Report

Nonlinear Distribution of Atenolol Between Plasma and Cerebrospinal Fluid

Francis M. Gengo,[1,3,4] Susan C. Fagan,[1] L. Nelson Hopkins,[1,2] Debby Wagner,[1] and David P. Schuster[1]

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Long Evans rats were given atenolol doses ranging from 0.27 to 5.4 mg/kg by intraperitoneal injection. Animals were dosed once every 2 hr for a total of five doses. Atenolol concentrations 1 hr after the last dose were measured from simultaneously obtained plasma and cerebrospinal fluid (CSF) samples. CSF concentrations of atenolol were not proportional to plasma concentrations. The ratio of CSF/plasma concentrations was higher (0.33) at lower plasma atenolol levels (<100 ng/ml) than at the higher atenolol plasma levels (0.05) ($P < 0.001$). The relationship between plasma and cerebrospinal fluid atenolol concentrations was best described by the sum of a Michaelis–Menten and linear function. Animals were also given atenolol doses and then subjected to global cerebral ischemia. The relationship of atenolol concentrations from plasma and CSF in these animals was linear, with a constant partition ratio of 0.02. Together these data show that atenolol partitioning between plasma and CSF is nonlinear and possibly an energy-dependent process.

KEY WORDS: atenolol; cerebral distribution; nonlinear pharmacokinetics.

INTRODUCTION

Cerebrovascular permeability to drugs has been shown to be dependent on the octanol:water partition coefficient if drug transfer between plasma and the central nervous system occurs by simple diffusion through an aporous lipid membrane (1,2). Differences in the octanol:water solubility ratio of beta-blockers have been associated with their distribution into the central nervous system (CNS) (3,4). Several authors have suggested that relatively water-soluble beta-blockers which would be predicted to distribute into the CNS less readily should produce lower CNS effects (5–7). However, comparison of the CNS effects produced by beta-blockers that differ primarily in their lipid solubility reveals little difference in CNS pharmacologic effect (8–11), which suggests that sufficient CNS levels are reached by all beta-blockers (12,13).

The CNS penetration of water soluble beta-blockers may not involve solely simple diffusion. The lipophilic beta-blocker propranolol has been shown to be actively sequestered by brain tissue via saturable cytoplasmic binding (15). Pardridge *et al.* (14) suggest that lipophilic amines such as propranolol and lidocaine are actively transported as well as dependent upon cerebral flow and plasma pH. If an active transport process for the CNS uptake of atenolol were operant, predictions of its CNS distribution based on passive diffusion underestimate the CNS atenolol exposure and therefore underestimate its potential to produce CNS effects. Atenolol was chosen as a study compound because of its water-soluble character (octanol:water distribution coefficient, 0.015) and low binding to plasma protein (10%) (14).

The purpose of this investigation is to determine whether the distribution of a water-soluble beta-blocker, atenolol, into the CNS is a simple linear process determined by the plasma concentration or also involves active process as for the more lipophilic amine drugs.

MATERIALS AND METHODS

A total of 40 Long Evans rats weighing between 200 and 250 g was studied. Thirty animals were given five intraperitoneal doses of atenolol as either 0.27, 0.54, 1.35, 2.70, 4.05, or 5.40 mg/kg. Doses were administered every 2 hr over a 10-hr period. One hour after the fifth dose, each animal was anesthetized with ether, blood was collected from the heart, and cerebrospinal fluid (CSF) was collected from the foramen magnum. Blood was centrifuged at 4000*g* for 5 min and plasma was harvested. A sample of cerebrospinal fluid was tested for occult blood and negative samples were frozen along with plasma at −18°C until analysis.

An additional 10 rats were similarly studied. After the final dose, each underwent a procedure to induce cerebral ischemia. Cerebral ischemia was induced using a modification of the four-vessel occlusion model of Pulsinelli and Bri-

[1] Dent Neurologic Institute, Buffalo, New York 14209.
[2] Department of Neurosurgery, State University of New York at Buffalo, Buffalo, New York 14222.
[3] Departments of Neurology & Pharmaceutics, State University of New York at Buffalo, Buffalo, New York 14222.
[4] To whom correspondence should be addressed at Division of Neuropharmacology, Dent Neurologic Institute, 3 Gates Circle, Buffalo, New York 14209.

