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Kinetic Studies on the Entry of *d*-Amphetamine into the Central Nervous System: I. Cerebrospinal Fluid[1]

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ABSTRACT

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After intravenous administration to rats, *d*-amphetamine undergoes a rapid distributive phase ($k \sim 0.99 \cdot min^{-1}$) during which the drug is lost from plasma. The rate of entry into the cerebrospinal fluid (CSF) is also rapid ($k \sim 0.58 \cdot min^{-1}$), suggesting that the drug moves directly from plasma to CSF. Entry of drug into CSF is mainly by diffusion. Neither active transport associated with CSF formation nor active transport independent of CSF formation is quantitatively important. After intracerebroventricular injection, *d*-amphetamine disappears from CSF relatively slowly ($k \sim 0.063 \cdot min^{-1}$). Egress of drug is mainly by diffusion ($k \sim 0.044 \cdot min^{-1}$), although active transport associated with bulk absorption does play a measurable role ($k \sim 0.019 \cdot min^{-1}$). At steady state, the concentration of free drug in plasma and in CSF is equivalent.

There is extensive literature on the pharmacology of *d*-amphetamine (Costa and Garattini, 1970; Lewander, 1977). Within this literature, many aspects of drug disposition and drug action have been described in detail (*e.g.*, Caldwell, 1976). However, one area that has received only minimal attention is that of pharmacokinetics. For example, there are few studies that describe the mechanisms or quantify the rates for *d*-amphetamine entry into the central nervous system.

An understanding of the pharmacokinetics of *d*-amphetamine is essential to an understanding of the sites and actions of the drug. Therefore, the present study describes the kinetic behavior of *d*-amphetamine in relation to the brain. The cerebrospinal fluid (CSF) is the focus of the study, because: 1) fluid compartments are generally more accessible than other compartments; 2) the contents of fluid compartments are normally more homogeneous than those of tissue compartments; and 3) data on CSF can be compared to previous data on blood and plasma (Simpson, 1978a,b).

Materials and Methods

Animals. Male Sprague-Dawley rats (Camm Research Institute, Wayne, NJ) weighing between 200 and 250 g were used in these studies. The rats are a pathogen-free strain that is Cesarean derived and barrier reared.

Recording of blood pressure and heart rate. Resting blood pressure and heart rate and drug-induced changes in blood pressure and heart rate were measured. Mean arterial blood pressure was monitored with a cannula (PE 50) installed in the left carotid artery. Blood pressure recordings were obtained using a pressure transducer (Hewlett Packard 1280B) and pressure amplifier (Hewlett Packard 8805C) connected to a thermal recording system (Hewlett Packard 7754A). Heart rate was monitored with two subdermal electrodes (active leads) in the chest wall and one subdermal electrode (reference lead) in a hind limb. Heart rate recordings were obtained by coupling a rate computer (Hewlett Packard 8812A) with a bioelectric amplifier (Hewlett Packard 8811A), both of which were connected to the thermal recording system.

During blood pressure and heart rate experiments, animals were anesthetized (pentobarbital sodium, 50 mg/kg i.p.). The anesthetic agent was administered 30 to 45 min before *d*-amphetamine. When animals were anesthetized, their body temperatures were maintained at 35–36°C (Thermistemp temperature controller, Yellow Springs Instrument Co., Yellow Springs, OH). All animals received atropine (25 mg/kg i.p.); this agent ensured that reflex neural activity would not obscure the peripheral sympathomimetic actions of *d*-amphetamine.

Perfusion of the ventricular system. Both pull and push-pull techniques were used; all methods were conventional (Myers, 1972). During push-pull experiments, the inflow cannula was installed in the lateral ventricle and the outflow cannula was installed in the cisterna magna. The inflow cannula (27-gauge needle) was implanted approximately 1 mm lateral to and 1 mm dorsal to bregma. The outflow cannula (27-gauge needle) was inserted midline through the atlantooccipital membrane. Cannulas were installed after animals had been anesthetized and secured in a stereotaxic instrument (David Knopf Instruments, Tujunga, CA.)

