poorly the amount of receptors occupied by the agent. If the CNS-related effects are due to blockade of the central β -receptors, as suggested by Frishman *et al.* (1979), the extent of β -receptor occupancy of drugs in the CNS is of crucial value in analyzing the effects.

Atenolol therapy is reported to be associated with a low frequency of CNS-related side effects (Cruickshank & Prichard, 1988; Dimănes *et al.*, 1990; Simpson, 1977; Zacharias *et al.*, 1979). In our subjects, receiving atenolol continuously, about 50% of rabbit lung β_1 -receptors and 3% of rat reticulocyte β_2 -receptors were occupied by atenolol in CSF (Table 2). The β -adrenoceptors are plasma membrane receptors located on cell surfaces and facing the extracellular fluid. Because the molecules appear to move freely between CSF and the brain extracellular water (Matsen & West, 1972), the receptor occupancy of drugs in CSF may be similar to that in the effect compartment, i.e. on the neuronal cells. The low β_2 -receptor occupancy of atenolol in CSF may thus be associated with the low incidence of central side effects. The hypothesis requires, however, further evidence to be proved.

Concentrations of atenolol in plasma measured with h.p.l.c. detecting racemic drug are very similar to those obtained from the RRA analysis detecting receptor-bound drug (Wellstein *et al.*, 1986). The similarity of results has been regarded as evidence that active drug metabolites and enantioselective kinetics do not contribute to the receptor binding and β -adrenoceptor-blocking effects of atenolol in man. Concentrations of atenolol in CSF are reported to be about 1/10 of those in plasma (Cruickshank *et al.*, 1980; Neil-Dwyer *et al.*, 1981; Taylor *et al.*, 1981). Our data (Table 3) agree with these data. Metoprolol is moderately lipophilic and crosses easily the blood-brain barrier (Cruickshank *et al.*, 1980; Dimănes *et al.*, 1990; Neil-Dwyer *et al.*, 1981). In several studies metoprolol has been associated with a higher incidence of CNS-related side effects than atenolol (Dimănes *et al.*, 1990). It has been suggested that differences in central side effects between atenolol and metoprolol could be due to a difference in their central β_2 -adrenoceptor blocking activity. Our study supports that hypothesis, because in our patients metoprolol occupied 19% and atenolol only 3% of rat reticulocyte

β_2 -receptors in lumbar CSF (Table 3 and 4). Whether more extensive β_2 -receptor binding of metoprolol in the CNS is really the cause of reported differences remains to be established.

Impairment of detection of flash fusion frequency following metoprolol administration has been attributed to the action of its 4-hydroxy-metabolite (Gengo *et al.*, 1985). The metabolite (Regårdh *et al.*, 1983) was in our assays of relatively low β_1 - and of very low β_2 -receptor binding affinity (Figures 1 and 2). Hydroxy-metabolites are also likely to be present in lower quantities than their parent compounds in the brain, because the hydroxy-substitute decreases lipophilicity. Thus, 4-OH-metoprolol could hardly produce any action upon the CNS by blocking central β -receptors. The metabolite can, however, produce various effects via mechanisms unrelated to β -receptor function.

The concentrations of metoprolol in CSF were similar to those in plasma (Table 4) in accordance with the literature (Cruickshank *et al.*, 1980; Neil-Dwyer *et al.*, 1981). The data from the earlier studies, however, are not fully comparable, because active metabolites and enantio-selective kinetics (Lennard *et al.*, 1983) can affect the concentrations of metoprolol measured with the RRA. When we analyzed in our laboratory plasma concentrations of metoprolol with h.p.l.c. detecting racemic metoprolol and with the RRA detecting the β_1 -receptor binding components of metoprolol, the concentrations measured with h.p.l.c. were up to 50% less than those measured with the RRA. Thus, metoprolol concentrations in our study are likely to be higher than those obtained by methods measuring racemic drug. It can be assumed, however, that drug concentrations measured with RRA are better correlated with effects than racemic drug concentrations measured with h.p.l.c. Firstly, metoprolol *in vivo* is not a racemic mixture, but consists of variable proportions of its levo- and dextro-enantiomers, which differ greatly from each other in their blocking properties. Secondly, racemic metoprolol concentration excludes the β_1 -receptor binding metabolites of metoprolol, which may contribute to drug effects in some subjects.

