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Leucine Modulates Peptide Transport System-1 Across the Blood-brain Barrier at a Stereospecific Site within the Central Nervous System

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Abstract—Previous results have shown that leucine injected into a cerebral ventricle (i.c.v.) can act as an allosteric regulator of peptide transport system-1 (PTS-1), the system that transports Tyr-Pro-Leu-Gly-NH_2 (Tyr-MIF-1) and the enkephalins out of the central nervous system (CNS). D-Leucine appeared more potent than L-leucine. In the current study, dose–response curves were constructed for each compound after both intravenous (i.v.) and i.c.v. injection. Based on ED50 values after i.c.v. injection, D-leucine was about 200 times more potent than L-leucine in its inhibition of PTS-1, thereby confirming stereospecificity of the allosteric site. D- and L-Leucine were also more potent when given i.c.v. than when given i.v., suggesting that the site is located on the CNS side of the blood–brain barrier (BBB). The finding that D-leucine was less potent than L-leucine when given i.v. is also consistent with a CNS site of action because the L-isomer of leucine has been shown to be preferentially transported into the brain. These findings agree with the previous suggestion that some of the neurotoxic effects of leucine may be mediated through PTS-1 and could help explain how D-amino acids can exert opiate-related effects on the CNS.

Leucine has recently been found to act as an allosteric modulator (Banks & Kastin 1986) of peptide transport system-1 (PTS-1), the system that transports selected opiate-related peptides from the central nervous system (CNS) to the peripheral circulation (Banks et al 1986; Banks & Kastin 1990). Because hyperleucinaemia and an excess of opiate peptides have been associated with similar neurotoxic lesions (Menkes et al 1954; Waisman et al 1962; Brandt et al 1980), it was suggested that some of leucine's toxicity might be mediated through its effects on PTS-1. Since D-leucine was more potent than L-leucine after intracerebroventricular (i.c.v.) injection, it seemed that the regulatory site might be stereospecific.

It is not known whether this regulatory site of action of leucine on PTS-1 is located on the brain or blood side of the blood–brain barrier (BBB). Such sites for other substances have often been found to occur on the blood side of the BBB (Rudman & Kutner 1978; Hervonen & Steinwall 1984; Brust 1986). The stereospecificity of the site should make it possible to determine whether it is located on the CNS or peripheral side of the BBB because the BBB is more permeable to L- than to D-leucine (Oldendorf 1973). We, therefore, compared the relative abilities of D- and L-leucine after i.c.v. and intravenous (i.v.) administration to alter PTS-1.

Materials and Methods

Male ICR mice, 25–30 g, from Charles River Laboratory (Wilmington, MA) were housed under 12 h light/12 h dark cycles for at least 48 h with food and water freely available. Mice were randomly assigned to the treatment groups. Leucine was purchased from Sigma Chemical Co. (St. Louis, MO) and administered in lactated Ringer's solution.

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Intravenous administration

Mice were anaesthetized with urethane and the right jugular vein exposed. A response curve over time was constructed after injection of 20 μmol/mouse (0·2 mL) of leucine (D- or L-) into the jugular vein 2, 5, 10, 20, 30, or 60 min before assessment of the brain to blood transport rate of [^{125}I]-Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2). The time of optimal response (10 min) was used to test L- or D-leucine in doses ranging from 0·006 to 20 μmol i.v.

Intraventricular administration

Mice were anaesthetized with urethane and part of the skull exposed by removal of the scalp. A hole was made into the skull and 100 nmol of leucine was injected into the lateral ventricle in a volume of 1 μL of lactated Ringer's solution 2, 5, 10, 20, 30, or 60 min before assessment of the brain to blood transport of [^{125}I]-Tyr-MIF-1. Dose-response curves were determined after injection of 0·001 to 130 nmol of either D- or L-leucine i.c.v. 30 min before determination of transport rate.

Intraperitoneal administration

The acute effect of a single dose of 200 μmol (2 mL) of D- or L-leucine given i.p. 60 min earlier on the transport of [^{125}I]-Tyr-MIF-1 was determined. In another study, the effect of chronic D- or L- leucine was determined after i.p. injections of 200 μmol (2 mL) at 0800 and at 1600 h for 4 days. On day five, mice received an injection at 0800 and at 1200 and were studied at 1300 h.

Transport of [^{125}I]-Tyr-MIF-1

The method of Noble et al (1967) for intraventricular injection as adapted for quantifying brain to blood transport (Banks & Kastin 1989) was used (Banks & Kastin 1986; Banks et al 1990). Mice were anaesthetized with urethane and one μL of lactated Ringer's solution containing 25 000

counts min^{-1} of radioiodinated Tyr-MIF-1 was injected into the lateral ventricle with the use of a one μL Hamilton syringe (Reno, NV). Mice were decapitated 10 min later and the amount of peptide available for transport was determined as previously described (Banks & Kastin 1989) in mice overdosed with anaesthetic. The results are expressed as the percent of the transport in control mice as previously shown (Banks & Kastin 1988). Because the procedure of i.v. injection, but not that of i.c.v. or i.p. injection, was associated with some inhibition in transport rate, time-matched injected controls were used in all i.v. experiments.

