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J. Pharm. Pharmacol. 1991, 43: 874–876
Communited June 18, 1991

Pharmacokinetics and brain entry of alaptide, a novel nootropic agent, in mice, rats and rabbits*

R. LAPKA, *Research Institute for Pharmacy and Biochemistry, Kouřimská 17, 130 60 Prague, Czechoslovakia*

Abstract—Pharmacokinetics of a novel nootropic agent, alaptide, have been examined in plasma and brain of mice, rats and rabbits following an intravenous dose (1 mg kg^{-1}). First-order equilibration rate constants between plasma and brain (k_{BO}) were calculated by a two-compartment model with a linked compartment (brain). Brain alaptide equilibrates rapidly with the central compartment in mice and rats due to the high k_{BO}/β ratio. In rabbits the equilibration is much slower ($k_{BO}/\beta \sim 1$). Partition coefficients between brain and plasma calculated from areas under the brain and plasma concentration-time curves, are 0·479, 0·549 and 0·864, in mice, rats and rabbits, respectively.

Alaptide, cyclo (1-amino-1-cyclopentanecarbonyl-L-alanyl), has been developed as a derivative of melanocyte-stimulating hormone-release inhibiting factor (MIF, L-prolyl-L-leucyl-glycinamide) in the search for neuropeptides with effects on learning and memory. It has been shown to increase avoidance latencies in a passive avoidance procedure in rats after oral administration and to attenuate amnesia induced in this type of experiment by electroconvulsive shock (Krejčí et al 1986a, b). Antiamnesic effects could be demonstrated in conditioned taste aversion experiments where amnesia was produced by repeated electroconvulsive shock treatment (Krejčí 1987). A beneficial effect on short-term memory was observed in a social recognition test (Hliňák et al 1990). Pharmacological and biochemical studies indicate that alaptide influences the dopaminergic system: an increase in homovanillic acid was found in the striatum of the rat (Krejčí et al 1986b; Dlohožková et al 1989). Alaptide attenuates the development of tolerance to neuroleptic drugs using the catalepsy test in rats and prevented the decrease of striatal homovanillic acid after the withdrawal of the neuroleptic agent (Valchář et al 1985).

For many drugs, the drug effect lags behind the drug plasma concentration, as the transfer of drug from blood to the site of action is not instantaneous (Maitre et al 1990). To study the pharmacodynamic characteristics of a drug in non-steady state situations a hypothetical drug effect compartment has been proposed (Sheiner et al 1979). The parameter characterizing this link model is the equilibration rate constant k_{EO}. Since of central interest in pharmacodynamic/pharmacokinetic investigation of centrally acting drugs is the relationship among the concentrations of a drug in the sampling compartment (mostly plasma), in the effector site (e.g. brain tissue, cerebrospinal fluid), and the effect, we have tried to model the relationship between plasma and brain alaptide concentrations in three animal species.

*Parts of this investigation were presented at the "33rd Psychopharmacological Conference", Jesenik Spa, 1991.

Materials and methods

Materials. [^{3}H]Alaptide was prepared by exchange reaction with ^3H_2O. The final product had a specific activity of 33·3 MBq mg^{-1} and radiochemical purity 99·7%.

Animal studies. Male NMRI mice (25 g), male Wistar rats (180–200 g) and male chinchilla rabbits (3·5–4 kg) were given 1 mg [^{3}H]alaptide kg^{-1} by intravenous bolus injection. At various times following the dose mice and rats were killed by decapitation under ether anaesthesia, blood was collected into heparinized test-tubes, and their brains removed. In rabbits, blood was obtained from the ear vein and cerebrospinal fluid (CSF) was obtained by cannulation of the posterior ventricle. At 1 and 6 h alaptide was also detected in rabbit brain homogenates. During the experiment the animals were housed in metabolic cages for urine collection. Plasma, brain homogenates, CSF and urine were kept at −20°C until analysis.

Radioactivity determination. Radioactivity in 100 μL of biological fluids was detected by liquid scintillation counting with external standardization on a Packard 2200 LSC.

Chromatography of urine. Twenty μL of urine was separated by thin layer chromatography (TLC) on Silica Gel 60 (Merck, Darmstadt, Germany) with unlabelled alaptide as a standard.

The plates were eluted in n-butanol:acetic acid:water (4:1:1, v/v/v). Alaptide was visualized by iodine vapour and radioactivity detected by autoradiography.