Propranolol is highly lipophilic and crosses very easily the blood-brain barrier (Cruickshank *et al.*, 1980; Neil-Dwyer *et al.*, 1981). Therefore, propranolol in plasma equilibrates rapidly with that in the brain following drug administration. The receptor binding of propranolol was extensive following an oral 40 mg dose both in CSF and plasma (Figures 3 and 4). Because propranolol is frequently administered in much higher doses, e.g. 80 mg twice or thrice daily, receptor binding in subjects receiving continuously high drug doses is likely to be near to complete receptor saturation.

The incidence of CNS-related side effects associated with propranolol therapy is reported to be considerably higher than that associated with atenolol (Zacharias *et al.*, 1977) or metoprolol therapy (Cruickshank & Prichard, 1989; Dimănes *et al.*, 1990). The mean rat reticulocyte β_2 -receptor occupancy of propranolol in CSF, at 4 h following a single dose, was 80%, whereas in subjects receiving continuously atenolol or metoprolol the receptor occupancy was 3% and 19%, respectively. The large difference in receptor occupancy could be responsible for the reported difference in the incidence of side

effects. It should be noted, however, that propranolol as well as the other drugs, can produce effects via mechanisms unrelated to β -receptor functions, e.g. by blocking central 5-HT-receptors or by interacting with brain lipids (Conant *et al.*, 1989; Cruickshank & Prichard, 1988; Kostis & Rosen, 1987; Patel & Turner, 1981).

Propranolol concentrations in CSF were only 1/10–1/40 of those in plasma. In spite of that the percentage of receptor occupancy in CSF was similar to that in plasma (Figures 3 and 4). Thus, the ability of propranolol to prevent binding of endogenous catecholamines to receptor sites appears to be similar in plasma and CSF. Similar occupancy in the peripheral and central organs, however, is not necessarily associated with similar inhibition of the catecholamine effect due to differences in catecholamine responses between the organs.

Our data on the relative concentrations of propranolol in CSF and plasma accords with the literature (Cruickshank *et al.*, 1980; Neil-Dwyer *et al.*, 1981; Taylor *et al.*, 1981). Data concerning propranolol concentrations, however, may be affected by active metabolites (Fitzgerald & O'Donnell, 1971; Rochester *et al.*, 1980) and enantioselective drug kinetics (von Bahr *et al.*, 1982), similar to those on metoprolol. Therefore, the concentrations of propranolol in CSF presented earlier in the literature and those presented in our study are not fully comparable.

The ratio of K_i -values (Table 6) determines the difference in binding affinity between receptor-subtypes, whereas the ratio of percents of β_1/β_2 -receptor occupancy (Table 7) determines the selectivity of antagonist activity. The relative role of factors affecting the selectivity of receptor binding of drugs *in vivo* (Table 8) varies from drug to drug. The high selectivity of atenolol in the CSF (Table 7) is caused obviously by its low CSF concentrations (Table 3) and by its low affinity to β_2 -receptors (Table 2). Therefore, atenolol at concentrations occurring

in vivo is unable to occupy a large fraction of β_2 -receptors in the CSF (Table 3). Other factors (Table 8) are probably of little importance, because atenolol lacks active metabolites, is minimally bound to plasma proteins and the kinetics of its enantiomers do not differ from each other (Wellstein *et al.*, 1986). The similar selectivity of metoprolol in the CSF and plasma (Table 7) is related primarily to its similar concentrations in these fluids (Table 4) and to its negligible binding to plasma proteins. Active metabolites are not likely to affect the selectivity of metoprolol, because the selectivity of its most important metabolite (Regårdh *et al.*, 1983) was roughly similar to its parent compound (Table 7). Stereoselective factors (Table 8) may affect the selectivity of metoprolol *in vivo* (Lennard *et al.*, 1983). Our data, however, were not appropriate for analysing the role of those factors in our samples. Propranolol (Table 6) and its metabolites (Fitzgerald & O'Donnell, 1971; Rochester *et al.*, 1980; Wellstein *et al.*, 1985b) are nonselective compounds. Therefore, most of the factors listed in Table 8 cannot affect the selectivity of propranolol *in vivo*. However, because propranolol shows *in vitro* a degree of β_2 -selectivity (Table 6), its receptor binding in clinical samples can show marked β_2 -selectivity at very low drug levels.

In conclusion, the rabbit lung β_1 - and rat reticulocyte β_2 -receptor occupancy of atenolol, metoprolol and propranolol quantitated accurately the amount of their antagonist activity in plasma and CSF samples. Because receptor occupancy measures directly the intensity of competitive receptor antagonism, the parameter may be of value in analyzing the mechanisms of various drug effects in man.

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