0022-3565/87/2403-0717$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 240, No. 3

Printed in U.S.A.

Transport of Diphenhydramine in the Central Nervous System[1]

MARK J. GOLDBERG, REYNOLD SPECTOR and CHAO-KUO CHIANG
Departments of Internal Medicine, Pharmacology and Anesthesia, University of Iowa College of Medicine, Iowa City, Iowa
Accepted for publication November 17, 1986

ABSTRACT

The transport and metabolism of diphenhydramine was studied *in vitro* in the isolated rabbit choroid plexus and *in vivo* in New Zealand white rabbits and Sprague-Dawley rats. *In vitro*, [^{14}C] diphenhydramine was accumulated by a saturable, energy-requiring system in choroid plexus. *In vivo*, 20 min after intraventricular injection into rabbits, [^{14}C]diphenhydramine was cleared from cerebrospinal fluid much more rapidly than [^{3}H]sucrose, a molecule transported in the central nervous system by simple diffusion. *In vivo*, employing the *in situ* rat brain perfusion technique, [^{14}C]diphenhydramine was cleared from the cerebral perfusion fluid as rapidly as [^{14}C]diazepam. However, the clearance of [^{14}C]diphenhydramine, but not [^{14}C]diazepam, was inhibited by the addition of 10 mM unlabeled diphenhydramine to the perfusate. These *in vivo* and *in vitro* results show that diphenhydramine, unlike diazepam, is transported between blood, brain and cerebrospinal fluid, in part, by saturable, carrier-mediated transport processes at both the blood-brain and blood-cerebrospinal fluid barriers.

The antihistamine diphenhydramine, which commonly causes sedation, readily passes from blood into the CNS (Spector et al., 1980; Glazko and Dill, 1949). Although there is little data on diphenhydramine concentrations in the CSF, studies in animals suggest that the brain concentration of diphenhydramine is several times greater than the plasma concentration after i.v. drug administration (Glazko and Dill, 1949; Witiak and Lewis, 1978). However, diphenhydramine, a weak base with a pK_a of approximately 9, is mainly ionized at physiologic pH (Casy, 1978). Although the nonionized form of diphenhydramine is highly lipid soluble and would be expected to cross the blood-brain barrier readily, only 2.5% of diphenhydramine is nonionized at pH = 7.4 (Casy, 1978; Rapoport, 1976; Ganellin, 1978). Thus, based on the physiochemical properties of diphenhydramine, passive processes (*e.g.*, simple diffusion) do not adequately explain the pharmacokinetics of diphenhydramine in the CNS (Rapoport, 1976; Bradbury, 1979).

Carrier-mediated (saturable) processes transport many substances between blood and the CNS (Rapoport, 1976; Bradbury, 1979). These processes exist at both the choroid plexus (the anatomical locus of the blood-CSF barrier) and the cerebral capillaries (the locus of the blood-brain barrier) (Rapoport, 1976; Bradbury, 1979). We hypothesized that, *in vivo*, the choroid plexus and cerebral capillaries might transport diphenhydramine by carrier-mediated processes. To investigate these possibilities, we performed the following experiments. First, we studied the isolated rabbit choroid plexus to see whether it contained a carrier-mediated transport system for diphenhydramine. We also studied the efflux of intraventricularly injected [^{14}C]diphenhydramine from the CNS and the influx of [^{14}C]diphenhydramine through the blood-brain barrier using the *in situ* rat brain perfusion technique (Takasato *et al.*, 1984). Finally, we determined the steady-state concentration of diphenhydramine in the CSF during the i.v. infusion of diphenhydramine. These studies showed that diphenhydramine is transported in the CNS, in large part, by carrier-mediated processes.

Received for publication August 15, 1986.
[1] This work was supported in part by National Institutes of Health Grant NS15073 (R.S.) and by the Veterans Administration (M.J.G.).

Methods

[^{14}C]Diphenhydramine (α-[^{14}C]benzhydryl; 50.1 mCi/mmol) was synthesized by Wizard Laboratories (Davis, CA). [^{3}H]Inulin (2.0 Ci/mmol), [^{3}H]sucrose (10 Ci/mmol) and [^{14}C]diazepam (54 mCi/mmol) were obtained from Amersham/Searle (Arlington Heights, IL). Crystalline diphenhydramine HCl was obtained from Sigma Chemical Co. (St. Louis, MO). The purity of the [^{3}H]inulin, [^{3}H]sucrose and [^{14}C] diazepam was confirmed (>98% in each case) by paper chromatography in two solvent systems suggested by the manufacturers. Chromatography of [^{14}C]diphenhydramine is described below. Other chemicals were obtained from commercial sources. Adult New Zealand white rabbits and Sprague-Dawley rats were used.

