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Report

Transport of Desglycinamide-Arginine Vasopressin Across the Blood–Brain Barrier in Rats as Evaluated by the Unit Impulse Response Methodology

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The pharmacokinetic characteristics of desglycinamide-arginine vasopressin (DGAVP) with respect to its transport across the blood–brain barrier (BBB) were studied with the use of serial CSF sampling in an individual animal and the unit impulse response methodology. Transport rate is determined as BBB clearance, the volume of plasma per unit time cleared of the peptide by BBB transport, and the extent of transport as the percentage of the administered dose transported into the central nervous system. Plasma kinetics of DGAVP were shown to be linear within the dose range studied (50–150 μg), plasma mean residence time (MRT) being 18 ± 4 min (mean ± SE; $n = 9$). Elimination of DGAVP from CSF after icv administration was linear, with an MRT of 10 ± 1 min ($n = 9$). After iv administration of 100 μg DGAVP, CSF concentrations were detectable for 90 min. Transport from plasma to the central nervous system was linear. The BBB transport clearance value was 1.0 ± 0.3 μl/min, and 0.026 ± 0.007% of the administered dose was transported into the central nervous system. Results demonstrate that, within the concentration range studied, DGAVP is transported across the BBB by passive diffusion, although to a very low extent.

KEY WORDS: desglycinamide-arginine vasopressin (DGAVP; AVP1-8); blood–brain barrier (BBB); transport; cerebrospinal fluid; unit impulse response methodology.

INTRODUCTION

Arginine vasopressin (AVP; AVP1-9) (Fig. 1) and its fragments display centrally mediated behavioral effects (1–4), in addition to the well-known peripheral antidiuretic and pressor activity. Vasopressin fragments and analogues are currently being studied for potential therapeutic application in mild memory disorders due to minor brain trauma, senile dementia, and Alzheimer's disease (5). The smaller fragments and analogues are virtually devoid of antidiuretic, pressor, and ACTH releasing properties but have retained their behavioral effects (6,7) or are even more potent than their parent peptide AVP (8).

Only limited attention has been paid to the pharmacokinetic profile of vasopressin-like peptides with respect to transport across the blood–brain barrier (BBB) (9,10), which is paramount to understanding the behavioral activities of these compounds. Recent literature reveals a vivid discussion on whether peptides can cross the BBB (11–14); nevertheless, substantial experimental evidence has accumulated lately, pointing to the fact that a variety of peptides is transported into the central nervous system upon peripheral administration (15–20).

In a previous study we used an *in vitro* BBB model to examine the transport characteristics of vasopressin and its fragments (21). These peptides were able to cross a monolayer of cerebrovascular endothelial cells to a measurable extent and were fairly stable to metabolic degradation by the endothelial cells. Molecular size had a significant effect on transport rate and transport was symmetrical and concentration independent, indicating that vasopressin and its fragments are transported across the *in vitro* BBB system, mainly by paracellular passive diffusion. Also, the existence of a carrier system in the BBB for transport of vasopressin-like peptides from brain to blood has been suggested (23). The results obtained with the *in vitro* model, however, failed to demonstrate the existence of such a carrier for DGAVP (21). Since BBB transport of peptides is generally extremely low, specific and sensitive *in vivo* methodologies are required to obtain reliable results. Conventional techniques have led to ambiguous results, mainly by lack of sensitivity of the analytical or pharmacokinetic procedures and large variability because of the need of many individual animals in single point methods.

We have recently developed a new experimental approach as an alternative for the conventional methodologies (22). With the use of the unit impulse response approach, complete BBB transport profiles can be obtained in one in-

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Fig. 1. The amino acid structure of arginine vasopressin.

dividual animal. This method has shown to be applicable over a wide range of transport rates (22). The use of CSF however offers a number of advantages. The use of CSF allows repeated sampling in an individual rat, thus enabling the determination of a complete transport profile in one individual animal as already suggested by Collins *et al.* (23), thereby avoiding the interanimal variation when each sample point represents one animal. Further, CSF concentrations, when compared with brain tissue concentrations, showed a better correlation with CNS drug effects than brain concentrations. For a number of centrally acting drugs, the CSF was pharmacokinetically indistinguishable from the effect compartment, implying that CSF concentrations and concentrations at the receptor site were alike (24,25) or that CSF concentration can be directly compared with brain parenchymal concentrations (26).