During push-pull experiments, the ventricular system was perfused with artificial CSF (Merlis, 1940). A Harvard infusion pump (Harvard Apparatus Co., Millis, MA) was used to maintain a constant rate of perfusion. The pump was connected to the ventricular cannulas by polyethylene tubing. The rate of perfusion was 8 μl/min.

Pull experiments were done identically to push-pull experiments

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with two exceptions: 1) a single cannula was installed (cisterna magna); and 2) the withdrawal rate was 2 μl/min.

Rate of CSF formation. The technique used was that of Heisey *et al.* (1962). According to this technique, an impermeant drug (inulin) is perfused through the ventricular system. At steady state, there will be a difference between the concentration of inulin that enters the system and the concentration that leaves the system. Dilution of inulin as it passes through the system is caused by newly formed CSF.

CSF formation is quantified by using the following equation:

$$R_{csf} = R_p ([I]_i - [I]_o)/[I]_o, \quad 1$$

in which

R_{csf} = rate of CSF formation (microliter/minute)
R_p = rate of perfusion (microliter/minute)
$[I]_i$ = concentration of inulin infused (M)
$[I]_o$ = concentration of inulin withdrawn (M)

In the present experiments, inulin ([*inulin-carboxyl*-^{14}C], 3.2 μCi/mg, New England Nuclear, Boston, MA) was assayed by liquid scintillation spectometry.

Clearance of drugs from CSF. The clearances of inulin and *d*-amphetamine were measured. Techniques for studying the two drugs were nearly identical.

By definition, clearance (C) is equal to the rate of loss of drug divided by the concentration of drug. When applied to inulin, clearance is expressed as:

$$C = (dI/dt)/[I] \quad 2$$

In a push-pull experiment, the rate of loss of inulin can be quantified as:

$$dI/dt = R_i[I]_i - R_o[I] \quad 3$$

in which:

R_i = rate of infusion (microliter/minute)
R_o = rate of withdrawal (microliter/minute)

Combining Equations 2 and 3 yields:

$$C = (R_i [I]_i - R_o [I]_o)/[I] \quad 4$$

Examination of Equations 1 and 4 reveals an important principle. When an impermeant drug is being studied and when infusion rate is equal to withdrawal rate, then rate of CSF formation will equal clearance of the impermeant drug. This principle derives from the fact that an impermeant drug (no diffusion and no transport) can be lost from CSF only by bulk absorption. At steady state, the rate of bulk absorption must equal rate of CSF formation.

There is one notable difference between clearance of inulin and clearance of *d*-amphetamine. As already explained, the former drug is impermeant and is lost from CSF by bulk absorption. The process of bulk absorption occurs distal to the fourth ventricle. Therefore, the denominator in Equation 4 should be $[I]_o$; that is, the concentration of inulin just distal to the fourth ventricle (*i.e.*, the cisterna magna). By contrast, *d*-amphetamine is lost from CSF both by bulk absorption and by other processes (see "Results"). Therefore, when applied to *d*-amphetamine, the equation describing clearance should employ the average concentration of *d*-amphetamine in the ventricles. Assuming that *d*-amphetamine diffuses through CSF and assuming that diffusion is a first order process, one can calculate the average concentration of *d*-amphetamine (A) in the ventricles as (Heisey *et al.*, 1962):

$$[A]_a = [A]_o + 0.37 ([A]_i - [A]_o) \quad 5$$

in which

$[A]_a$ = average concentration of *d*-amphetamine in the ventricles
$[A]_i$ = concentration of *d*-amphetamine infused (M)
$[A]_o$ = concentration of *d*-amphetamine withdrawn (M)

Thus, clearance of *d*-amphetamine can be calculated as:

$$C = (R_i[A]_i - R_o [A]_o)/[A]_a \quad 6$$

in which the meaning of the various symbols is implicit in the foregoing discussion.

