Renal and Cerebrospinal Fluid Formation Pharmacology of a High Molecular Weight Carbonic Anhydrase Inhibitor

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ABSTRACT

To achieve selective inhibition of cytosolic and membrane-bound carbonic anhydrase (CA II and CA IV, respectively), we synthesized a polymer of molecular weight 3500 from polyoxyethylene bis acetic acid and aminobenzolamide. The new compound, designated F (for Florida) 3500, is stable, water soluble and nontoxic. It is excreted largely unchanged by glomerular filtration. The $K_i$ at 37°C against CA II is 0.14 μM and against CA IV 4.0 μM, some 20 times more than the parent aminobenzolamide and about three times more than acetazolamide or methazolamide. F 3500 does not penetrate red cells and is confined to extracellular fluid. Relations of dose to renal HCO$_3^-$ excretion was studied by i.v. injection into rats. Peak effects were reached with 100 mg/kg, eliciting 40 mM HCO$_3^-$ in urine; this is taken to be the effect of inhibiting CA IV. This compares to a peak of 103 mM HCO$_3^-$ after 3 mg/kg aminobenzolamide, which agrees with previous data on other low molecular weight sulfonamides and defines the effect on CA II and CA IV. We conclude that both isozymes are necessary for normal full renal reabsorption of HCO$_3^-$.

We studied the effect of perfusing F 3500 (100–4000 μM) through the ventriculo-cisternal system of rats on cerebrospinal fluid (CSF) formation. F 3500 was also given intravenously at 100 mg/kg. CSF formation was unaffected. Low MW sulfonamides by either route lowered CSF formation 30 to 50%. It appears either that membrane-bound enzyme (CA IV) is not accessible from the CSF or blood side or that it plays little or no role in CSF formation.

Since the definitive finding of CA in membranes of secretory tissues, i.e., kidney (Maren and Ellison, 1967; reviewed in Maren, 1980) the question has arisen whether the cytosolic (CA II) or the membrane-bound enzyme (CA IV) or both are responsible for the catalysis of the hydroxylation of CO$_2$ which leads to acidification of the urine and reabsorption of filtered HCO$_3^-$.

Closely connected to this problem is that of disequilibrium pH in proximal tubular urine, well discussed in relation to CA by Tinker et al. (1981).

In attempts to solve this question specifically related to renal function and fluid secretion, macromolecular inhibitors of CA have been synthesized, using dextrans of varying molecular weight. In one case amino-dextrans were linked to carbonic anhydrase (CA II and CA IV, respectively), we found that a dextran-bound inhibitor of MW 6700 had a profound effect inhibiting HCO$_3^-$ reabsorption in the proximal tubule of the isolated perfused kidney, no different from the permeant low MW compound acetazolamide. Lucci et al. (1983), using in vivo micropuncture, obtained similar results, comparing dextran-bound inhibitor with the low MW 2-succinylamino-1,3,4-thiadiazole-5-sulfonamide. In contrast, Karlmark et al. (1979) obtained negative results using the dextran linked aminobenzolamide described above. This discrepancy is discussed by Lucci et al. (1983); additionally, it is likely that Karlmark et al. (1979) used an inadequate dose of their polymer, in view of the lower sensitivity of CA IV to all the sulfonamide inhibitors (Maren et al., 1993).

Further evidence on cytosolic vs membrane-bound carbonic anhydrase was adduced in experiments using mice genetically deficient in cytosolic CA II. These mice showed a normal urinary electrolyte output except for higher (by 1 unit) pH and lower titratable acid. Their urinary electrolyte response to 25 mg/kg methazolamide appeared the same as for normal mice (Brechue et al., 1991). Because of inevitable uncertainties in physiological work using mice, the experiments were repeated with further precautions in sampling; now it was found that in the critical analysis of HCO$_3^-$ output, the deficient mice excreted about one-half the amount of the normal (K.D. Hess and W.F. Brechue, unpublished data). The data were not corrected for glomerular filtration rate, but we are left with the tentative conclusion that there is a contribution to renal acid-base balance from both cytosolic and membrane-bound carbonic anhydrase.

ABBREVIATIONS: CA, carbonic anhydrase; CAI, carbonic anhydrase inhibitor; MW, molecular weight; CSF, cerebrospinal fluid.

