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RAPID COMMUNICATION

Orally Administered cyclo(His-Pro) Reduces Ethanol-Induced Narcosis in Mice

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BANKS, W. A., A. J. KASTIN AND J. B. JASPAN. *Orally administered cyclo(His-Pro) reduces ethanol-induced narcosis in mice.* PHARMACOL BIOCHEM BEHAV **43**(3), 939-941, 1992.—Cyclo(His-Pro) (cHP) is an endogenous, enzymatically resistant, biologically active peptide. We examined its ability to be absorbed after oral administration. cHP radioactively labeled with ^{125}I (I-cHP) was fed to adult mice, and blood and tissue samples were taken 15-90 min later. Radioactivity quickly appeared in blood at levels about one half to one fourth those previously found after IV injection. The highest concentrations were in the kidney and liver, but the testes, muscle, lung, and brain also contained more radioactivity than was accounted for by their vascular spaces. Between 25-32% of the radioactivity recovered from blood 30 min after feeding eluted on high-performance liquid chromotography in the position of intact peptide. Oral cHP reversed ethanol-induced narcosis, an effect previously found to occur within the brain. These results show that cHP can be absorbed orally in amounts sufficient to affect the CNS.

Brain Blood-brain barrier Gastrointestinal absorption Ethanol Peptide Sleep

CYCLO(His-Pro) (cHP) is a highly stable, biologically active peptide produced by numerous tissues including the CNS. cHP was originally described as a degradation product of thyrotropin-releasing hormone (TRH), but only about 4% of cHP is produced in this way (11,13). Thus, rather than being a metabolic product cHP is largely an independently produced peptide that seems to have its own profile of actions, including the ability to reverse ethanol-induced narcosis (14). cHP is about 200 times more potent when given into the lateral ventricle of the brain (ICV) than when given IV, indicating a site of action within the brain (4).

cHP is resistant to enzymatic degradation, perhaps because of its cyclization. This raises the possibility that cHP might be effective orally. Other peptides, including TRH (9), Pro-Leu-Gly-NH_2 or MIF-1 (7,12), and an analog of luteinizing hormone-releasing hormone (LHRH) (6) have been shown to exert effects after oral administration. Delta sleep-inducing peptide (3) and β-casomorphin (15) are absorbed by the gastrointestinal tract in the neonate as are some small peptides in the adult (5). We, therefore, determined whether cHP could be absorbed in intact form after oral administration in quantities sufficient to produce a biologic effect.

METHOD

Iodination

cHP purchased from Sigma Chemical Co (St. Louis, MO) was radioactively labeled with ^{125}I (I-cHP) by the chloramine T method. Products were separated by high-performance liquid chromatography (HPLC) on a C-18 column. Specific activity was about 200 Ci/g.

Oral Administration

Male ICR mice (Charles River Laboratories, Wilmington, MA) weighing 25-35 g were housed in a 12L : 12D climate-controlled animal facility with food and water freely available. Mice were fed 1.1(10^7) cpm of I-cHP in a volume of 20 μl lactated Ringer's solution containing 1% bovine serum albumin (BSA). Five minutes before removal of blood from the carotid artery, mice were anesthetized with IP urethane. Mice were then decapitated, portions of the organs weighed, and radioactivity determined in a gamma counter. Blood from the carotid artery was centrifuged at 4,000 × *g* at 4°C for 10 min and radioactivity in the serum determined. Results for serum were expressed as cpm/ml and for organs as 10^3(cpm/g or-

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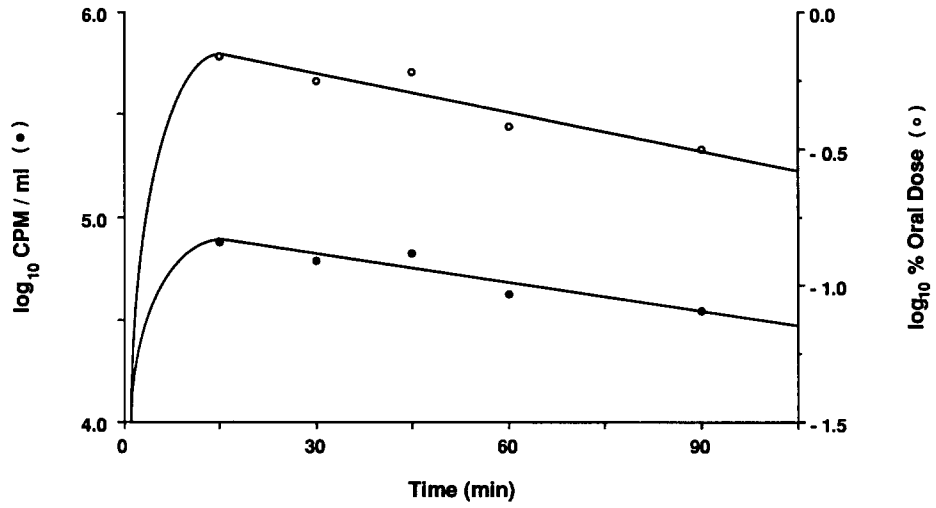

FIG. 1. Relationship between concentration of radioactivity in blood and time after oral feeding of I-cHP.

gan)/(cpm/ml serum) = μl/g. The values used to correct for vascular space were those previously reported (2,8).

Identification of Circulating Radioactivity

Serum obtained from the carotid artery 30 min after feeding I-cHP was lyophilized, reconstituted in distilled water, and submitted to analysis by reversed-phase HPLC on a C-18 column.