Recovery of *d*-[^{3}H]amphetamine. *d*-[^{3}H]Amphetamine (15.6 Ci/mmol, generally labeled, New England Nuclear Corporation) was administered intravenously, after which it was recovered from plasma or CSF. For plasma studies, blood was collected in heparinized tubes. Samples were centrifuged, after which aliquots were assayed by liquid scintillation spectrometry. For CSF studies, samples were collected by using either pull or push-pull techniques (see above); CSF samples were also assayed by liquid scintillation spectrometry. No effort was made to separate *d*-[^{3}H]amphetamine from its metabolites, because: 1) collection periods were usually short (10 min or less) compared to plasma T½ for the drug (~35 min; Simpson, 1980); and 2) the principal metabolite of *d*-amphetamine in the rat is *p*-hydroxyamphetamine (Dring *et al.*, 1970), an agent whose entry into CSF is likely to be restricted. Aliquots of plasma or CSF (<100 μl) were added to 10 ml of a scintillation mixture. This mixture was composed of: naphthalene, 60 g; 2,5-diphenyloxazle (PPO), 4 g; 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP), 0.2 g; methanol, 100 ml; ethyleneglycol, 20 ml; and 1.4-dioxane to a final volume of 1 liter.

In a series of preliminary experiments, the authenticity of *d*-[^{3}H] amphetamine in biological fluids was confirmed. Aliquots of plasma and CSF from animals injected with *d*-[^{3}H]amphetamine were submitted to a dual analysis: liquid scintillation spectrometry and extraction with subsequent gas chromatographic assay. The results indicated that at least 90% of the recovered radioactivity was associated with *d*-amphetamine. This finding confirms that *d*-amphetamine metabolites were not obscuring the data. The finding also indicates that tritium in *d*-amphetamine was not exchanging with endogenous substances (*e.g.*, water).

Administration of drugs. The drugs used were: *d*-amphetamine sulphate (gift from Smith Kline and French Laboratories, Philadelphia, PA), chlorisondamine chloride (gift from Ciba Pharmaceutical Co., Summit, NJ) and atropine (Mann Research Laboratories, New York, NY). The vehicle for all injections was saline; the volume of fluid administered varied with body weight (1 mg/kg). All drug dosages are expressed as the free base.

Analysis of data. Data are presented as the mean ± S.D. The number of observations for each data point is provided in the text. Statements about statistical significance refer to use of the Student's *t* test; significance implies a P-value of .01 or less. Curves that have been fitted to data points were obtained by linear regression analysis.

Results

Rate of CSF formation. In a series of 10 animals, the rate of CSF formation was ~2.3 ± 0.1 μl/min.

The effect of hemodynamic changes on CSF formation was examined under three conditions: 1) after administration of *d*-amphetamine (1 mg/kg i.v.); 2) after administration of chlorisondamine (1 mg/kg i.v.); and 3) after administration of both. Under all three conditions, hemodynamic status changed markedly but CSF formation was unchanged.

A representative experiment illustrating the effects of *d*-amphetamine and chlorisondamine on blood pressure, heart rate and CSF formation is shown in figure 1. The administration of chlorisondamine, a ganglionic blocking agent, produced a lowering in blood pressure and heart rate but no concomitant changes in CSF formation. The subsequent administration of *d*-amphetamine produced increases in blood pressure and heart rate but no changes in CSF formation.

The foregoing data indicate that CSF formation remains relatively constant when hemodynamic status varies between two extremes: 1) loss of all efferent sympathetic activity due to ganglionic blockade and 2) supranormal sympathetic activity due to administration of *d*-amphetamine.