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We wished to pursue this matter with *in vivo* experiments in rats, which were not possible with the dextran compounds due to histamine release, bleeding, anaphylaxis and general toxicity. Accordingly, we covalently bonded aminobenzolamide to a carboxy derivative of polyethylene glycol, which is widely regarded as nontoxic, giving a high MW carbonic anhydrase inhibitor (CAI) which we call F 3500 (for Florida and its MW).

Two systems were explored: the renal excretion of $\text{HCO}_3^-$ in the intact rat, and the formation of CSF in the rat, measured by ventriculo-cisternal perfusion (Vogh et al., 1987). The latter system has the great advantage of having secretory cells directly exposed to drug solutions. We compared the effects of F 3500 with low MW sulfonamides, aminobenzolamide itself, methazolamide, benzolamide, dorzolamide and the trimethylpyridinium derivative of benzolamide. Work in this laboratory has shown that CSF formation is partially dependent on formation and secretion of $\text{HCO}_3^-$, synthesized from $\text{CO}_2$ and catalyzed by CA (Vogh et al., 1987, and papers cited therein).

![Diagram of compounds](image)

**Materials and Methods**

Benzolamide (CL 11,366) was obtained from the American Cyanamid Co., Pearl River, NY. Aminobenzolamide (CL 13,475) was synthesized by a modification of the method of Vaughan *et al.* (1956). The starting material, 2-amino-1,3,4-thiadiazole-5-sulfonamide, was made by acid hydrolysis of its 2-acetyl derivative, acetazolamide. The amino compound was then reacted with N-acetyl sulfanilyl chloride to give the acetyl derivative of aminobenzolamide that was then deacetylated for 45 min at 100°C in 1 N HCl to give aminobenzolamide. We thank Dr. Margaret Deyrup for making some of the batches. Methazolamide was also obtained from American Cyanamid. Dorzolamide was obtained from Merck Research Laboratories, West Point, PA. The trimethylpyridinium derivative of benzolamide was synthesized by C. Supuran according to Supuran and Clare, 1995.

F 3500 (Fig. 1) was synthesized from aminobenzolamide and polyoxyethylene bis acetic acid (MW 3350, Sigma Chemical Co., St. Louis, MO) using the carbodiimide-linking reaction described fully in Conroy *et al.* (1996). As noted there, the best preparations have a residual molar concentration of 0.015% or about 1 part in 7000 of the original CL 13,475, although commonly there was 10 times this
amount. This estimate was made by analysis of the free aromatic amine, according to the method of Bratton and Marshall (1939), which was modified by increasing the pH of the trichloroacetic/sodium citrate buffer to pH 4 by the addition of 5N NaOH. F 3500 is a water-soluble, waxy crystalline material and due to unreacted COOH groups, is acidic with pH of 3.3. The separation of unreacted CL 13,475 from polymer that had a MW only 10 times that of free drug requires special separation techniques (Conroy et al., 1996). However, the residual molar concentration of free drug is not sufficient to elicit inhibition of CA isoenzymes, in particular CA II, at concentrations of polymer used for inhibition of CA IV.

Dose-response data on HCO₃⁻ excretion for aminobenzolamide and F 3500 were obtained in white female rats (Sprague Dawley, obtained from Harlan Sprague-Dawley, Indianapolis, IN) weighing 250 to 300 g. They were initially anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg). The femoral vein was cannulated for supplemental anesthesia doses and for injection of drug at zero time. At 1 hr a small abdominal incision was made, the bladder drawn out, urine evacuated and the bladder replaced. The same procedure was done at 2 and again at 3 hr. Total CO₂ in urine was determined with the Kopp-Natelson microgasometer using 0.1 ml of fluid. pH was determined with conventional glass micro-electrode. In addition, the renal clearance of F 3500 was studied both in the rat and rabbit (New Zealand White, obtained from Big D Rabbitry, Dade City, FL) after i.v. administration of 25 to 250 mg/kg. All procedures were conducted in facilities accredited by the American Association for Accreditation of Laboratory Animal Care.

CSF flow was measured in anesthetized rats (sodium pentobarbital) during ventriculo-cisternal perfusion with blue dextran, as described in earlier publications from this laboratory (Vogh et al., 1987).