The aim of the present study was to establish the *in vivo* BBB transport profile of desglycinamide-arginine vasopressin (DGAVP; AVP1-8), currently one of the most frequently applied peptides to study centrally mediated effects of vasopressin-like peptides, using the unit impulse response methodology, and to test for carrier-mediated transport of DGAVP from brain to blood.

METHODOLOGY

BBB transport can be characterized by the time course of cumulative cleared plasma volume caused by that transport. Extensive theoretical and mathematical considerations have been published elsewhere (22,27). This model-independent procedure can be summarized as follows: in a group of rats the CSF concentration time profile is individually registered after intracerebroventricular administration of the peptide (unit impulse response). Each profile is fitted to an exponential function. The coefficient and exponent values are used to assess the mean curve (weighing function). In the second group of animals, peptide is administered intravenously and individual CSF concentration–time profiles are determined (response function).

The input function which describes the cumulative amount of peptide transported across the BBB as a function of time is obtained by deconvolution of the individual response function with the mean weighing function. From the input function, the cumulatively cleared plasma volume as a function of time $V(t)$ is calculated according to

$$V(t) = \Sigma_i \frac{I(t_i) - I(t_{i-1})}{C_p[t_{i-1} - t_i]} \quad (1)$$

where $C_p[t_{i-1} - t_i]$ is the logarithmic average of the plasma concentration of the peptide during the time interval between subsequent sampling events and $I(t)$ is the cumulative amount of drug transported into the central nervous system as a function of time. If transport across the BBB is governed by passive diffusion $V(t)$ is a linear function; the slope is a clearance value which is model independent and not influenced by the plasma concentration time profile and hence an adequate parameter for characterizing BBB transport rate. In case of nonlinearity, the existance of a potential carrier system and its kinetic parameters (V_{max}, K_m) can also be assessed from the input profile obtained by deconvolution (22).

MATERIALS AND METHODS

Chemicals and Peptides

DGAVP dicitrate (batch TH1390-AK111) was a gift of Organon International, Oss, The Netherlands. All chemicals used were of analytical grade and dissolved in freshly prepared Milli-Q-water (Waters Millipore, Millford, MA).

Animals and Surgery

Adult male SPF rats of Wistar descent, weighing 180–200 g and maintained on a commercially available diet (standard laboratory diets RMH-TH, Hope Farms, Woerden, The Netherlands), were used throughout the study. One day before the experiment the animals were anesthetized with a 150-μl intramuscular injection of Hypnorm (Janssen Pharmaceutica, Goirle, The Netherlands) and mounted in a stereotactic instrument. A 2-cm midsagittal incision was made to expose the skull and the periost was removed. For ventricular cannulas a hole was drilled using a 1-mm dental burr, 1.5 mm lateral and 0.8 mm rostral from the bregma point. A polyethylene cannula (i.d., 0.28 mm; o.d., 0.61 mm), with a broader part at 3.2 mm from the cannula tip serving as a depth marker, was inserted into the lateral ventricle and immediately fixated with a drop of cyanoacrylate glue. To avoid CSF leakage, the upper tip of the cannula was sealed until a few minutes before the start of the experiment. For cannulation of the cisterna magna, a hole was drilled on the sagittal midline, immediately rostral to the interparietal-occipital bone suture, using a 1-mm dental burr.