Rate of *d*-amphetamine egress from the circulatory system. At various times (fig. 2) after intravenous administra-

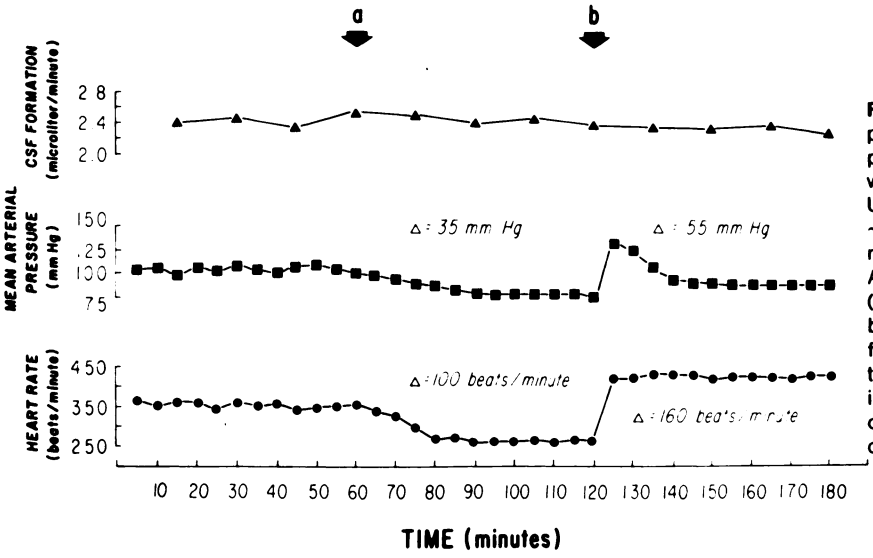

Fig. 1. The effects of increased and decreased sympathetic activity on heart rate, mean arterial blood pressure and CSF formation. The experimental animal was a rat (anesthetized, pretreated with atropine). Under control conditions, average heart rate was ~360 beats/min, mean arterial pressure was ~110 mm Hg, and rate of CSF formation was ~2.4 μl/min. After intravenous administration of chlorisondamine (1 mg/kg; a), heart rate decreased (~100 beats/min), blood pressure decreased (~35 mm Hg) but CSF formation did not change. After intravenous administration of *d*-amphetamine (1 mg/kg; b), heart rate increased (~160 beats/min), blood pressure increased (~55 mm Hg) but CSF formation did not change.

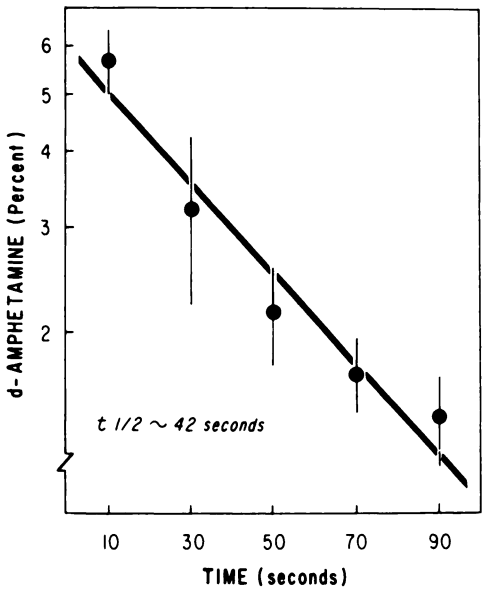

Fig. 2. Rate of *d*-amphetamine egress from the circulatory system. At various times after intravenous administration of *d*-amphetamine, animals (n = 5) were sacrificed and the percentage of injected *d*-amphetamine remaining in plasma was determined (see "Materials and Methods"). As the figure shows, egress of drug was very rapid ($T_{1/2}$ ~ 42 sec).

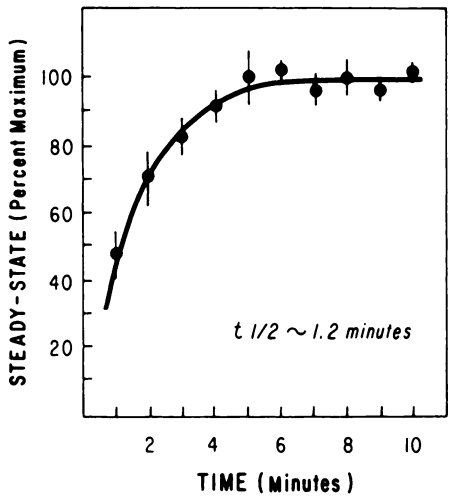

Fig. 3. Rate of *d*-amphetamine entry into CSF. At various times after intravenous administration of *d*-amphetamine, aliquots of CSF were assayed for drug (see "Materials and Methods"). The rate of entry of *d*-[^{3}H]amphetamine into CSF was the same in the presence and in the absence of a large excess of unlabeled *d*-amphetamine. Therefore, the data have been cumulated and presented as percentage of steady state. $T_{1/2}$ for drug entry into CSF was ~1.2 min.

