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Distribution, Survival and Biological Effects in Mice of A Behaviorally Active, Enzymatically Stable Peptide: Pharmacokinetics of Cyclo(Leu-Gly) and Puromycin-induced Amnesia

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RAINBOW, T. C., J. B. FLEXNER, L. B. FLEXNER, P. L. HOFFMAN AND R. WALTER. *Distribution, survival and biological effects in mice of a behaviorally active, enzymatically stable peptide: Pharmacokinetics of cyclo(Leu-Gly) and puromycin-induced amnesia.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 787–793, 1979.— Cyclo(Leu-Gly), the enzymatically resistant diketopiperazine formally derived from the C-terminal dipeptide sequence of oxytocin, exhibits activity in several behavioral systems. The distribution of cyclo(Leu-^{14}C(U)Gly) in brain, and the time course of the disappearance of this labeled peptide from brain and plasma after subcutaneous injection into mice have been studied. The intact peptide was distributed equally in the five cerebral areas studied, for up to 96 hours after injection. Two exponential components were determined for peptide disappearance rates in plasma and brain; peptide half-lives in plasma up to 10 hr and from 24–96 hr after injection were, respectively, 0.8 and 33 hr; in brain, 1.0 and 42 hr. The peptide was found to accumulate in brain intracellular space to some degree. The time course of distribution of labeled cyclo(Leu-Gly) in subcellular fractions of mouse brain was also examined, and the concentration of peptide in the synaptosomal fraction was significantly correlated with the degree of protection against puromycin-induced amnesia of a maze-learning test. The results obtained not only confirm that cyclo(Leu-Gly) penetrates brain tissue intact and remains intact after peripheral administration in order to exert its behavioral effects, but, moreover, suggest an intriguing dynamic relationship between peptide concentration in the synaptosomal fraction and behavioral activity.

Memory Cyclo(Leu-Gly) Puromycin amnesia Pituitary peptides Time course Site of action

THE neurohypophyseal hormones, as well as some of their analogs and fragments, have been demonstrated to produce effects on certain aspects of memory [5, 9, 11, 30, 33, 35–37]. For example, many of these peptides, after systemic or intracerebral administration, inhibit extinction of active and passive avoidance responses in rats [5] and attenuate the amnesia caused by puromycin in mice [9, 20, 33] and CO_2 in rats [27]. Some of these peptides also have been reported to facilitate [19,28] or inhibit [35,36] the development of tolerance to and/or dependence on morphine in rodents, and it has been postulated that similar adaptive changes may underlie both the development of tolerance to various drugs and learning or memory [23]. The time-dependency of the effect of post-training peptide injections [11] on attenuation of puromycin-induced amnesia is also consistent with the view that peptides modify processes of memory and that their effect is not due to long-lasting general arousal of some CNS mechanism. The available evidence—albeit indirect—supports the hypothesis that the peptides act directly at sites in the CNS [5, 15–17, 21, 30].

In the present study our objective was to further evaluate the sites of peptide action in the CNS by determining, after subcutaneous (SC) injection into mice of a mnemonically active peptide, (a) its regional and subcellular distribution in the brain; (b) its half-life in plasma, brain and subcellular fractions of brain, and (c) the relationship between the degree of its protective effect against puromycin-induced amnesia and its concentration in particular subcellular frac-

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tions. For these purposes we have chosen cyclo(Leu-Gly), a diketopiperazine formally derived by ring closure from the C-terminal dipeptide of oxytocin [33], that has been shown to be among the neurohypophyseal fragments that modify memory processes [9, 11, 28, 33, 35]. Cyclo(Leu-Gly) has the advantages that it is completely resistant to degradation both *in vitro* and *in vivo* in several species for at least 22 hr [12,14] and that it readily penetrates the blood-CSF barrier [14].

EXPERIMENT 1: REGIONAL DISTRIBUTION IN MOUSE BRAIN AND HALF-LIFE IN BRAIN AND PLASMA OF CYCLO(LEU-^{14}C(U)GLY) AFTER SC INJECTION

Method

Male ICR mice (27–35 g; West Jersey Biological Supply) were used in all the biochemical and behavioral experiments. They were housed 4 to a cage at room temperature with free access to food and water and were assigned at random to an experimental group. Cyclo(Leu-^{14}C(U)Gly), with a specific activity of 106 Ci/mole, was from the same batch used in previous studies [12,14]. The peptide was administered subcutaneously (SC) in 0.1 ml of Kreb's bicarbonate buffer. In the present experiment, 1 μmole (0.17 mg) of peptide was given; this amount injected immediately after training results in full protection against the amnesic effects of puromycin [9]. However the specific radioactivity of the injected peptide was varied in studies of the time course of regional uptake of peptide into brain. One μCi/μmole was used for the 10, 30 and 60 min time points; 3 μCi/μmole for 4, 7 and 10 hr; and 5 μCi/μmole for the 24, 48 and 96 hr time points.