Choroid plexus accumulation, release and binding studies *in vitro*. The ability of isolated rabbit choroid plexuses to accumulate [^{14}C]diphenhydramine was measured by methods previously described (Spector and Goldberg, 1982). Briefly, individual choroid plexuses (weighing about 6 mg) were obtained from the brains of New Zealand white rabbits (1.5–2.0 kg) that were killed with i.v. pentobarbital. Each choroid plexus was placed in 3 ml of artificial CSF (Merlis, 1940) containing 5 mM glucose, [^{14}C]diphenhydramine and, in some cases, other substances. The incubations were carried out in a metabolic

ABBREVIATIONS: CSF, cerebrospinal fluid; CNS, central nervous system; T/M, tissue/medium.

shaker at 37°C or 1°C under 95% O_2-5% CO_2 (95:5) for various times. At the end of the incubation, each choroid plexus was wiped on a glass slide, weighed and homogenized in 0.5 ml of water. The radioactivity in tissue homogenates and media was determined, and the T/M radioactivity ratios were calculated by dividing the disintegrations per minute per gram of choroid plexus by the disintegrations per minute per milliliter of medium (Spector and Goldberg, 1982). The release of [^{14}C] diphenhydramine from choroid plexuses that had been preincubated for 5 min in artificial CSF containing 4 μM [^{14}C]diphenhydramine was also measured (Spector and Goldberg, 1982).

The ability of the isolated choroid plexus homogenates to bind [^{14}C] diphenhydramine was determined by three methods (Lorenzo and Spector, 1973). First, the ability of the isolated choroid plexus to release previously accumulated [^{14}C]diphenhydramine was measured as described above. Second, isolated rabbit choroid plexuses (that had been incubated in 4 μM [^{14}C]diphenhydramine for 2 min at 37°C) were homogenized in 1 ml of artificial CSF in scintered glass homogenizing tubes at 4°C. Duplicate aliquots of homogenate (0.1 ml) and supernatant (0.1 ml), obtained after centrifugation at 50,000 × *g* for 15 min at 4°C, were assayed for ^{14}C activity. Third, binding of [^{14}C]diphenhydramine was determined by equilibrium dialysis. Briefly, 0.75 ml of either 1) buffer (modified artificial CSF containing 0.2% sodium azide, pH 7.4), 2) choroid plexuses homogenized in buffer (about 250-mg choroid plexus in 0.5 ml of buffer) or 3) fresh rabbit serum were placed in segments of Spectrapor #1 dialysis tubing. Dialysis was performed against 7 ml of buffer containing [^{14}C]diphenhydramine at 37°C for 4 hr, which allowed for complete equilibration of ^{14}C in buffer.

Efflux studies. In order to measure the efflux of diphenhydramine from the CSF compartment, New Zealand white rabbits (2.3–3.1 kg) were injected intraventricularly with 0.2 ml of artificial CSF containing 7 μCi of [^{14}C]diphenhydramine and 21 μCi of [^{3}H]sucrose after induction of anesthesia with sodium thiopental (Spector, 1981). After the injection, the hole in the skull above the right lateral ventricle was sealed with bone wax, the skin was sutured and the rabbit rapidly regained consciousness. After 20 min, the rabbit was killed with an overdose of thiopental, the heart was severed, 1 ml of cisternal CSF was withdrawn and the brain was removed as rapidly as possible. The choroid plexuses were removed, weighed and homogenized in 1.5 ml of 0.6 N perchloric acid. The left and right halves of the brain were each weighed and homogenized in 10 ml of 0.6 N perchloric acid. Duplicate 0.2-ml portions of the various brain and choroid plexus homogenates or the withdrawn CSF were assayed for ^{14}C and ^{3}H. The ^{3}H and ^{14}C distintegrations per minute per gram or milliter of the various brain and choroid plexus samples and CSF were calculated, as well as the ^{14}C/^{3}H ratios in the tissues divided by the same ratio in the injected solution. The recovery of the injected ^{3}H and ^{14}C was calculated for the left and right halves of whole brain, the choroid plexus and an assumed volume of CSF of 3 ml (Spector, 1981).

Diphenhydramine influx into CSF. To determine the influx of i.v. injected diphenhydramine into the CSF, six rabbits were given a loading dose of diphenhydramine hydrochloride i.v. (2.15 mg/kg in 5 ml of saline over 2 min), followed by a maintenance dose of 15.12 mg/kg in 30 ml of saline infused at a constant rate over 3 hr. Venous blood samples for diphenhydramine determinations were collected from an ear vein at 1.5 hr. At 3 hr, each rabbit was sacrificed by an overdose of thiopental, and blood and CSF specimens for diphenhydramine determination were obtained by cardiac and cisternal puncture, respectively. Diphenhydramine was measured in plasma and CSF by a gas chromatographic assay (Spector *et al.*, 1980).