tion of *d*-amphetamine (labeled plus unlabeled drug to produce a dose of 1 mg/kg) to rats (group n = 5), plasma was collected and assayed for drug. As previously reported (Simpson, 1978a), there is a rapid distributive phase during which *d*-amphetamine is lost from the circulation. The data in figure 2 indicate that the $T_{1/2}$ for egress is ~42 sec, yielding a rate constant of ~0.99 · min^{-1}.

Rate of *d*-amphetamine entry into CSF. In a series of five animals, the entry of *d*-[^{3}H]amphetamine (~100 μg/kg intravenous administration) into CSF was quite rapid (fig. 3). A similar result was obtained when *d*-[^{3}H]amphetamine was administered with an excess (10 ×) of unlabeled *d*-amphetamine. By analyzing the rate of approach to an asymptote, one can calculate that *d*-[^{3}H]amphetamine entered CSF in the control condition with a $T_{1/2}$ of ~1.2 min and a rate constant of ~0.58 · min^{-1}. The simultaneous adminstration of unlabeled *d*-amphetamine did not significantly change these values.

Rate of *d*-amphetamine egress from CSF. Two techniques were used to study the egress of *d*-amphetamine from CSF: 1) a push-pull experiment to determine clearance of drug and 2) a pull experiment to determine rate of loss of drug.

During push-pull experiments, *d*-[^{3}H]amphetamine (1×10^{-5} M) was perfused through the ventricular system (see "Materials and Methods"). In a series of five animals, clearance was calculated (Equation 4) to be 10.1 ± 2 μl/min.

In pull experiments, a bolus of *d*-[^{3}H]amphetamine (~2.3 μg, 10 μl) was injected into the lateral ventricle, after which samples of CSF were collected for 30 min (fig. 4.) Analysis of the data reveals that *d*-[^{3}H]amphetamine disappeared from CSF with a $T_{1/2}$ of ~11 min and a rate constant of ~0.063 · min^{-1}.

The effect of unlabeled *d*-amphetamine on the clearance and on the rate of disappearance of *d*-[^{3}H]amphetamine was determined. In clearance experiments, *d*-[^{3}H]amphetamine was perfused through the ventricular system with a 10-fold excess of unlabeled *d*-amphetamine. Similarly, in rate of disappearance experiments, *d*-[^{3}H]amphetamine was injected into the ventric-

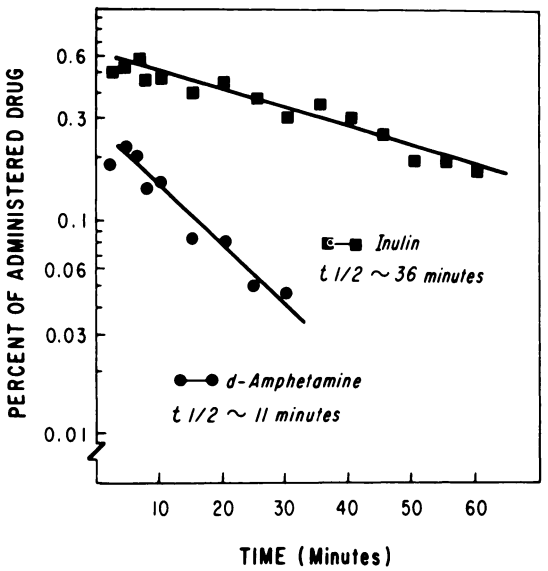

Fig. 4. Rate of drug egress from CSF. At various times after intracerebroventricular injection of either inulin or *d*-amphetamine, aliquots of CSF were assayed for drug (see "Materials and Methods"). The rate of egress of *d*-[^{3}H]amphetamine from CSF was the same in the presence and in the absence of a large excess of unlabeled *d*-amphetamine. Therefore, the data have been cumulated and presented as percentage of administered drug per microliter of CSF. $T_{1/2}$ for disappearance of *d*-amphetamine (~11 min) differed considerably from $T_{1/2}$ for disappearance of inulin (~36 min).