Drug concentrations in tissues, fluids and perfusates were determined using enzyme inhibition in the CO₂ hydration assay. Thirty min after the i.v. injection of F 3500 in the anesthetized rat, tissues were perfused intravascularly through the heart with 750 ml of saline to remove drug from the extracellular tissues. All tissues (5–25 mg) were homogenized in 0.2 ml water and boiled briefly before assay to release bound drug. Choroid plexus was exposed after perfusion experiments by lateral incisions in the brain, rinsed in situ, excised, then briefly washed again in 0.5 ml saline before analysis for drug. Standard curves were constructed using individual CA isoenzymes and authentic inhibitor standards at 0°C using barbital buffer (Maren, 1966; Maren et al., 1990). Equilibrium between enzyme and the drugs were achieved within 4 min of mixing. The I₅₀ for inhibitors is the molar concentration of drug in the assay that halves the catalytic rate of CO₂ hydration. Kᵢ is related to I₅₀ by the relationship Kᵢ = I₅₀ · 1/n EQ, where Kᵢ is the molar amount of enzyme in the assay. Additional Kᵢ measurements were made at 25°C for all inhibitors and these with the 0°C data were extrapolated to 37°C using the Arrhenius relationship (Conroy and Maren, 1995) to obtain Kᵢ values that were physiologically relevant (table 1).

The uptake of F 3500, p-aminohippurate, benzolamide and aminobenzolamide and into kidney slices from the rat was studied at 1 hr at 25°C in 100% O₂ according to the method of Cross and Taggart (1950) as used in Conroy et al. (1996). Drug concentrations were 1 mM and contained 10 mM acetate to facilitate active uptake. The uptake of F 3500 into red cells was measured by incubation for 1 hr of F 3500 (150 μM) with intact canine red cells according to Maren et al. (1961).

Results

Activity against CA; pharmacology of F 3500 and aminobenzolamide. Table 1 shows the activity of F 3500, aminobenzolamide and four other sulfonamides against CA II (cytosolic) and CA IV (membrane-bound) enzyme. As previously reported (Maren et al., 1993), the sulfonamides all appear as less active against CA IV than CA II by some 20-fold (table 1). This also extends also to F 3500. As with previous high MW sulfonamides described in the introduction, the polymer synthesis involves some loss of inhibitory activity, but the resulting compound is still reasonably active even against CA IV (Kᵢ = 4.0 μM) and more so against CA II (Kᵢ = 0.14 μM).

Aminobenzolamide (the ligand for F 3500) is briefly described in Maren (1967). The sulfonamide pK is 9, and the amide proton has pK of 3. Accordingly, its properties are akin to benzolamide, which is the same structure but lacking the p-NH₂ group. Aminobenzolamide is a stable white powder (M.P. = 247–248°C) soluble in water at neutral pH and poorly lipid soluble. F 3500 is a crystalline waxy solid, freely soluble in water and insoluble in most organic solvents (except CH₂Cl₂) and with acidic groups as described above. As noted under Materials, F 3500 contains on a molar basis 0.015 to 0.17% of unreacted aminobenzolamide, varying with individual batches.

The entry of F 3500 into red cells was studied in vitro as previously described for a variety of low MW sulfonamides (Maren et al., 1961). In that study all the compounds completely saturated the red cells. In 1 hr aminobenzolamide was also shown to fully saturate the red cells (about 50 μM corresponding to the CA concentration). Similar experiments with F 3500 yielded 0.6 μM in the red cells. It seems that any drug found may correspond to the penetrance of the trace impurity of aminobenzolamide that would give 0.03 μM in the red cells, when read off the aminobenzolamide curve, or 0.6 μM as noted above when read off the F 3500 curve.

After i.v. injection of F 3500 at 25 to 250 mg/kg into the rat (n = 5) or rabbit (n = 4), the volume of distribution was 190 to 290 ml/kg body weight (rat) and 80 to 150 ml/kg (rabbit), corresponding roughly to the volume of extracellular fluid. These were determined by extrapolating first order decay curves to zero time. The plasma half-life (t₁/₂) is 13 to 30 min (rat, n = 6) and 20 to 35 min (rabbit). From the standard expression for renal clearance, using the means of these data:

\[
\text{Clearance} = \frac{\ln 2 \cdot \text{vol. dist.}}{t_{1/2}}
\]

\[
= \frac{0.7 \cdot 240 \text{ ml/kg}}{22 \text{ min}}
\]

\[
= 7 \text{ ml/min per kg}
\]
This is in the range of glomerular filtration rate in the rat (Smith, 1951). In the rat, clearance was 2 ml/min kg−1; GFR is usually cited as 3 ml/mg kg−1 (Growdon et al., 1971). Over a period of 3 hr (rat), 50 to 75% of F 3500 was excreted as such; no aminobenzolamide (as analyzed for free aromatic amine) was found. This is because the very small amount injected as impurity or formed in vivo is all sequestered in the red cell. That molar concentration of aminobenzolamide in red cells was 0.5 to 3.0 mM from doses of 33 to 250 mg/kg of F 3500. These amounts of aminobenzolamide were 0.17% the molar equivalent of injected F 3500, the same as the molar percent of aminobenzolamide impurity in the polymer.