Diphenhydramine transport through the blood-brain barrier. The *in situ* brain perfusion technique of Takasato *et al.* (1984, 1985) was employed, except that adult Sprague-Dawley rats instead of Osborne-Mendel rats were used, and a slightly different bicarbonate-based balanced salt solution (Merlis, 1940) containing 5 mM glucose was employed (Spector *et al.*, 1986; Merlis, 1940). The perfusion rate was 63 μl/sec. Briefly, this technique involves cannulating the external carotid artery of pentobarbital-anesthetized rats and tying off all branches of the internal carotid artery, especially the pterygopalatine branch, which normally siphons substantial blood from the internal carotid artery (Takasato *et al.*, 1984, 1985; Spector *et al.*, 1986). At time 0, the common carotid artery is ligated and the perfusion of the internal carotid artery begun at a constant rate. The perfusion is continued with the bicarbonate-based balanced salt solution at 37°C. Eight seconds are required for the perfusate to reach the brain and are subtracted from the time of the perfusion used in the calculations below. At the end of the perfusion, the rat's head is decapitated and the telencephalon (forebrain) on the perfused side is removed, weighed, homogenized in water and assayed for radioactivity. In all experiments, greater than 200 times as many microcuries of [^{3}H]inulin as [^{14}C]diphenhydramine or [^{14}C]diazepam were included in the perfusate; [^{3}H]inulin was used as a measure of the i.v. space as described by Takasato *et al.* (1984, 1985).

Perfusate flow through the perfused hemisphere was measured using [^{14}C]diazepam, with the assumptions that all the [^{14}C]diazepam was completely extracted during the passage of the perfusate through the brain capillaries and that there was no back diffusion of [^{14}C]diazepam from brain into blood during the perfusion (Takasato *et al.*, 1984, 1985). With these assumptions, flow is constant (Takasato *et al.*, 1984, 1985) and is described by

$$F_P = \frac{C_B}{T \times C_P}, \quad (1)$$

where F_P = regional cerebral perfusion flow (milliliters per gram per second), C_B = brain concentration of [^{14}C]diazepam (disintigrations per minute per gram), T = time in seconds and C_P = the concentration of [^{14}C]diazepam in the perfusate (disintegrations per minute per milliliter).

As described under "Results," [^{14}C]diphenhydramine was extracted as rapidly as [^{14}C]diazepam and could also be used as a measure of cerebral perfusion flow.

Chromatography of [^{14}C]diphenhydramine. The purity of the [^{14}C]diphenhydramine was stated to be greater than 95% by the manufacturers. We confirmed these results using silica gel G thin-layer chromatography. After spotting, the plates were developed in absolute ethanol-1 N ammonium hydroxide (100:5) (Solvent System A) and 1-butanol-15 N ammonium hydroxide (100:2) (Solvent System B) (12). The location of unlabeled diphenhydramine (50 μg) was detected with UV light.

In the five rabbits given [^{14}C]diphenhydramine intraventricularly, the nature of the ^{14}C in the withdrawn CSF was determined; 88 ± 4% (S.E.M., $n = 5$) of the ^{14}C in the CSF cochromatographed with unlabeled diphenhydramine on the thin-layer chromatographic plate in Solvent System A described above.

The nature of the accumulated ^{14}C within isolated choroid plexuses (that had accumulated ^{14}C from the medium containing 4 μM [^{14}C] diphenhydramine for 5 min) was determined by extraction and thin-layer chromatography of the radioactivity. In brief, at the end of the incubation, the choroid plexuses were homogenized in 500 μl of water containing unlabeled diphenhydramine. Duplicate samples were alkalinized with 6 M potassium hydroxide and extracted into 4 ml of heptane. The organic layer was then acidified with hydrochloric acid, after which the aqueous layer was removed, alkalinized with 6 M potassium hydroxide and extracted into 2 ml of heptane. After removal, the heptane was evaporated to dryness and reconstituted in methanol. Fifty-microliter aliquots were spotted on silica gel G thin-layer plates and developed in Solvent Systems A and B. To assess recovery and serve as controls, [^{14}C]diphenhydramine and unlabeled diphenhydramine were added to homogenates of choroid plexus cooled to 1°C. These samples were then carried through the procedure described above. In all these assays, duplicate aliquots of homogenates, supernatants and appropriate controls were assayed for ^{14}C activity. With these methods, 91% of the ^{14}C accumulated by the isolated choroid plexus was associated with unlabeled diphenhydramine on the thin-layer chromatography plates ($n = 3$). These results were identical with controls; *i.e.*, 91% of the ^{14}C in the controls cochromatographed with unlabeled diphenhydramine with comparable overall recovery of ^{14}C.

Assays. ^3H and ^{14}C scintillation spectroscopy was performed as previously described (Spector and Goldberg, 1982). The volume of all samples was adjusted to 1.05 ml with water, and 2.5 ml of Scintiverse were added to make a gel. Samples were assayed in a Packard Scintillation Spectrometer.