ular system with a 10-fold excess of unlabeled *d*-amphetamine. In neither case did excess *d*-amphetamine alter the kinetic behavior of *d*-[^{3}H]amphetamine.

Clearance and rate of disappearance of [^{14}C]inulin were also determined. As explained earlier, rate of CSF formation is equivalent to clearance of [^{14}C]inulin. Therefore, clearance of the drug is ~2.3 ± 0.1 μl/min (see above). Rate of disappearance of inulin was determined by methods identical to those just described for *d*-[^{3}H]amphetamine (fig. 4). $T_{1/2}$ for disappearance of the drug was ~36 min, yielding a rate constant of ~0.019 · min^{-1}.

Plasma:CSF ratio of *d*-[^{3}H]amphetamine. Ten minutes after intravenous injection of *d*-[^{3}H]amphetamine (labeled plus unlabeled drug to produce a dose of 1 mg/kg) to rats (n = 4), samples of plasma and CSF were collected. Comparison of plasma and CSF concentrations of *d*-[^{3}H]amphetamine revealed a plasma: CSF ratio of ~2.

Discussion

Theory and assumptions. There are relatively few studies that provide detailed kinetic analyses of drug entry into or egress from the CNS. The literature on *d*-amphetamine is especially limited. These matters suggest that any presentation of kinetic data should be explicit about assumptions, and it should assess the reasonableness of these assumptions.

The present study attempts to describe quantitatively *d*-amphetamine entry into CSF. The analysis of the kinetic behavior of *d*-amphetamine is based upon three assumptions: 1) all processes are first order; 2) all processes can be treated as though they were unidirectional and irreversible; and 3) overall rate constants can be fractionated into their individual rate constants. The validity of the first assumption is implicit in the data (*e.g.*, figs. 2, 3 and 4). The validity of the third assumption will be examined shortly.

An analysis of *d*-amphetamine entry into CSF is based on the assumption that, shortly after drug administation, flux from plasma to CSF can be treated as though it were unidirectional and irreversible. This assumption seems to be reasonable. The apparent rate constant for *d*-amphetamine moving into CSF (k_i ~ 0.58 · min^{-1}) is much greater than that for its movement out of CSF (k_o ~ 0.063 · min^{-1}). In addition, *d*-amphetamine is not an endogenous substance. Therefore, immediately after its intravenous administration, plasma concentration of drug is infinitely high relative to CSF concentration of drug. By definition, net flux (F) is related to the appropriate rate constants multiplied by plasma concentration ($[A]_p$) and CSF concentration ($[A]_{csf}$) of *d*-amphetamine, *i.e.*, $F = k_i [A]_p - k_o [A]_{csf}$. A comparison of k_i and k_o, and of $[A]_p$ and $[A]_{csf}$, indicates that, immediately after intravenous administration, *d*-amphetamine flux into CSF is essentially unidirectional and irreversible. Therefore, k_i can be viewed as a close approximation of the authenic rate constant describing the process of *d*-amphetamine entry into CSF.

Mechanisms and rates for *d*-amphetamine entry into CSF. The data support two conclusions: 1) *d*-amphetamine enters CSF mainly by diffusion and 2) a substantial fraction of the diffusing drug moves directly from plasma to CSF. These conclusions are based on the following considerations.