The distribution in tissues of rat was measured 30 min after i.v. injection of 100 mg/kg of F 3500 (see “Materials and methods” for drug analysis). The concentration of F 3500 in plasma was 46.6 μM. By contrast very low levels of drug were found in washed tissues, consistent with the inability of F 3500 to cross membranes. The concentrations were (μM): lung 0.6, muscle 0.6, kidney 2.6, liver 3.6, spleen 0.3, heart 1.9, brain 0.5 and red cells <0.7. Clearly, F 3500 is excluded from intracellular fluid. It should be noted this small accumulation may represent the component of free aminobenzolamide in the polymer, in which case the concentration values would be 10 times lower, reflecting the difference in the molecular weight of the two compounds.

F 3500 appeared nontoxic after i.v. injection to rat and rabbit in doses as high as 250 mg/kg. Aminobenzolamide itself was studied in rat after intravenous injection of 3 to 6 mg/kg. The volume of distribution was initially ~400 ml/kg and later nearly 1000 ml/kg. This is not understood. The t1/2 was variable, averaging about 40 min. Renal clearance was about 4 ml/min, somewhat less than GFR (Smith, 1951). In the rabbit 10 mg/kg yielded volumes of distribution of 220 ml/kg, t1/2 of 20 min, and clearance of 8 ml/min.

Renal HCO3−. The hallmark of renal CA inhibition, as is well known, is HCO3− excretion. After a full dose of a classic inhibitor, such as acetazolamide or ethoxzolamide, renal HCO3− increases from virtually 0 to 10 to 20 μEq/min per kg body weight. This is essentially the same in all mammalian species examined: mouse, rat, rabbit, dog, man (Maren, 1969). The corresponding HCO3− concentration in urine is 100 to 200 mM.

Figure 2 shows the dose-response relations between aminobenzolamide or F 3500 and urinary HCO3− concentration in the rat. The peak effect for the former, achieved at 3 mg/kg, is 105 mM HCO3−, which corresponds to excretion rate of 20 μeq/min per kg at urine flow of 0.2 ml/min kg−1 (not shown) and is the same effect as the “classic” inhibitors as given just above. The peak effect for F 3500, achieved at 100 mg/kg, is considerably less, 40 mM, corresponding to 7 μEq/min per kg (not shown). Comparing the least doses for full effects, the small molecule (on weight basis) is 33 times as active. This may be compared with 12-fold difference in Ki in vitro against CA II and 28 fold difference in vitro against CA IV (table 1).

Our most significant finding is that what appears to be complete inhibition of the membrane-bound enzyme (by F 3500, with full access to luminal surface) produces only 40% of the effect observed with inhibition of all renal CA, membrane-bound and cytosolic. Presumably, the two enzymes work in concert; we discuss this in the “Discussion.”

CSF flow. In this system, the choroid plexus is exposed to the drug perfusate. Flow is measured by the dilution of a marker in the perfusate. In the anesthetized rat, CSF flow is 2.80 ± 0.12 μl/min, as measured by ventricular-cisternal perfusion (Vogh et al., 1987). Table 2, in agreement with earlier work, shows that a high i.v. dose of methazolamide reduces this to 1.6 μl/min. F 3500 at 100 mg/kg had no effect by this route. Of the five drugs tested in the CSF perfusion system, benzolamide, dorzolamide and the trimethylypyridinium adduct of benzolamide were all effective when perfused at 10−4 M but not at 10−5 M. Methazolamide showed modest activity at 10−3 M. Some details of these tests are given below.