Results

The time course of [^{14}C]diphenhydramine uptake (T/M ratios) by the isolated choroid plexus is shown in figure 1. With concentrations of 4 μM [^{14}C]diphenhydramine in the medium, T/M ratios increased to approximately 8 at 1 min and to over 20 at 10 min.

The effect of various potential inhibitors of [^{14}C]diphenhydramine uptake by the isolated choroid plexus at 2 and 5 min, compared with control T/M ratios, is shown in table 1. The choroid plexus uptake of [^{14}C]diphenhydramine was significantly inhibited by diphenhydramine (1 and 10 mM) in the medium. The uptake was also inhibited by other weak bases (nicotine and tolazoline), but not by the weak acid, probenecid. The uptake of diphenhydramine was inhibited by dinitrophenol and iodoacetate and, to a greater extent, by incubation at 1°C.

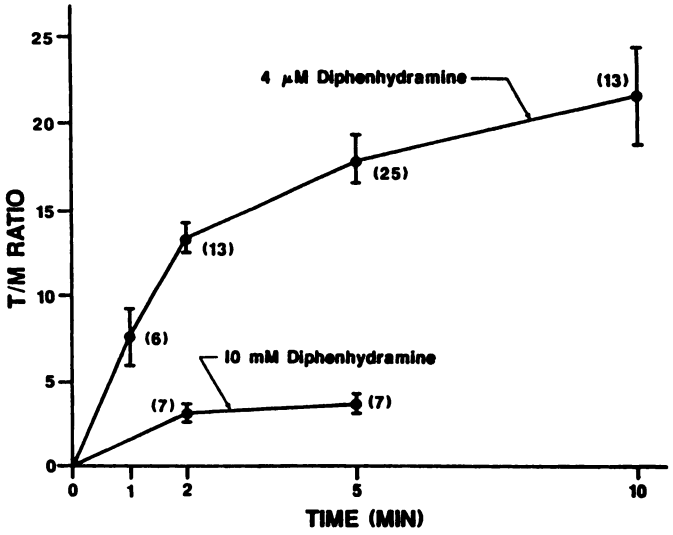

Fig. 1. Uptake of diphenhydramine as a function of time. Choroid plexuses were incubated in artificial CSF containing 4 μM [^{14}C]diphenhydramine for various times at 37°C under 95% O_2-5% CO_2 in a metabolic shaker. At the end of the incubation, T/M ratios were determined. Values are means ± S.E.M., with the number of experiments in parentheses.

The choroid plexus uptake of [^{14}C]diphenhydramine from artificial CSF was due only in part to binding. By the centrifugation technique, 61 ± 2% (S.E.M.; n = 5) of the [^{14}C]diphenhydramine in the choroid plexus homogenates was found in the supernatant. Also, choroid plexuses that were preincubated in 4 μM [^{14}C]diphenhydramine for 5 min released, at progressively longer times, an increasing percentage of the ^{14}C into 3 ml of release medium (maintained at 37°C under 95% O_2-5% CO_2 in a metabolic shaker) (table 2). When diphenhydramine (10 mM) was added to the release medium to cause displacement of [^{14}C]diphenhydramine from tissue binding sites and to prevent reuptake of [^{14}C]diphenhydramine, a greater percentage of ^{14}C was released, with ultimately 95% of the radioactivity associated with the choroid plexus being released after 5 min of release incubation (table 2). However, when the release experiments were carried out at 4°C, a reduced percentage of ^{14}C was released (table 2). By equilibrium dialysis, 31% (n = 2) of the [^{14}C]diphenhydramine was not bound by choroid plexus homogenates at 37°C. Thus, greater than 95% of the accumulated [^{14}C]diphenhydramine in the choroid plexus was not irreversibly bound within the choroid plexus. A substantial portion, however, may be reversibly bound, as suggested by the equilibrium dialysis experiments.

The recoveries (in percentages) of [^{14}C]diphenhydramine and [^3H]sucrose in CSF and brain 20 min after intraventricular injection are shown in table 3. Assuming a 3-ml volume of CSF, 100% of [^3H]sucrose was recovered. As expected, the majority (75%) of the injected [^3H]sucrose remained in the CSF (Spector, 1981). However, less than half the injected [^{14}C]diphenhydramine was recovered. Almost all the recovered [^{14}C]diphenhydramine was found in the right brain, and less than 1% of the injected dose remained in the CSF. These results suggest that, compared with [^3H]sucrose, [^{14}C]diphenhydramine is rapidly transferred out of CSF (table 3).