There are three mechanisms by which *d*-amphetamine might enter CSF: active transport associated with CSF formation, active transport independent of CSF formation and passive diffusion. The first two categories are not quantitatively important. The rate constant for *d*-amphetamine entry into CSF (~0.58 · min^{-1}) is so large compared to the rate constant for CSF formation. (~0.017 · min^{-1}) that the latter value contributes negligibly to the former. [The rate constant for CSF formation can be estimated as follows. Rate of CSF formation was ~2.3 μl/min (see above). The volume of the rat ventricular system ranges from 100 to 200 μl. Therefore, the rate constant for CSF formation ranges from 0.023 to 0.012 · min^{-1}, with an average value of 0.017 · min^{-1}.] Active transport independent of CSF formation is also unimportant. The fact that a large excess of unlabeled *d*-amphetamine did not alter *d*-[^{3}H]amphetamine entry into CSF makes an active transport mechanism unlikely.

Entry of *d*-amphetamine into CSF is mainly by diffusion. As already explained, the rate constant for entry into CSF is ~0.58 · min^{-1}. This value is close to that of the rate constant for *d*-amphetamine egress from the circulation (~0.99 · min^{-1}). These data suggest, but do not prove, that *d*-amphetamine moves directly from plasma to CSF, as opposed to moving from plasma to brain tissue to CSF.

Mechanisms and rates for *d*-amphetamine egress from CSF. There are three mechanisms by which *d*-amphetamine

TABLE 1

Rate constants for *d*-amphetamine entry into and egress from cerebrospinal fluid

The various mechanisms for drug movement and the determination of rate constants are described in the text.

	Entry	Egress
Diffusion	0.56·min^{-1}	0.044·min^{-1}
CSF formation	0.017·min^{-1}	
Bulk absorption		0.019·min^{-1}
Active transport		
Cumulative rate constant	0.58·min^{-1}	0.063·min^{-1}

might escape from CSF: active transport associated with bulk absorption, active transport independent of bulk absorption and passive diffusion. Of these three, only one is quantitatively unimportant. The fact that large excesses of unlabeled *d*-amphetamine did not alter either the rate of disappearance or the clearance of *d*-[^{3}H]amphetamine suggests that there is no active transport that is independent of bulk absorption.

The data on inulin can be used to determine both the mechanisms and rates for *d*-amphetamine egress from CSF. For example, the clearance of inulin, a drug that leaves CSF solely by bulk absorption, was ~2.3 μl/min. This is only a fraction of the clearance for *d*-amphetamine (~10.1 μl/min). A comparison of the data indicates that *d*-amphetamine must escape mainly by diffusion and only to a lesser extent by bulk absorption.

The individual contributions that bulk absorption and diffusion make to the overall process of *d*-amphetamine egress can be quantified. By definition, an overall rate constant (K) is the algebraic sum of its individual rate constants. Thus, K equals the rate constant for diffusion (k_d) plus the rate constant for bulk absorption (k_b). As determined experimentally, K is approximately 0.063 · min^{-1}. The value for k_b is equal to the rate constant for disappearance of inulin, *i.e.*, approximately 0.019 · min^{-1}. For reasons that are obvious, the rate constant for disappearance of inulin is equal to the k_b for any drug injected into CSF. Finally, the value for k_d can be obtained by subtraction and is approximately 0.044 · min^{-1}. The several rate constants describing *d*-amphetamine entry into and egress from CSF are summarized in table 1.

The diffusion constants describing movement into and out of CSF differ considerably. Nevertheless, at steady state the concentrations of free drug in plasma and in CSF are equivalent. In the rat, approximately one-half of the plasma concentration of *d*-amphetamine is protein bound (Baggot *et al.*, 1972). This means that, at steady state, the ratio of plasma concentration (free plus protein bound drug) to CSF concentration (only free drug) should be approximately 2. This is the result that was obtained.

The present study has considered the kinetics of single, acute injections of *d*-amphetamine. The applicability of the findings to multiple or chronic injections of *d*-amphetamine remains to be determined. However, there is reason to believe that chronic injections of the drug may alter its disposition (Kuhn and Schanberg, 1978). Therefore, continued study of the kinetics of *d*-amphetamine may help to explain its varied actions, particularly those that pertain to tachyphylaxis and tolerance.

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