Binding of alaptide to bovine serum albumin (BSA). [^{3}H]Alaptide (0·1–10 μg mL^{-1}) dissolved in isotonic Krebs-Ringer phosphate buffer, pH 7·4, was dialysed against 4% BSA in the same buffer for 20 h at 37°C (Verner et al 1984).

Pharmacokinetic analysis. The pharmacokinetics of plasma alaptide can be described by the sum of two exponential terms. Since the time course of brain alaptide is not identical with the calculated profile in the peripheral compartment based on data in plasma, we added the third (brain) compartment to the model (Fig. 1). The distribution volume, V_B, of this compartment was set to 0·0145, 0·0075 and 0·0039 L kg^{-1} in mice, rats and rabbits, respectively (Boxenbaum 1984). Based on plasma data the parameters of the model (V_C, k_{10}, k_{12}, k_{21}, β-hybrid elimination constant) were calculated using non-linear regression with the program MULTI (Yamaoka et al 1981). Constants characterizing the linked model (k_{1B}, k_{BO}) were computed using fixed parameters of the two-compartment model and brain alaptide concentrations (Colburn 1981).

The partition coefficient between brain and plasma was calculated from the ratio of the areas under the brain concentration vs time curve (AUC_B) and the plasma concentration vs time curve (AUC_P) (Gibaldi & Perrier 1975; Gonzales et al 1975; Hedaya & Sawchuk 1989). The area terms were calculated by the linear trapezoidal rule and extrapolated to infinity using the final concentration divided by the terminal rate constant.

Results

No evidence could be found for the formation of any alaptide metabolites in the urine of any of the animal species. Thus alaptide concentrations could be determined directly by liquid scintillation counting.

Alaptide binding to BSA is low; at concentrations from 0·05 to 5 μg mL^{-1} only 7·5% on average was bound to albumin in a concentration-indepent manner.

Fig. 2 shows the time course of plasma and brain/CSF alaptide concentrations in mice, rats and rabbits after an intravenous bolus. In separate experiments we compared alaptide concentrations in the brain homogenate and CSF of the rabbit. Almost identical values were found, therefore only CSF data were recorded thereafter in rabbits and brain concentrations in mice and rats. Brain or CSF concentrations of alaptide peaked from 0·5 h (mice) to 2 h (rabbits).

Table 1 summarizes various parameters of the model described in Materials and methods. The constant k_{BO} characterizes the output from the brain compartment and is the driving force for brain concentrations.

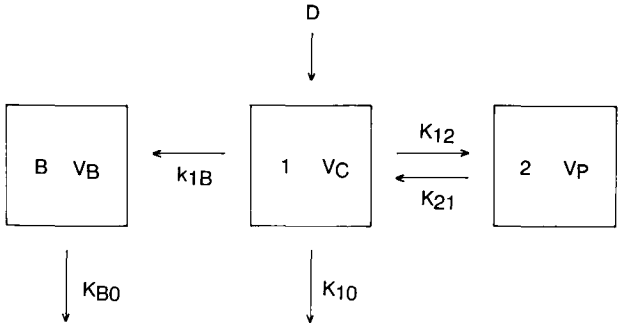

FIG. 1. Pharmacokinetic model for curve-fitting of plasma and brain concentrations. B represents brain compartment, 1 central compartment, and 2 peripheral compartment.