A stainless-steel cannula (o.d., 0.8 mm; i.d., 0.5 mm), with a copper disk at 8 mm from the lower tip serving as a depth marker, was inserted in the hole under a 45° angle. To prevent CSF leakage, a removable stainless-steel stopper was inserted into the cannula. In the parietal bone another hole was drilled for placement of a stainless-steel screw (1.2-mm diameter). The cannulas were anchored to this screw using dental cement (Simplex Rapid, Austenal Dental, Harrow, U.K.). On the day of the experiment, polyethylene cannulas were implanted in the left common carotid artery and in the right external jugular vein under light ether anaesthesia. After surgery the animals were given a 2-hr recuperation period before the start of the experiment.

Experimental Procedure

For icv administration, the cannula of the lateral ventricle was opened and connected to a piece of polyethylene tubing containing a solution of the peptide in saline. The open end of the tubing was connected to a motor-driven syringe pump and a fixed volume of 10 μl was administered

by switching on the pump for a fixed time (5–10 sec). The total volume of drug solution administered was corrected for the dead volume of the cannula. At regular time intervals, CSF samples (10–25 μl) were drawn from the cisterna magna cannula and collected in preweighted polystyrene tubes. Blood samples of 200 μl were drawn and heparinized. Plasma was obtained by centrifugation. CSF and plasma were stored at −20°C until DGAVP analysis. The objective was to sample for at least three half-lives of the peptide (based on CSF concentrations), being approximately 90 min.

To investigate dose dependence of DGAVP elimination from CSF, three different icv dosage regimens were given (0.05, 0.1 and 1 μg) and CSF concentration–time profiles were determined. To avoid excessive influence of intraindividual variability, each animal received the same dose three times on 1 day with a 3-hr interval between the successive experiments and exactly the same time schedule was maintained for each animal. For each dose three animals were used. In order to avoid variation due to factors such as stress and hydration, all animals were handled in a strictly standardized fashion, and before and during the experiments, the animals had free access to water.

At the end of the experiment the position of the cannula in the lateral ventricle was verified by injection of 5 μl of 0.5% Evans blue solution in saline. Then the rat was decapitated, the skull opened, and the brain removed and dissected by a paramedian coronal section. Blue discoloration of the entire ventricular system indicated a correct cannula position.

For iv administration the jugular vein cannula was connected to a motor-driven syringe pump containing a solution of the drug in saline. A fixed volume of 500 μl was infused in 1 min. Plasma kinetics were determined by iv administration of 50, 100, and 150 μg DGAVP in saline. Each dose was given to three animals.

At regular intervals, blood samples were obtained. To obtain BBB transport profiles, 100 μg DGAVP was administered iv to six rats and CSF and blood samples were obtained. Samples were stored at −20°C until analysis.

DGAVP Analysis

Concentrations of DGAVP in CSF and plasma were determined using a sensitive and specific radioimmunoassay (28). Cross-reactivity with endogenous AVP and DGAVP metabolites was lower than 6%. Detection limit was approximately 200 pg per ml in CSF and plasma. Interassay variation of the procedure was shown to be less than 15%.

Data Analysis

Basic plasma and CSF kinetic parameters were calculated by using model-independent pharmacokinetic analysis.

To check for dose-dependent elimination from plasma or from CSF, one-way analysis of variance of dose versus elimination clearance was performed.

The weighing function was calculated by means of modeling the CSF data following icv administration to an exponential function with negative exponent, using an extended least-squares optimization procedure (SIPHAR pharmacokinetic modeling software package, SIMED S.A., (Creteil, France). The mean weighing function was obtained from the individual constants and exponents. The response function was obtained from CSF concentration–time data after iv dosing. Subsequently, each response function was subjected to the point-area deconvolution with the mean weighing function, and after $I(t)$ was calculated, $V(t)$ was computed according to Eq. (1). Point-area deconvolution was performed by a custom made APL * PLUS program (STSC Inc., Rockville, MD) on a microcomputer. $V(t)$ was calculated from $I(t)$ and the respective plasma concentration time curve, using a specially designed spreadsheet application.