Tyr-MIF-1 was radioiodinated with ^{125}I by the chloramine-T method and the reaction halted by sodium metabisulphite. The monoiodinated form was isolated by HPLC and contained 5·6 fmol of peptide per 25 000 counts min^{-1}.

Statistics

Analysis of variance (ANOVA) was followed by Duncan's multiple range test. Regression lines were computed by the least squares method and the ED50 values derived from these.

Results

Intravenous administration

ANOVA showed a statistically significant effect of 20 μmol leucine on transport: $F(12,68)=2·16$, $P<0·05$ (upper panel, Fig. 1). Duncan's multiple range test showed a significant ($P<0·05$) effect for this dose of D-leucine at 5 min and for

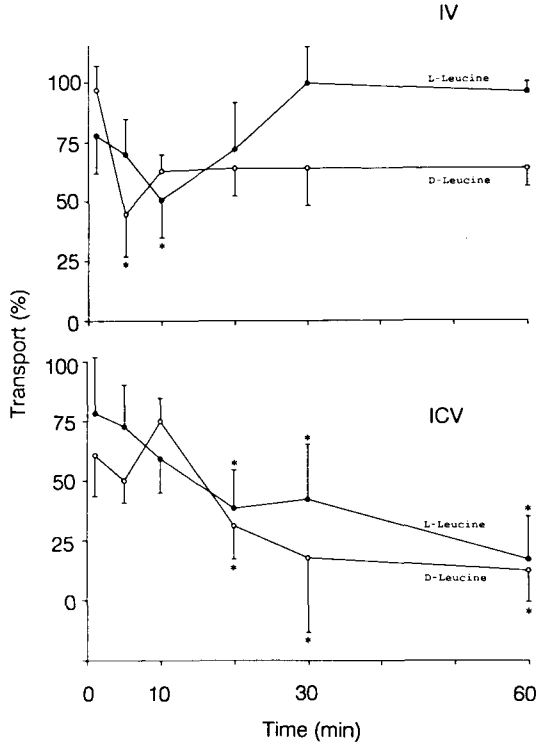

FIG. 1. Effect of D- (○) or L-leucine (●) on the transport rate of [^{125}I]-Tyr-MIF-1 by PTS-1. A dose of 20 μmol leucine was given as an intravenous bolus (upper panel) or a dose of 100 nmol was given intraventricularly (bottom panel). The abscissa indicates time elapsed between administration of leucine and radioactively labelled Tyr-MIF-1. 6–8 mice were used per point. * $P<0·05$ in comparison with controls (100%).

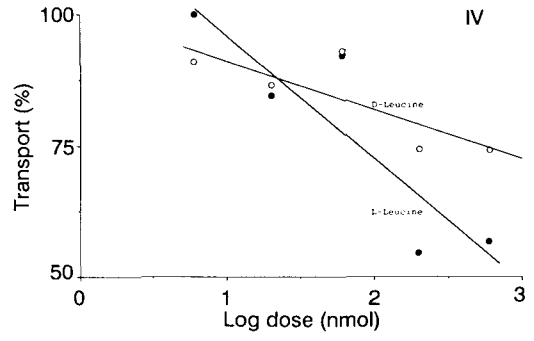

FIG. 2. Effect of varying doses of D- (○) or L-leucine (●) given intravenously on the transport rate of [^{125}I]-Tyr-MIF-1 by PTS-1. Each point represents about 5 mice.

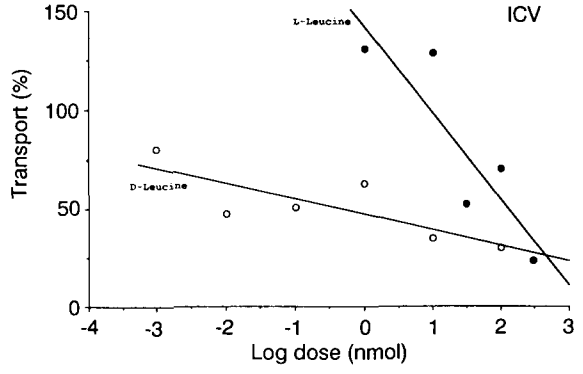

FIG. 3. Effect of varying doses of D- (○) or L-leucine (●) given intraventricularly on the transport rate of [^{125}I]-Tyr-MIF-1 by PTS-1. Each point represents about 5 mice.