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erley involving prior occlusion of the vertebral arteries and temporary occlusion of both common carotid arteries (16).

After the final dose, both common carotid arteries were clasped tightly to occlude the carotid arteries. The clasps remained in place for 30 min, while the animals were closely monitored for signs of neurologic deficit. The clasps were then removed, allowing reperfusion of the carotid circulation for 30 min. Cerebrospinal fluid and plasma samples were then collected at the time of sacrifice.

Chromatography. CSF and plasma samples were assayed using a procedure which is a modification of a previously published assay (17). Modifications which enabled analysis of atenolol concentrations included using a mobile phase of 20/1/79 acetonitrile/tetrahydrofuran/water and an excitation wavelength of 225 nm.

Sample preparation differed in that the alkalinized samples were extracted directly into methylene chloride with no protein precipitation step. Back extraction of the methylene chloride into 0.1% acetic acid resulted in fewer interferences than the evaporation of methylene chloride as in the previous assay procedure. Standard curves were prepared daily. Quality-control samples were prepared in bulk and frozen. Aliquots were removed as required and processed with each run. The quality-control samples and some of the standards that were processed for the CSF assay were prepared in normal saline. All of the samples for the plasma assay were prepared in plasma. Day-to-day and within-day variability was less than 8 and 5%, respectively. The limit of detection was 15 ng/ml for the plasma assay and 5 ng/ml for the CSF assay.

Data Analysis. Plasma and cerebrospinal fluid atenolol concentrations were pooled and fitted to an expression where CSF concentrations would be a linear function of plasma concentrations.

$$C_{csf} = C_{plasma} \cdot K_d \quad (1)$$

where C_{csf} is the atenolol concentration in CSF, C_{plasma} is the atenolol concentration in plasma, and K_d is the distribution coefficient. This can be rearranged to yield

$$C_{csf}/C_{plasma} = K_d \quad (2)$$

Plasma and cerebrospinal fluid concentrations were also fitted to an expression where CSF concentrations would be the result of both a linear distribution process and a nonlinear distribution process.

$$C_{csf} = (C_{plasma} \cdot K_d) + \frac{D_{max} \cdot C_{plasma}}{K_m + C_{plasma}} \quad (3)$$

where D_{max} is the maximum distribution capacity of atenolol into CSF by other than simple diffusion. D_{max} is a term in the Michaelis–Menten equation analogous to what has previously been described as the maximal transport capacity (T_{max}). T_{max}, however, is generally reserved for data which provide more information about the direction of discrete transport processes (16). K_m is the plasma concentration where ½D_{max} is achieved. This can be rearranged to yield

$$\frac{C_{csf}}{C_{plasma}} = K_d + \frac{D_{max}}{(K_m + C_{plasma})} \quad (4)$$

All parameters were estimated using nonlinear least-squares regression (PCNONLIN).

RESULTS

Cerebrospinal fluid samples from 3 of the 30 animals in Group 1 were found to contain blood and all data from these animals were eliminated for subsequent analysis. Cerebrospinal fluid and atenolol plasma concentrations from the remaining 27 animals are shown in Table I. Plasma atenolol concentrations resulting from the six different doses ranged from 56.9 to 1532.0 ng/ml. In 10 rats (2 from each study day) plasma atenolol concentrations measured 2 and 4 hr prior to the time of sacrifice were within 5% of atenolol plasma concentrations measured at the time of sacrifice. The ratio of CSF/plasma atenolol concentrations was not consistent over this plasma concentration range. The ratio was statistically higher for plasma concentrations under 250 ng/ml than was