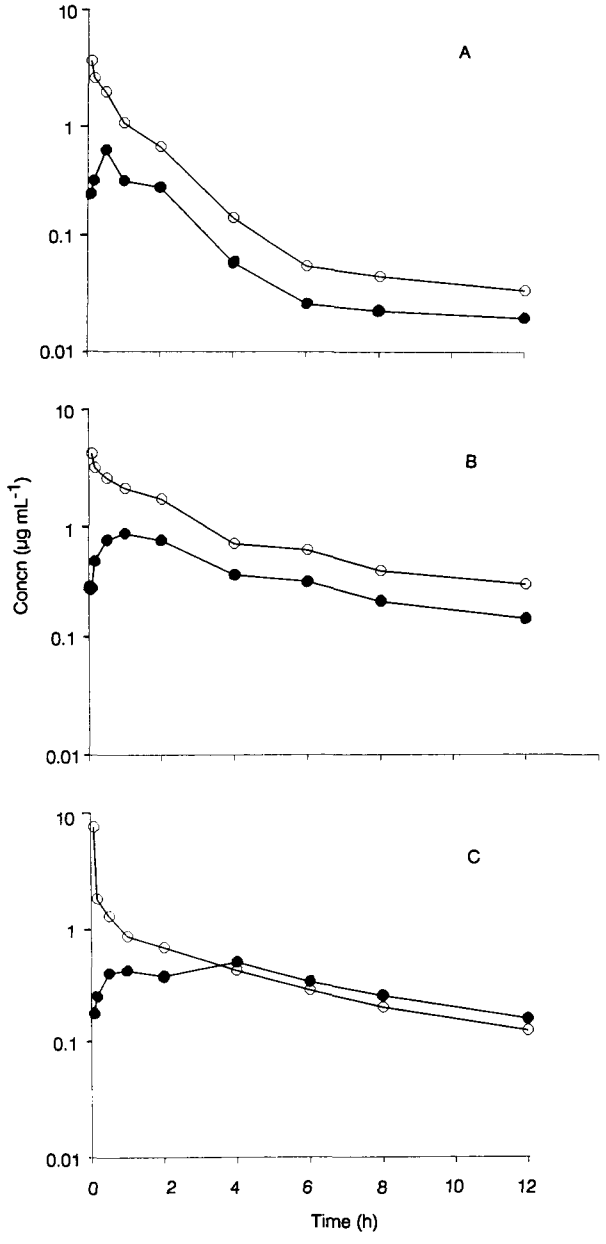

FIG. 2. Mean plasma (○) and brain (●) alaptide concentrations following intravenous bolus injection (1 mg kg^{-1}) to mice (A, n = 4), rats (B, n = 4), and rabbits (C, n = 3).

Discussion

By giving a drug as an intravenous bolus, we can estimate the rate of blood-brain equilibration, a parameter that estimates the onset properties of the drug effect. Sheiner et al (1979) have shown that the model-predicted drug concentration at the site of action (i.e. brain) is independent of the transfer rate constant (k_{1B}), which relates the amount in the brain to the concentration of drug in the central compartment. This means that the brain amount is a function of the pharmacokinetic profile in plasma and the rate constant k_{BO}.

Inspecting the brain and plasma concentration-time profiles in different species, we were surprised by the species-dependent

Table 1. Pharmacokinetic parameters of alaptide in three animal species.

		Parameter							
Species	n	V_C (L kg^{-1})	k_{10} (h^{-1})	k_{12} (h^{-1})	k_{21} (h^{-1})	k_{1B} (h^{-1})	k_{BO}(h^{-1})	β(h^{-1})	AUC_B/AUC_P
Mouse	4	0·783 (6·6)	0·607 (17·1)	0·266 (33·5)	0·168 (86·9)	0·0268 (19·8)	4·19 (23·9)	0·109 (112)	0·479
Rat	4	0·733 (5·0)	0·177 (24·9)	0·281 (26·0)	0·190 (71·1)	0·0110 (58·2)	2·05 (70·7)	0·0569 (99·3)	0·549
Rabbit	3	0·0290 (96·2)	7·49 (55·8)	16·2 (22·2)	0·905 (55·9)	0·0393 (62·3)	0·245 (15·5)	0·229 (17·0)	0·864

In mice and rats n represents the number of animals in each pooled concentration-time point and parameters are presented as their final value (% asymptotic coefficient of variation). In rabbits n denotes the number of animals and parameters are presented as mean parameter (% coefficient of variation).

changes: relative brain (with respect to plasma) concentrations followed the order of the size of the species. We therefore tried to assess brain:plasma partition coefficients at steady-state, by using the model-independent method based upon AUC ratios. The cause of nonequal partition coefficients in different species may be due to several factors, such as binding to blood proteins and tissue components, metabolism of the drug, diffusion in and out of the tissue, and bulk flow of the CSF (Gonzales et al 1975). Since binding of alaptide to plasma proteins as well as its metabolic clearance are negligible, one can speculate that the most significant reason for species-dependent partition coefficients is the uneven diffusion in and out of the brain, which may reflect the efficiency of a transporter system. However, the exact nature of such a phenomenon is unknown.

My thanks are due to Dr S. Šmolík for the preparation of [^{3}H]alaptide, Mrs M. Vinařová for technical assistance, and Dr M. Fišerová for the cannulation of rabbits.

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