L-leucine at 10 min. Based on this information, 10 min was selected as the time point used for the dose-response curves.

The dose-response curves for i.v. D- and L-leucine are shown in Fig. 2 and are based on results with doses from 6 to 600 nmol. The curves were not reliably linear at doses above this range, with transport rates increasing above maximal inhibition at some doses. The ED50 for D-leucine was 263 μmol and for L-leucine was 0·924 μmol.

Acute i.p. administration of D-, L-leucine, or vehicle (0·9% NaCl) did not alter transport rate. Chronic administration twice a day for 5 days did not alter either transport rate or body weight.

Intraventricular administration

ANOVA showed a statistically significant effect for the administration of 100 nmol leucine: $F(12,80)=2·37$, $P<0·05$ (bottom panel, Fig. 1). Duncan's multiple range test showed that both D- and L-leucine produced statistically significant ($P<0·05$) inhibition of transport at 20, 30, and 60 min. Based on these results, 30 min was selected as the time at which the dose-response curve relationship was tested. The ED50 for D-leucine was 0·381 nmol and the ED50 for L-leucine was 80·2 nmol (Fig. 3).

Discussion

These results confirm previous findings (Banks et al 1986; Banks & Kastin 1986) that the amino acid leucine, when given i.c.v., can inhibit the brain to blood transport of [^{125}I]-

Tyr-MIF-1 as mediated by PTS-1. Previous results had suggested that D-leucine given i.c.v. could inhibit PTS-1 at a lower dose than could i.c.v. L-leucine (Banks & Kastin 1986). This was confirmed here by the finding of a lower ED50 for D- than for L-leucine. In addition, either D- or L-leucine can inhibit transport when the route of administration is i.v. Therefore, the leucine-sensitive allosteric regulatory site for PTS-1 does exhibit stereospecificity.

It was found that the ED50 with i.c.v. injection was lower than the ED50 with i.v. injection for either D- or L-leucine. This makes it likely that the site of action for modulation of PTS-1 is located within the CNS. Similarly, the lack of effect with i.p. administration makes it less likely that the site of action is readily accessible from this route. A higher potency after CNS administration, however, is only presumptive proof that the site of action is in the CNS. Substances given centrally can enter the peripheral circulation by reabsorption of cerebrospinal fluid, diffusion across the membranes that comprise the BBB, or by saturable transport mechanisms. As a result, central administration may sometimes mimic the effects of an i.v. infusion (Davson et al 1987a). Nevertheless, more potent effects with CNS administration can, in general, be considered strong evidence for a CNS site of action.

Location of the regulatory site on the CNS side of the BBB would also explain the otherwise paradoxical finding that, when assessed by ED50 values, D-leucine is more potent than L-leucine when they are given i.c.v. but less potent when given i.v. This is because the BBB preferentially transports L-leucine into the CNS (Oldendorf 1973) and also transports L-leucine out of the CNS (Davson et al 1982). Therefore, when given i.v., L-leucine is able to enter the CNS more readily than D-leucine and so can achieve higher doses within the CNS where the regulatory site is presumably located. When given centrally, however, brain to blood transport would decrease concentrations within the CNS more rapidly than if exit were exclusively by reabsorption of CSF (Davson et al 1987b) so that relatively less is available at the regulatory site within the CNS.

A CNS site of action is consistent with the findings of others (Cusick et al 1978; Hutchison et al 1983) showing that the neurotoxic effects of a diet deficient in valine are due to the transport of leucine by the BBB. Valine and leucine, both branched-chain amino acids, are transported by a common system across the BBB so that a decrease in the serum concentration of one allows increased transport of the other (Oldendorf 1973). This explains why a diet deficient in valine, but not in valine and leucine, produces increased concentrations of leucine in the brain (Hutchison et al 1983) and neurotoxicity (Cusick et al 1978). Valine, unlike leucine, does not affect PTS-1 when given i.c.v. (Banks & Kastin 1986).

A potent effect for D-leucine on PTS-1 is also consistent with previous findings. Some D-amino acids, including D-leucine, can exert opiate-like effects on analgesia and food intake (Bodnar & Butler 1983; Albonetti et al 1985). These effects could be explained by the ability of D-leucine to inhibit PTS-1, a system which transports some opiate peptides from the brain to the blood, thereby allowing the accumulation of these opiate peptides within the CNS. This action might be involved in those conditions in which D-amino acids accumulate, such as with some genetic mutations or in renal disease (Nagata et al 1989). A CNS site of action is also consistent with the possibility that the neurotoxicity of leucine in newborns (Menkes et al 1954; Waisman et al 1962) might be mediated through PTS-1 (Banks & Kastin 1986).

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