Table I. Atenolol Concentrations

Plasma (ng/ml)	CSF (ng/ml)	Ratio
	Nonischemic group	
56.9	27.7	0.48
66.4	27.8	0.42
74.7	27.3	0.36
91.5	28.5	0.31
92.5	41.2	0.44
92.6	8.2	0.09
97.8	27.0	0.27
101.9	12.0	0.12
130.0	11.2	0.09
146.3	24.7	0.16
160.2	43.4	0.27
199.6	53.7	0.27
204.2	48.3	0.28
210.1	24.9	0.23
257.8	24.9	0.090
425.7	28.7	0.067
439.2	71.6	0.162
507.3	38.5	0.075
519.1	17.1	0.032
542.7	30.7	0.056
639.0	35.3	0.055
648.0	59.0	0.091
652.2	35.9	0.054
765.3	78.8	0.102
951.5	81.3	0.085
1012.8	52.7	0.052
1532.0	59.9	0.039
	Ischemic group	
114	5.0	0.04
137	6.8	0.05
183	9.1	0.05
228	13.7	0.06
380	34.0	0.09
526	47.3	0.09
686	54.8	0.08
886	79.0	0.09
1174	35.0	0.03

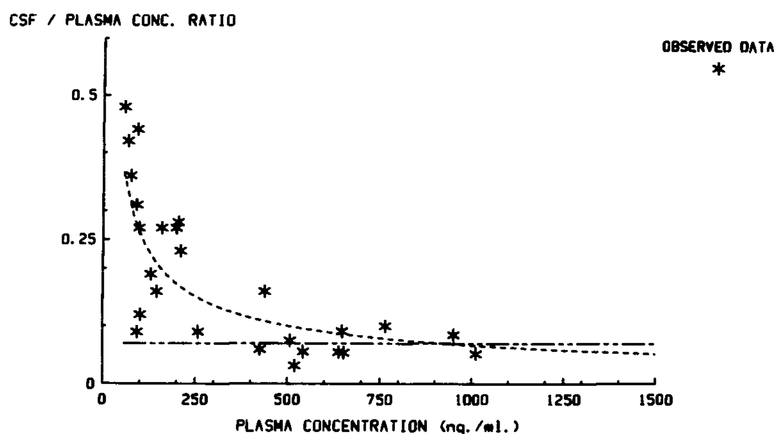

Fig. 1. Atenolol CSF/plasma ratio at various plasma atenolol concentrations. The lines represent estimates based on data fitted to Eq. (1) (——–—) and Eq. (3) (---).

observed for plasma concentrations above 250 ng/ml ($P < 0.001$, two-tailed t test). These data were fitted to Eqs. (1) and (3). The data were not well described by Eq. (1), which yields a K_p estimate (CSF/plasma) of 0.06 ± 0.008 (mean ± SD) and a poor fit ($R^2 = 0.36$) (SSR 15686). These same data were better described by Eq. (3), which yields estimates of K_d, D_{max}, and K_m of 0.02 ± 0.01, 39 ± 17, and 49 ± 70, with a significantly better fit than achieved with Eq. (1) ($R^2 = 0.81$) (SSR 6631) ($F = 2.4, P < 0.05$). The observed data and calculated data based on these estimates are expressed as the CSF/plasma ratio for various plasma atenolol concentrations [Eqs. (2) and (4)] in Fig. 1.

Cerebrospinal fluid and plasma atenolol concentrations from the 10 animals subjected to global cerebral ischemia (Group 2) are also given in Table I. The ratio of CSF/atenolol plasma concentrations is constant over the entire range of plasma concentrations as shown in Fig. 2. These data were well described by Eq. (1), which yields a K_d estimate of 0.02 ± 0.02.

DISCUSSION

The data presented here suggest that the distribution of the hydrophobic beta-blocker atenolol between blood and cerebrospinal fluid is mediated by more than passive diffusion. For passive diffusion to be the only process responsible for atenolol distribution between plasma and CSF, the relationship of CSF to plasma would be well described by a simple linear function. This was not the case. The ratio of CSF:plasma atenolol concentration varied systematically with plasma concentrations. The relationship was well described by the sum of a passive diffusion process and a simultaneous nonlinear distribution process. Further study will be necessary to determine whether the nonlinear distribution is the result of a decreased uptake of atenolol into CSF or an increased clearance of atenolol from CSF at high plasma concentrations.