Employing the *in situ* rat brain perfusion technique, cerebral perfusion flow (measured by [^{14}C]diazepam extraction) was 38.5 ± 3.6 (S.E.M.; n = 7) μl/g of forebrain per min (table 4). After the 7-sec perfusion, the mean [^{14}C]diazepam/[^3H]inulin ratio in forebrain divided by the same ratio in the injectate was 51 (table 4). The extraction of [^{14}C]diphenhydramine was almost identical (table 4). However, the addition of 10 mM (but not 1 mM) diphenhydramine hydrochloride to the perfusate decreased the [^{14}C]diphenhydramine/[^3H]inulin ratio to 38 % of control (table 4). Unlike the result with [^{14}C]diphenhydramine, the addition of diphenhydramine hydrochloride (10 mM) to the

TABLE 1

Uptake of [^{14}C]diphenhydramine by the isolated choroid plexus

Choroid plexuses were incubated for various times in artificial CSF containing 4 μM [^{14}C]diphenhydramine under various conditions. At the end of the incubation, the T/M ratios were determined.

Experimental Condition	T/M Ratio (2 Min)	Percent Control	T/M Ratio (5 Min)	Percent Control
Control; [^{14}C]diphenhydramine, 4 μM	13.45 ± 0.86* (13)	—	17.93 ± 1.32 (25)	—
Diphenhydramine, 0.1 mM	—	—	16.07 ± 2.96 (8)	90
Diphenhydramine, 1 mM	—	—	8.48 ± 1.41 (8)	47*
Diphenhydramine, 10 mM	3.08 ± 0.43 (7)	23*	3.75 ± 0.51 (7)	21*
Nicotine, 10 mM	6.13 ± 1.29 (4)	46*	—	—
Tolazoline, 10 mM	4.77 ± 0.72 (4)	36*	—	—
Probenecid, 1 mM	—	—	23.41 ± 3.49 (7)	131
Dinitrophenol, 1 mM, + iodoacetate, 1 mM	—	—	9.00 ± 1.07 (4)	50*
1°C	4.18 ± 0.54 (4)	31*		

* Values are means ± S.E.M., with the number of determinations in parentheses.
* $P < .05$ when compared with appropriate control (Dunnett's test).

perfusate did not significantly alter the extraction of [^{14}C] diazepam (table 4) and thus did not change cerebral perfusion flow. Choline chloride (10 mM) decreased [^{14}C]diphenhydramine extraction slightly but not significantly (table 4).

After i.v. infusion of diphenhydramine over 3 hr into conscious rabbits, the concentrations of diphenhydramine in plasma at 1.5 and 3 hr and in CSF at 3 hr were 804 ± 92, 806 ± 166 and 909 ± 116 ng/ml (S.E.M., $n = 6$), respectively. When rabbit plasma was dialyzed against medium containing [^{14}C] diphenhydramine, 47% ($n = 2$) of the diphenhydramine was unbound. Similar equilibrium dialysis studies done on plasma collected at 3 hr from four of the six rabbits given diphenhydramine i.v. revealed that 55 ± 1% (S.E.M.; $n = 4$) of diphenhydramine in plasma was unbound.

Discussion

There are four principal findings in these studies. First, the concentration of diphenhydramine in the CSF during the i.v. infusion of diphenhydramine (at steady state) is approximately twice the unbound concentration of diphenhydramine in the plasma. Our hypothesis about the mechanism by which this occurs is discussed below.

Second, the choroid plexus contains an uptake system for [^{14}C]diphenhydramine that depends on two processes. Greater than 50% of the uptake (with a medium concentration of 4 μM) was by a saturable process and presumably depended on energy production sensitive to dinitrophenol and iodoacetate (table 1). Approximately 30% of the accumulation was by a nonsaturable process and was not inhibited even at 1°C. Nonspecific binding of diphenhydramine within or on the choroid plexus accounts for a portion of the accumulation because diphenhydramine was bound by the choroid plexus as determined by equilibrium dialysis. However, a substantial portion of this binding was not irreversible as determined by the release and centrifugation studies. The accumulation was also not due to metabolism of [^{14}C]diphenhydramine by the choroid plexus. If the pH values of the intracellular and extracellular spaces of choroid plexus were 7.0 and 7.3, respectively, a T/M ratio of 1.6 would be expected (based on pH partitioning) because the pK_a of diphenhydramine is 9.0 and the intracellular space of the choroid plexus represents 60% of its volume (Rapoport, 1976; Lorenzo and Spector, 1973; Johanson, 1978). However, in these studies, a T/M ratio of approximately 20 was obtained at near equilibrium, indicating that other factors besides pH partitioning (active transport, for example) must be operating. Because the system in choroid plexus that accumulates [^{14}C]diphenhydramine was inhibited by other weak bases (*e.g.*, nicotine and tolazoline) but not by probenecid, a drug that inhibits the weak organic acid transport system of choroid plexus (Lorenzo and Spector, 1973), we conclude that a part of the accumulation of [^{14}C]diphenhydramine by the choroid plexus was due to a specific active transport mechanism.