To our surprise, F 3500 was ineffective, even at the high concentration (4 × 10−3 M) used. This is 1000× the Ki for CA IV at physiological temperature. No drug was found in red cells or brain tissue, and virtually all of perfused drug was recovered in the outflow perfusate. The choroid plexus contained 5.3 μM of drug as aminobenzolamide, which reflects the aminobenzolamide impurity (see “Materials and materials”) which had been actively taken up. We make the assumption, from our studies of F 3500, that this compound does not enter the cytosol. Using the Ki of 0.012 μM for CA II and 0.14 for CA IV (table 1) and the standard relation between fractional inhibition and free drug (taken as 5.3 μM) present,

\[ i = \frac{I_{\text{free}}}{I_{\text{free}} + K_i} \]

it is calculated that inhibition (i) is about 0.998 for CA II and 0.973 for CA IV. We shall return to the interpretation of this in the “Discussion.”

Methazolamide was effective at 10−3 M but not at lower concentration. It has relatively low activity against both CA II and CA IV (table 1). This compound is not actively taken up by secretory cells (Maren, 1969), and table 2 shows a low concentration in choroid plexus, compared to dorzolamide and benzolamide. We observed similar low or no activity.
when this drug was tested topically for lowering of IOP (Maren et al., 1987). After the \(10^{-3}\) M perfusion, 50 \(\mu M\) was found in the red cells, which represents 22% of the administered dose.

Benzolamide was effective at \(10^{-3}\) to \(10^{-4}\) M but not \(10^{-5}\) M in the perfusate, which is readily explained by its uptake into the choroid plexus and high activity against the enzymes. At \(10^{-3}\) M the choroid plexus contained 127 \(\mu M\) and at \(10^{-4}\) M, 11 \(\mu M\). This agrees with the uptake of benzolamide in kidney (Maren, 1967) and previous work on CSF flow (Broder and Oppelt, 1969). This value of 127 \(\mu M\) in choroid plexus, using \(K_i\) from table 1, generates fractional inhibition (\(i\) in above equation) well over 0.99 (or 99%) for both enzymes.

Dorzolamide was very effective at \(10^{-4}\) M but not at \(10^{-5}\) M. This is expected, in view of its activity in lowering flow of the aqueous humor when administered topically (reviewed by Maren, 1995). Table 2 shows a concentration of 13 \(\mu M\) after \(10^{-4}\) M infusion in the choroid plexus, comparable to 10 \(\mu M\) in the ciliary process when 1 drop is applied to the cornea at 55 mM resulting in a lowering of eye pressure (unpublished data from this laboratory). As for benzolamide, \(i\) against both enzymes is well over 0.99.

The quaternary compound trimethylpyridinium benzolamide is also effective at \(10^{-4}\) M in lowering CSF flow (table 2). As with benzolamide, its high degree of ionization prefers active uptake into secretory cells, and it shows good activity against the enzymes (table 1). The uptake into kidney and other secretory tissues of completely ionized sulfonamides (quaternary compounds) and highly ionized ones such as benzolamide should be recognized and evaluated. In these situations inhibition is not restricted to CA IV. Compounds of this nature achieve inhibition of both membrane and cytotoxic enzymes.

Because of the presence of free carboxy groups that confer charge to F 3500, its active uptake and that of aminobenzolamide was studied in the rat kidney. Aminobenzolamide gave a slice to medium ratio of 2.4, typical of charged anions (Despopolos, 1965), but F 3500 gave a S/M ratio of 0.6 which indicates no active uptake.

Table 2 also shows brain concentrations in some of the experiments. The only i.v. treatment that yielded inhibitory amounts was methazolamide, attesting to the ready diffusibility of this drug (Maren, 1967). By the perfusion route, \(10^{-3}\) M benzolamide and dorzolamide yielded small concentrations in brain, compared to that in perfusion fluid or choroid plexus.

### Discussion

This section is divided into three parts, corresponding to the "Results," covering pharmacology of F 3500; renal carbonic anhydrase inhibition and effects of ventriculo-cisternal perfusion of F 3500 and other sulfonamides on CSF flow.

**Pharmacology of F 3500.** It is of particular importance that this compound is nontoxic. Unlike previous high MW CA inhibitors (Tinker, et al., 1981), it can be administered systematically. Judged by work in rabbit and rat, its pharmacological properties are akin to those of inulin (MW 5000). In the rabbit, F 3500 does not enter red cells, is distributed in extra-cellular fluid, has a renal clearance of 4 to 8 ml/min and a plasma \(t_{1/2}\) of 20 to 40 min. The low concentrations of the drug in tissues bear out these findings. About 50 to 75% of administered drug is recovered unchanged in the urine. No aminobenzolamide was found in the urine after F 3500.