RESULTS

Sensitivity of the radioimmunoassay was satisfactory to obtain concentration–time profiles of DGAVP, in both CSF and plasma, for 90 min after iv or icv administration.

There was no significant effect of the administered dose of DGAVP on plasma clearance [$F(2,6) = 1.48$; $P = 0.30$] (Fig. 2). Therefore, the parameters from the three dosage groups were pooled to determine the mean kinetic parameters as shown in Table I.

CSF elimination clearance was independent of icv dose (Fig. 3) [$F(2,6) = 1.90$; $P = 0.23$] and hence the kinetic parameters for CSF elimination were calculated from pooled dose data (Table I). Subsequently, the weighing function for each individual animal was determined. When fitted functions were compared using Akaike values, monoexponential functions gave better fits than biexponential elimination functions. Coefficients of variation in the fitted parameters ranged between 5 and 15%. Mean coefficient was 2853 ± 944 ng/ml, and mean exponent 0.097 ± 0.013 min^{-1} (mean ± SE; $n = 6$).

Figure 4 shows a typical example of CSF and plasma concentration time profiles after peripheral administration of 100 μg DGAVP. The BBB input functions obtained by deconvolution reached plateau values 45–90 min after iv administration. When $V(t)$ was calculated according to Eq. (1), linearity was obtained and regression analysis was used to determine the slope value. The resulting BBB transport characteristics of DGAVP are summarized in Table II and Fig. 5.

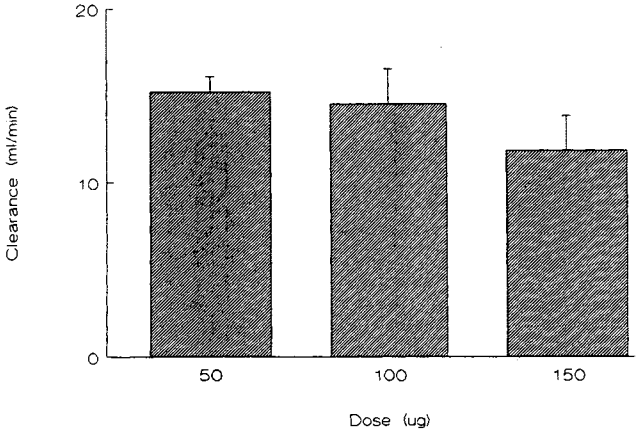

Fig. 2. The effect of the iv dose of DGAVP on plasma elimination clearance values.

Table I. Pharmacokinetic Parameters of DGAVP in Plasma After iv Administration and After icv Administration in CSF (Mean ± SE; $n = 9$)[a]

Parameter	Plasma	CSF
MRT (min)	18 ± 4	10 ± 1
Cl_{ss} (ml/min)	13 ± 1	0.05 ± 0.01
$V_{d,ss}$ (ml)	278 ± 57	0.7 ± 0.2

[a] MRT, mean residence time; Cl_{ss}, plasma clearance at steady state; $V_{d,ss}$, volume of distribution at steady state.

DISCUSSION

In this study we reveal the pharmacokinetic characteristics of DGAVP in rats with special reference to transport phenomena of DGAVP into and from the central nervous system.

DGAVP is relatively stable against enzymatic degradation (30), resulting in a relatively long residence time in plasma when compared with other peptides. Plasma clearance was dose independent (Fig. 2), indicating linear elimination kinetics in the dose range studied, which is in accordance with previous findings (31). Residence times and volumes of distribution are also in the same range as reported earlier (29).