The third finding reported herein is that the efflux of [^{14}C] diphenhydramine from the CSF of the rabbit occurs at a much more rapid rate than efflux of [^{3}H]sucrose. A portion of the [^{14}C]diphenhydramine was taken up by the brain; however, the total recovery of [^{14}C]diphenhydramine in the CNS after i.c.v. injection was less than half the total recovery of [^{3}H]sucrose. Studies have shown that water-soluble nonmetabolizable substances such as sucrose slowly diffuse out of the CSF through the extracellular space of brain and leave the CSF by bulk flow through the channels of the arachnoid villi to reach the dural venous sinuses or the cervical lymph (Bradbury, 1979; Levin *et al.*, 1970; Prockop *et al.*, 1962). The fact that diphenhydramine (MW 255 daltons) leaves the CSF at a much faster rate than sucrose suggests that diphenhydramine leaves the CSF by other

TABLE 2

Percentages of [^{14}C]diphenyhydramine released from isolated choroid plexuses preincubated in [^{14}C]diphenhydramine

The release of [^{14}C]diphenhydramine from choroid plexus preincubated for 5 min in 4 μM [^{14}C]diphenhydramine was measured.

Time	Control[a]	Diphenhydramine, 10 mM[a]	4°C
15 sec	41 ± 2[b] (5)	—	—
30 sec	58 ± 5 (5)	87 ± 2 (5)	—
1 min	63 ± 4 (8)	87 ± 2 (5)	35 ± 5 (5)
2 min	65 ± 4 (8)	94 ± 1 (4)	38 ± 3 (5)
5 min	82 ± 2 (4)	95 ± 2 (4)	—

[a] Values were measured at 37°C.
[b] Values are means ± S.E.M., with the number of incubations in parentheses.

TABLE 3

Recovery in percentages of intraventricularly injected [^{14}C] diphenhydramine and [^{3}H]sucrose

Five rabbits were given [^{14}C]diphenhydramine and [^{3}H]sucrose intraventricularly. After 20 min, they were killed, and the percentage of recovered ^{14}C and ^{3}H in CSF, right brain, left brain and choroid plexuses were determined.

Tissue	[^{14}C]Diphenhydramine	[^{3}H]Sucrose
Right brain	36.6 ± 4.2[a] (1.70 ± 0.21[b])	20.6 ± 2.9
Left brain	3.9 ± 1.8 (0.52 ± 0.08)	6.1 ± 0.2
Choroid plexus	1.2 ± 0.3 (1.45 ± 0.17)	0.8 ± 0.2
CSF	0.7 ± 0.1 (0.01 ± 0.00)	74.7 ± 6.8
Total recovered	42.5 ± 4.4	102.3 ± 4.8

[a] Values are means ± S.E.M.
[b] In parentheses is the ratio of the disintegrations per minute of [^{14}C]diphenhydramine to [^{3}H]sucrose per gram of tissue or milliliter of fluid divided by the same ratio in the intraventricular injectate.

TABLE 4

Cerebral perfusate flow measured with [^{14}C]diazepam or [^{14}C]diphenhydramine

Rat brains were perfused at 63 μl/sec for 7 sec with perfusate containing 1.5 × 10^{4} dpm/ml of [^{14}C]diphenhydramine or [^{14}C]diazepam (0.13 μM), 8 × 10^{6} dpm/ml of [^{3}H] inulin and, in some casees, unlabeled diphenhydramine or choline chloride. At the end of the perfusion, the ^{14}C/^{3}H ratios in brain were divided by the same ratio in the perfusate, and the perfusate flows were calculated.

Condition	[^{14}C]Diazepam		[^{14}C]Diphenhydramine	
	Ratio (*N*)	Flow (*N*)	Ratio (*N*)	Flow (*N*)
		μl/g/sec		μl/g/sec
Control	50.7 ± 5.5[a] (7)	38.5 ± 3.6 (7)	51.5 ± 3.9 (8)	39.1 ± 3.0 (8)
Diphenhydramine, 1.0 mM	—	—	63.6 ± 8.0 (8)	49.7 ± 4.2 (8)
Diphenhydramine, 10.0 mM	69.4 ± 6.1 (8)	46.2 ± 5.7 (8)	19.7 ± 2.8* (8)	11.2 ± 2.3 (8)*
Choline Chloride, 10 mM	—	—	47.6 ± 12.5 (8)	30.7 ± 6.9 (8)

[a] Values are means ± S.E.M., with the number of determinations in parentheses.
* $P < .05$ when compared with [^{14}C]diphenhydramine control; Dunnett's test.