As a carbonic anhydrase inhibitor, F 3500 shows good activity, albeit 12-fold less potent than the parent aminobenzolamide against CA II measured at 37°C (table 1), 20-fold at 0°C (not shown). It is not surprising that in the larger molecule there is some hindrance to the mobility of the \(-\text{SO}_3\text{NH}_2\) group with respect to attachment to the critical Zn site in the center of the molecule. However, activity in the \(\mu M\) range of dissociation constants should be adequate, with large doses, to yield pharmacologic results if the compound has access to the secretory tissue.
Renal $\text{HCO}_3^-$ excretion. Full doses of low MW CA inhibitors produce urine of 100 to 200 mM $\text{HCO}_3^-$ in all mammalian species (Maren, 1967; 1969). Figure 2 shows that this is produced by 3 mg/kg aminobenzolamide in rat; a higher dose has no further effect. By contrast, the peak effect for F 3500 (requiring 100 mg/kg) is only 40 mM $\text{HCO}_3^-$. These results differ from those of Tinker et al. (1981) and Lucci et al. (1983) who found no difference between effects of dextran-bound inhibitors and acetazolamide in isolated perfused kidney or in vivo micropuncture. However, our experiments are the first using the intact animal.

This agrees in principle with the findings cited previously on mice lacking cytosolic enzyme. In the second experiment, methazolamide elicited only half of the normal effect on renal mice lacking cytosolic enzyme. In the second experiment, first using the intact animal. Lucci et al. (1983) had also postulated, on the basis of micropuncture pH measurements using a dextran-bound sulfonamide inhibitor (MW 6000) and its low MW precursor, a 2-fold role for carbonic anhydrase: mediation of proton formation in the cytosol and bicarbonate dehydration leading to $\text{CO}_2$ reabsorption in the lumen. The basis for this was a proximal pH disequilibrium after the low MW compound but no such effect with the high MW compound. Inexplicably, however, in that study and differing from our results, low and high MW drugs gave the same inhibition of $\text{HCO}_3^-$ reabsorption. This is possibly explained by instability of the dextran-bound inhibitor (given at very high concentration) releasing the potent and permeable low MW sulfonamide (Geers, et al., 1985).

CSF. The original purpose of these experiments was to use F 3500 to distinguish between effects of CA inhibition in cytosol and in membranes. It was expected that a ventriculocisternal perfusion of F 3500 as high as $4 \times 10^{-8}$ M in contact with membranes of choroid plexus would have a pharmacological effect in reducing flow, as do other inhibitors (table 2); this was not observed. As was the case with red cells and tissues, the small amount of drug detected in choroid plexus (5.3 μM) was assumed to represent aminobenzolamide that had been actively uptaken from the CSF perfusate. By comparison with other sulfonamides of similar potency (tables 1 and 2) which do reach the cytosol from blood or CSF side, this concentration is not adequate to inhibit the choroid plexus cytosol to the extent of lowering flow. Indeed such was not observed, despite the fairly high degree of inhibition (1), as shown above. Thus we are left with the dilemma: either F 3500 does not reach the secretory site in the membranes or the membrane component is unimportant in the secretory process in this tissue. Clearly, bathing the tissue with the high concentrations ($4 \times 10^{-3}$ M, $1000 \times K_i$ for CA IV) of F 3500 is ineffective.

It is significant that i.v. F 3500 at 100 mg/kg also fails to

### Table 3

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<th>[HCO$_3^-$] concentration in kidney (mM) and urine; effect of full and luminal CA inhibition$^a$</th>
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<td>$\text{HCO}_3^-$ is progressively reduced by $H^+$ secretion in cell (CA II) and reabsorption of $\text{H}_2\text{CO}_3 \rightarrow \text{CO}_2$ in lumen (CA IV)</td>
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lower CSF flow, while methazolamide, a far more permeant drug, at 25 mg/kg is very effective (table 2). The two compounds have similar activities against the enzymes. Again, there appears failure of F 3500 to reach the active site of secretion.

The other four sulfonamides in the perfusion fluid did reduce CSF flow. From their structures (fig. 1) and previous work (Maren, 1967, 1995) it is clear that they have good activity against the enzymes and do enter cells. There is a relation between in vitro activity against CA and physiological effect. Notably, the weakest inhibitor, methazolamide, shows no activity at 10^{-4} M, although the strongest, dorzolamide and benzolamide, are maximally active at this concentration (table 2).

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