Elimination of DGAVP from CSF appeared to be rapid (Table I), MRT being 10 min, and could best be characterized with a monoexponential distribution and elimination function. It should be noted that variation in the 0.05-μg dose group is much larger compared with the other groups (Fig. 3). This is probably due to the fact that the low CSF concentrations (approximately 1 ng/ml) were near the detection limit of the RIA, resulting in an inevitable amount of extra variation when compared with both other groups. To avoid excessive influence of within-animal variation, the mean clearance values of the three successive experiments were used for statistical testing. This showed no significant influence of DGAVP icv dose on the CSF elimination clearance, implying that transport from CSF to blood is dose independent in the dose range studied. This is in agreement with the results obtained in our recently developed *in vitro* BBB model (21). Banks and Kastin proposed the existence

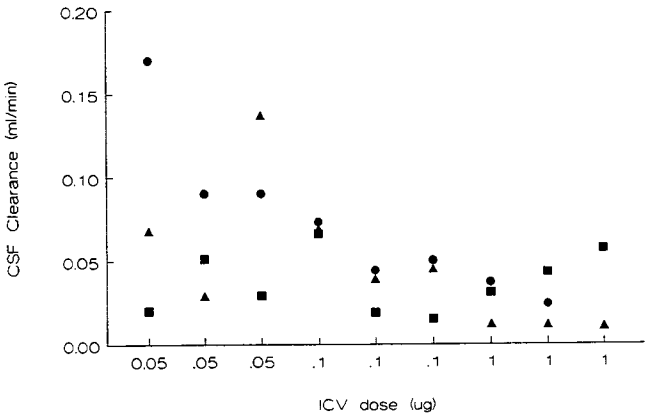

Fig. 3. The effect of the icv dose of DGAVP on CSF elimination clearance values; (■ first administration, ▲ second administration, ● third administration).

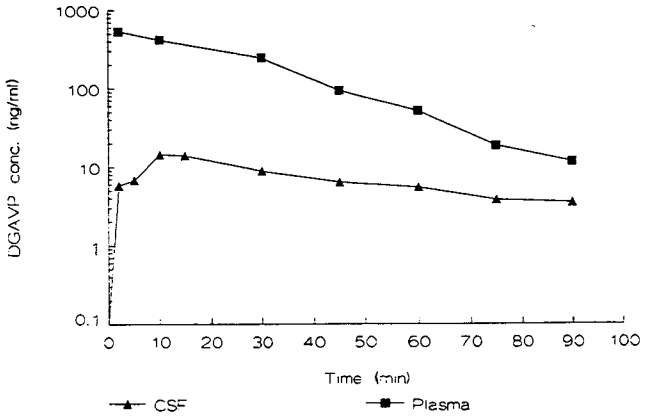

Fig. 4. Representative plasma and CSF concentration time profiles after iv administration of 100 μg DGAVP; (■ plasma concentration, ▲ CSF concentration).

of a carrier for arginine-vasopressin based on experiments in mice and they showed that vasopressin-like peptides could interact with brain to blood transport of vasopressin (27). The present study, however, does not give evidence for such a carrier-mediated transport of DGAVP in the dose-range studied. Direct comparison of the results from both studies is hazardous, however, since there are differences in species, the peptide used, and the experimental procedure. Competition experiments will be required to test for a possible carrier for DGAVP from brain to blood in rats.

The CSF concentration-time profiles can be individually modeled to a monoexponential function. Between the individual animals variation arises mainly from the values for the coefficients. The same phenomenon has been reported for other compounds using this methodology (22), and this result may be attributed to different physiological status (blood pressure, heart rate) of the animals studied, giving rise to differences in CSF mixing and pulsations (32,33). Nevertheless, the use of at least six animals for determination of the mean weighing function results in adequate accuracy as shown in the present study.

After iv administration of DGAVP, CSF elimination was markedly prolonged (Fig. 4) compared with icv administration, illustrating that blood to CSF transport is the rate-limiting step in CSF elimination. When plasma concentration decreases, the driving force for blood to CSF transport disappears; usually the input functions reached plateau values

Table II. Individual and Mean Parameters Characterizing BBB Transport of DGAVP (Mean ± SE; $n = 6$)

Rat No.	Amount transported (% of dose)	BBB clearance (μl/min)	Corr. coeff.[a]
ST44	0.060	1.96	0.97
ST45	0.025	1.15	0.98
ST46	0.021	1.56	0.98
ST47	0.023	0.72	0.98
ST48	0.025	0.20	0.94
ST60	0.004	0.42	0.95
Mean	0.026	1.0	
SE	0.007	0.3	

[a] Correlation coefficient of the individual $V(t)$ relationships.