routes, *e.g.*, passage through the blood-brain and/or blood-CSF barriers, which impede the passage of water-soluble molecules unless there are specialized transport systems (Bradbury, 1979). In fact, diphenhydramine was transported from CSF much faster than penicillin, which is known to be transported from CSF almost completely by an active transport mechanism in choroid plexus (Spector and Lorenzo, 1974). Because diphenhydramine is, to a great extent, ionized at physiologic pH, simple diffusion of diphenhydramine through the blood-brain or blood-CSF barriers can explain only a portion of this efflux. *In vivo*, certain quaternary ammonium compounds (*e.g.*, nicotine and N-methylnicotinamide) leave the CSF rapidly *in vivo*, and, because the choroid plexus accumulates these compounds by a saturable, energy-dependent process *in vitro*, the choroid plexus has been proposed to be the site of carrier-mediated transport of these quaternary ammonium compounds out of CSF (Schanker *et al.*, 1962; Tochino and Schanker, 1965). Similarly, because 1) diphenhydramine is rapidly transferred out of CSF *in vivo* (table 3) and 2) the choroid plexus accumulates diphenhydramine *in vitro* by a saturable, energy-dependent process, we conclude that the choroid plexus may be a site for carrier-mediated transport of diphenhydramine out of CSF. However, an alternative explanation (see below) is that diphenhydramine (not bound to histamine receptors or nonspecific binding sites in brain) is transported out of CSF and the extracellular space of brain through the saturable transport system in the cerebral capillaries.

The fourth finding was the extremely rapid but saturable clearance of diphenhydramine from blood into rat brain. The clearance of diphenhydramine was comparable to the clearance of diazepam, which is known to be completely cleared from perfusate (and retained in brain) during the *in situ* rat brain perfusion technique (Takasato *et al.*, 1984, 1985). Diphenhydramine behaved similarly, except that unlabeled diphenhydramine (10 mM) greatly decreased penetration of [^{14}C]diphenhydramine but not [^{14}C]diazepam into brain. Other weak bases that are predominantly ionized at pH = 7.4 (*e.g.*, amphetamine and nicotine) also readily pass through the blood-brain barrier (Wilson *et al.*, 1971; Oldendorf, 1977). We suggest that diphenhydramine and these other weak bases readily pass through the blood-brain barrier (to varying degrees) by two mechanisms: first, by simple diffusion of the nonionized lipid-soluble form, and second, by a saturable process (possibly facilitated diffusion of the ionic form) (Pardridge *et al.*, 1984). However, only one carrier for weak bases at the blood-brain barrier has been clearly documented, *i.e.*, for choline (K_t about 0.4 mM) (Cornford *et al.*, 1978), although Pardridge *et al.* (1984) provided data that suggested that amphetamine and lidocaine transfer through the blood-brain barrier is saturable. Choline, however, did not significantly inhibit diphenhydramine transport through the blood-brain barrier (table 4).

In conclusion, diphenhydramine enters the brain from blood extremely rapidly and, conversely, is readily transported from the CSF into the blood. Saturable transport systems for diphenhydramine exist at both the blood-brain barrier and the choroid plexus. At steady plasma diphenhydramine concentrations, the concentration of diphenhydramine in CSF is slightly higher than the concentration in plasma, notwithstanding the fact that approximately 50% of the diphenhydramine in plasma is bound to plasma proteins. These findings suggest that the choroid plexus actually transfers diphenhydramine predominantly from blood into CSF and that there is rapid bidirectional saturable transfer of diphenhydramine at the blood-brain barrier. If the choroid plexuses were transporting diphenhydramine from CSF into blood, one would anticipate (given an equilibrative transport system for diphenhydramine at the blood-brain barrier), a lower concentration of diphenhydramine in CSF, not a higher concentration, especially when plasma protein binding is taken into account. Thus, the choroid plexus in the case of diphenhydramine appears to act as it does in the case of morphine, which is transferred from plasma into CSF by the choroid plexus (Wang and Takemori, 1972). However, unlike diphenhydramine, morphine is poorly transported through the blood-brain barrier (Oldendorf, 1977). Although our hypothesis explains the data, alternative explanations are possible; further experimental work is necessary to exclude them.

Acknowledgments

The authors wish to thank Dale Kinzenbaw for his excellent technical help and Sam Meyer for her preparation of the manuscript.

References

BRADBURY, M.: The Concept of a Blood-Brain Barrier, John Wiley and Sons, New York, 1979.

CASY, A. F.: Chemistry and structure-activity relationships of synthetic and antihistaminics. *In* Histamine II and Anti-Histaminics: Chemistry, Metabolism and Physiological and Pharmacological Actions. Handbuch der Experimentallen Pharmakologgic, ed. by Mauricio Rocha e Silva, vol. 18, part 2, p. 175, Springer-Verlag, New York, 1978.

CORNFORD, E. M., BRAUN, L. D. AND OLDENDORF, W. H.: Carrier mediated blood-brain barrier transport of choline and certain choline analogs. J. Neurochem. **30:** 299–308, 1978.