It is not likely that the present data are the result of non-steady-state conditions, since the plasma and CNS half-lives of atenolol in rats have been reported to be 70–90 min (18) and the dosing period was 10 hr. It is also unlikely that these data are the result of reduced cerebral perfusion or reduced cerebral oxygen consumption secondary to beta-blockade resulting from the atenolol doses that were used (19).

The data reported here are consistent with previously published data for the lipid-soluble beta-blocker, propranolol (14,15). Also, clinical reports for water-soluble betablockers

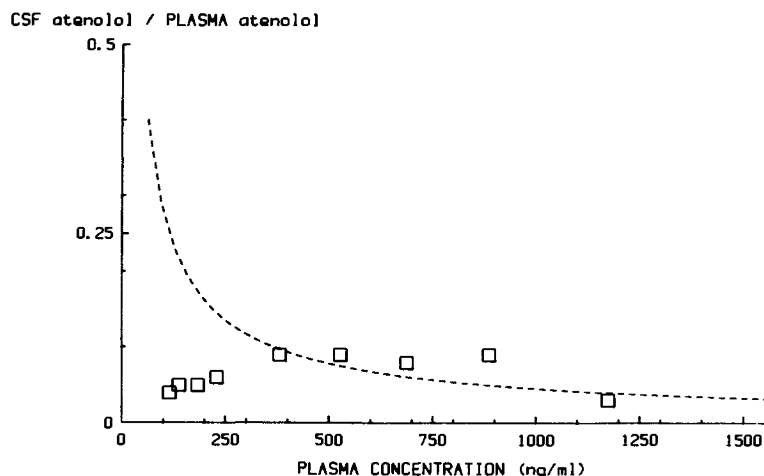

Fig. 2. Atenolol CSF/plasma ratio in animals subjected to cerebral ischemia. The line is the same as shown in Fig. 1 based on data fitted to Eq. (3).

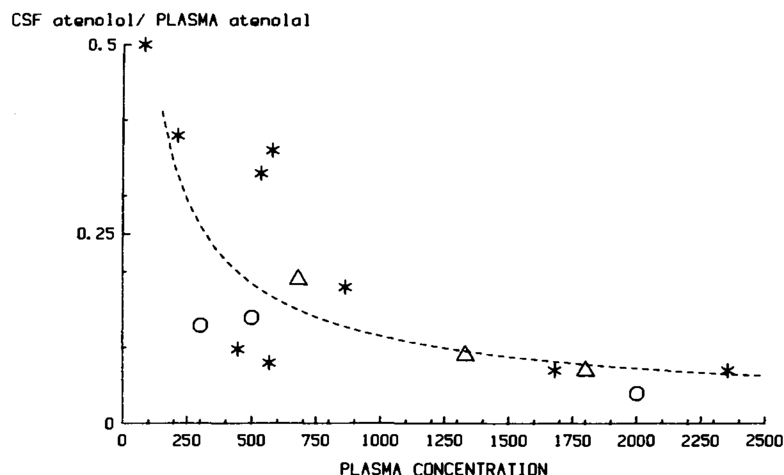

Fig. 3. Atenolol CSF/plasma ratio at various plasma concentrations reported from human studies. The fitted line represents estimated CSF/plasma ratios based on Eq. (4). Data are taken from Refs. 2, 3, and 4.

such as atenolol are consistent with the present findings and show a nonlinear relationship (2–4). Combining data from several published reports, a similar nonlinear relationship between the CSF:plasma atenolol ratio and plasma atenolol concentrations can be demonstrated (Fig. 3), which is well described using Eq. (4). These findings may account for clinical reports showing the CNS actions of water-soluble beta-blockers to be similar to the actions of lipid-soluble agents (8,9).

The nonlinear relationship between plasma and CSF may be an energy-dependent process since cerebral ischemia decreases the CSF:plasma atenolol concentration ratio. The resultant relationship between plasma and CSF atenolol concentrations in the animals subjected to cerebral ischemia was what would be expected by passive diffusion. Although speculative, the data are consistent with the possibility that atenolol uptake into the CSF is an energy-dependent process at lower plasma concentrations and that, under conditions of decreased energy production, only passive diffusion takes place.

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