Ethanol Narcosis

Male mice were feed 100 μl lactated Ringer's solution containing 1% BSA with or without 2 mg cHP. Five to 10 minutes later, mice were given 5.5 g ethanol/kg body weight IP as a solution of 50% ethanol in isotonic NaCl. Mice were placed on their backs after the onset of narcosis and the time to regain the righting reflex measured. Mice that did not achieve narcosis or that did not right by 180 min were excluded from analysis.

Statistics

Means are presented with their standard errors and compared by analysis of variance (ANOVA).

TABLE 1

UPTAKE BY VARIOUS TISSUES OF INGESTED I-cHP

Organ	I-cHP	I-cHP$_{corrected}$*
Brain	48.9 ± 7.8	37.4 ± 7.8
Liver	1071.7 ± 400.7	980.7 ± 400.7
Kidney	3160.0 ± 383.1	3002.0 ± 383.1
Testes	308.0 ± 17.4	286.1 ± 17.4
Lung	862.7 ± 274.0	684.7 ± 274.0
Muscle	355.3 ± 21.5	350.2 ± 21.5

Results are reported as means ± SE (n = 3). Results are expressed as μl/g; *corrected for vascular space.

RESULTS

Figure 1 shows the level of radioactivity in blood over time. Table 1 shows the mean level of radioactivity in the tissues at 15, 30, and 45 min.

Three major peaks eluted by HPLC. The first peak eluted in the position of free iodine and contained 68% of the total radioactivity found under the peaks, the second peak eluted in the position of I-cHP and contained 25% of the radioactivity, and the third peak eluted in the position of the stereoisomer of I-cHP (16) and contained 7% of the radioactivity.

Figure 2 shows that cHP reduced sleep time by 28.6%, a statistically significant difference, $F(1, 10) = 6.36, p < 0.05$. Excluded a priori from analysis were three mice in the group

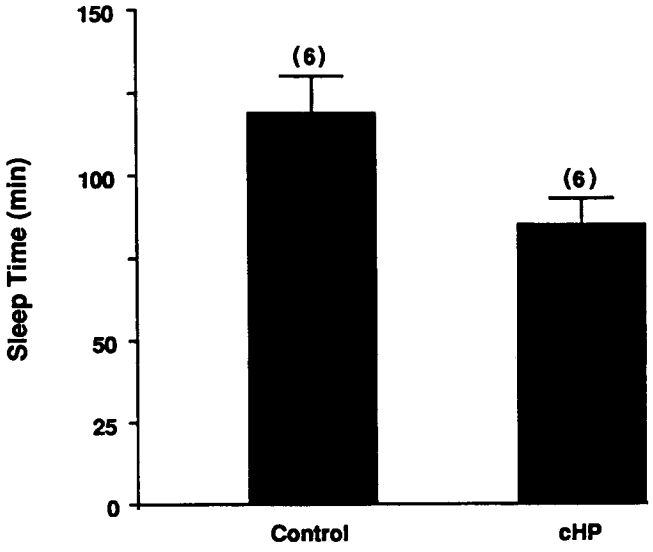

FIG. 2. Effect of oral cHP on sleep time of ethanol-induced narcosis. A statistically significant difference occurred between the two groups, $F(1, 10) = 6.36, p < 0.05$.

not receiving cHP and one mouse in the group receiving cHP that had sleep times exceeding 180 min.

DISCUSSION

The results show that cHP can be absorbed orally as an intact peptide to enter various tissues including the brain and so can exert a biologic effect. About 0.5% of the oral dose is in each ml of arterial serum 30 min after ingestion of I-cHP compared with about 1-2% of an IV dose. Therefore, it can be estimated that 25-50% of the oral dose may be absorbed into the circulation. Analysis by HPLC showed that 32% of the radioactivity circulating at 30 min eluted with intact peptide as compared with about 60% after IV administration. Together, these results suggest that oral administration may produce circulating levels of intact cHP that are about 20% of those achieved with an IV dose.

The finding that about half as much of the radioactivity eluted by HPLC as intact I-cHP after oral as compared with IV administration suggests that half the radioactivity reaching the general circulation represented I-cHP that was degraded by the stomach, intestines, or by first pass though the liver. Some of the radioactivity recovered from blood eluted in the position of the stereoisomer of the form of I-cHP injected. Conversion between these two forms is reversible and pH dependent (16). Therefore, it is likely that conversion occurred in the acidic environment of the stomach.

Radioactivity was found in all tissues examined. The pattern of distribution was similar that seen after parenteral administration (10), suggesting that cHP given orally can reach its sites of action after ingestion. The liver and kidney had the highest concentration, perhaps reflecting disposal by these organs. The brain had a low, but measurable uptake, indicating restriction, but not exclusion, by the blood-brain barrier. This is consistent with the ability of other small peptides to cross the blood-brain barrier to a modest extent but sufficient to exert effects directly on the brain (1).

The reduction by cHP in the sleep time of ethanol-induced narcosis also demonstrates that significant amounts of cHP can be absorbed orally. Previous studies have shown that about 200 times more cHP is needed to reverse narcosis when given IV than when given ICV (4). This indicates that the effect of cHP is mediated through the brain, an organ noted here to restrict entry of I-cHP. It is likely, therefore, that if enough cHP can be administered orally to achieve an effect within the brain then oral absorption should also be adequate to achieve concentrations that would be effective in peripheral tissues.

The findings show that cHP is absorbed after oral administration to adult mice. cHP can be absorbed intact in amounts sufficient to affect brain function.

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