GANELLIN, C. R.: Chemistry and structure-activity relationships of H_2-receptor antagonists. *In* Histamine II and Anti-Histaminics: Chemistry, Metabolism and Physiological and Pharmacological Actions. Handbuch der Experimentallen Pharmakologgic, ed. by Mauricio Rocha e Silva, vol. 18, part 2, p. 251, Springer-Verlag, New York, 1978.

GLAZKO, A. J. AND DILL, W. A.: Biochemical studies on diphenhydramine (Benadryl). J. Biol. Chem. **179:** 403–408, 1949.

JOHANSON, C. E.: Choroid epithelial cell pH. Life Sci. **23:** 861–868, 1978.

LEVIN, V. A., FENSTERMACHER, J. D. AND PATLAK, C. S.: Sucrose and inulin space measurements of cerebral cortex in four mammalian species. Am. J. Physiol. **219:** 1528–1533, 1970.

LORENZO, A. V. AND SPECTOR, R.: Transport of salicylic acid by the choroid plexus in vitro. J. Pharmacol. Exp. Ther. **184:** 465–471, 1973.

MERLIS, J. K.: The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. Am. J. Physiol. **131:** 67–72, 1940.

OLDENDORF, W. H.: Distribution of drugs to the brain. *In* Psychopharmacology in the Practice of Medicine, ed. by M. E. Jarvik, pp. 167–175, Appleton-Century-Crofts, New York, 1977.

PARDRIDGE, W. M., SAKIYAMA, R. AND FIERER, G.: Blood-brain barrier transport and brain sequestration of propranolol and lidocaine. Am. J. Physiol. **16:** R582–R588, 1984.

PROCKOP, L. D., SCHANKER, L. S. AND BRODIE, B. B.: Passage of lipid-insoluble substances from cerebrospinal fluid to blood. J. Pharmacol. Exp. Ther. **135:** 266–270, 1962.

RAPOPORT, S. I.: Blood-Brain Barrier in Physiology and Medicine, Raven Press, New York, 1976.

SCHANKER, L. S., PROCKOP, L. D., SCHOU, J. AND SISODIA, P.: Rapid efflux of some quaternary ammonium compounds from cerebrospinal fluid. Life Sci. **1:** 515–521, 1962.

SPECTOR, R.: Penetration of ascorbic acid from cerebrospinal fluid into brain. Exp. Neurol. **72:** 645–653, 1981.

SPECTOR, R., CHOUDHURY, A. K., CHIANG, C.-K., GOLDBERG, M. J. AND GHONEIM, M. M.: Diphenhydramine in Orientals and Caucasians. Clin. Pharmacol. Ther. **28:** 229–234, 1980.

SPECTOR, R. AND GOLDBERG, M. J.: Active transport of nicotine by the isolated choroid plexus in vitro. J. Neurochem. **38:** 594–596, 1982.

SPECTOR, R. AND LORENZO, A. V.: The effects of salicylate and probenecid on the cerebrospinal fluid transport of penicillin, aminosalicylic acid and iodide. J. Pharmacol. Exp. Ther. **188:** 55–65, 1974.

SPECTOR, R., SIVESIND, C. AND KINZENBAW, D.: Pantothenic acid transport at the blood-brain barrier. J. Neurochem., in press, 1986.

TAKASATO, Y., MOMMA, S. AND SMITH, Q. R.: Kinetic analysis of cerebrovascular isoleucine transport from saline and plasma. J. Neurochem. **45:** 1013–1020, 1985.

TAKASATO, Y., RAPOPORT, S. I. AND SMITH, Q. R.: An in situ brain perfusion technique to study cerebrovascular transport in the rat. Am. J. Physiol. **247:** H484–H493, 1984.

TOCHINO, Y. AND SCHANKER, L. S.: Active transport of quaternary ammonium compounds by the choroid plexus in vitro. Am. J. Physiol. 208: 666–673, 1965.
WANG, J. H. AND TAKEMORI, A. E.: Studies on the transport of morphine into the cerebrospinal fluid of rabbits. J. Pharmacol. Exp. Ther. 183: 41–48, 1972.
WILSON, C. O., GISVOLD, O. AND DOERGE, R. F.: Textbook of Organic Medicinal and Pharmaceutical Chemistry, 6th ed., J. B. Lippincott Company, Philadelphia, 1971.
WITIAK, D. T. AND LEWIS, N. J.: Absorption, distribution, metabolism, and elimination of antihistamines. *In* Histamine II and Anti-Histaminics: Chemistry, Metabolism and Physiological and Pharmacological Actions. Handbuch der Experimentallen Pharmakologgic, vol. 18, part 2, p. 513, Springer-Verlag, New York, 1978.

Send reprint requests to: Reynold Spector, M.D., Department of Internal Medicine, University of Iowa Hospitals, Iowa City, IA 52242.