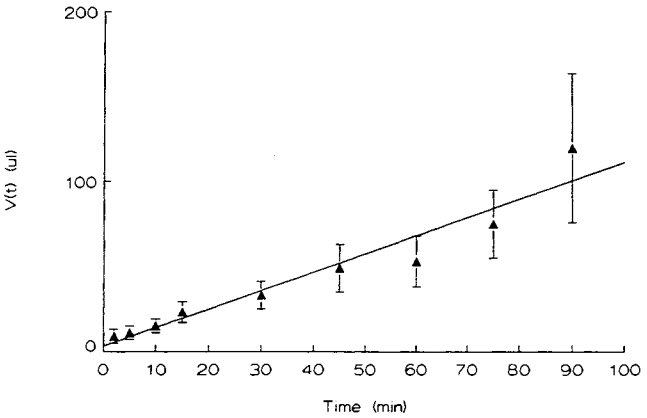

Fig. 5. Mean $V(t)$ relationship (mean SE; $n = 6$).

between 45 and 90 min after peripheral administration of DGAVP. Only about 0.03% of the administered dose is transported into the CNS in that time.

The resulting $V(t)$ functions were linear in time, and the BBB clearance values were very low but significantly different from zero ($P < 0.02$) (Table II). Both findings provide evidence that DGAVP is transported into the CNS by passive diffusion, which is in accordance with earlier results obtained with the *in vitro* BBB model (21). It has been shown that high picogram or low nanogram amounts in the central nervous system are required to evoke behavioral effects (8). From the presented data in Table II it can be calculated that a peripheral dose of the order of magnitude of tens of micrograms should be adequate to illicit a pharmacological response. This figure is in accordance with the empirical doses for iv or sc dosages found in the literature, which gave rise to a detectable behavioral effect of AVP or DGAVP (8,34).

From the previous *in vitro* experiments (35), it could be concluded that the most likely route for DGAVP transport is of paracellular nature, however, from the present *in vivo* results, no conclusion can be drawn on whether paracellular or transcellular pathways are involved. Recent work of Zlokovic *et al.* demonstrated that vasopressin interacted with the blood–brain barrier transport of delta sleep inducing peptide, indicating possible involvement of carrier mediation for vasopressin transport (36). In the present study, using a nanomolar plasma concentration range, transport of DGAVP into the central nervous system does not give evidence of relevant involvement of carrier mediation. It should be noted, however, that in the case of complete saturation, a carrier-mediated system will respond in a linear fashion and it is possible that desaturation and nonlinear effects may occur in a lower concentration range. It is apparent though, that, in contrast to what has been discussed and accepted as a paradigm for a long time, peptides can indeed enter the CNS by crossing the BBB. Several previous studies have failed to demonstrate brain and or CSF uptake after peripheral administration due to inadequate or unsensitive methodologies, for instance, after using single-passage techniques (37–39). However, with the technique presented in this paper, the low CNS transport of peptides can be determined and quantified. Other studies also show examples of adequate approaches by which transport of peptides, peptide-like compounds, or large hydrophilic compounds from blood to CNS was demonstrated. Heinzmann *et al.* (40) were even able to show extravasation in brain tissue of hydrophilic molecules up to a molecular weight of 150 kDa when using very sensitive detection techniques. The methodology we used is based on a multipassage approach with individual assessment of CNS transport, thereby avoiding interanimal variation.

In conclusion, our studies, using the unit-impulse response methodology, unequivocally demonstrate that DGAVP is transported across the blood–brain barrier and that transport is linear within the concentration range studied. Moreover, the experimental approach outlined in the present study can be expected to be suitable for other peptides as well.

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