At specified times after injection of radioactive peptide, mice were sacrificed by cervical dislocation. Brains were removed and dissected into the following areas: brain stem, hippocampus plus related entorhinal cortex, diencephalon, corpusstriatum and cerebral cortex. Brain areas from individual mice were homogenized in 6% trichloroacetic acid (TCA). Blood (0.5 ml) was collected into a heparinized syringe after cardiac puncture, treated with 6% TCA, and both blood and tissue samples were centrifuged at low speed to remove precipitated proteins. Aliquots (4 ml) of the supernatant were then added to 11 ml of counting solution (PCS, Amersham-Searle) and radioactivity determined, after correction for quenching, by liquid scintillation counting. The protein precipitates contained no detectable radioactivity.

For calculations, dpm/g tissue were converted to pmole peptide/g tissue, based on the specific radioactivity of the injected material, since all radioactivity represented intact peptide (see below). The values for pmole peptide/g tissue were first corrected for 3% contamination by blood as determined by the method of Purdy and Bondy [26]. The extracellular space (ECS) was considered to be 12% of the tissue volume [3,4] and to be in equilibrium with plasma. The calculated amount of peptide in the ECS was subtracted from total peptide in brain, and the result taken as peptide in the intracellular space (ICS).

To determine the presence of intact cyclo(Leu-^{14}C(U)Gly) in the brain 96 hr after its SC injection, each of 4 mice received SC 70 μCi($\sim$0.7 μmole) of the compound. The mice were decapitated 96 hr later, the 4 brains were homogenized in 8 ml of 0.4 N perchloric acid and the homogenate was centrifuged at low speed. The supernatant was adjusted to pH 6.5 with KOH, and after centrifugation, the supernatant was stored overnight at 4°C and then dried under N_2 at 35°C. The residue was dissolved in 100 μl of ethyl alcohol (total cpm=3280) and streaked on a silica gel thin-layer chromatography plate. The plate was developed with the solvent system chloroform: methyl alcohol: acetic acid (85:10:5, v/v/v). Radioactive material was located by use of a Packard Radiochromatogram Scanner (Model 7201), and its mobility was compared to that of a control sample of cyclo(Leu-^{14}C(U)Gly) run in the same solvent system.

Results

Previous experience indicated that cyclo(Leu-Gly) was not degraded by brain for up to 4 hr *in vivo* or 22 hr *in vitro* [14]. Results obtained in the present study by thin layer chromatography of the radioactive material from mouse brain at 96 hr after SC injection of cyclo(Leu-^{14}C(U)Gly) supported and extended these data. Scanning of the chromatogram revealed a single peak with an R_f of 0.93, the same as that found for authentic cyclo(Leu-^{14}C(U)Gly); there was no radioactivity detectable at the area where Gly would be expected. We conclude that the intact peptide survived in the brain for at least 4 days.

Table 1 gives the time course of appearance and disappearance of labeled cyclo(Leu-Gly) in plasma and cerebral cortex. The peak concentration of peptide was observed in plasma 10 min after injection; in cortex, at 30 min after injection. Peptide concentrations in cortex were indistinguishable from those found in the other brain areas examined except 10 min after injection, when no detectable peptide appeared in the ICS of cortex, diencephalon or striatum, while that of brain stem and hippocampus plus entorhinal cortex contained 729 and 171 pmoles/g, respectively. In all instances 10 min after injection the ECS appeared to have the same concentration of peptide as plasma based on the assumption that its volume was 12% of tissue volume [3].

As shown in Fig. 1, the disappearance rates of labeled cyclo(Leu-Gly) from plasma and cerebral cortex could each be resolved into 2 exponential components. The correlation between concentration of peptide and time after injection was significant in all instances (for plasma up to 10 hr, $r=-0.979$, $r^2=0.958$, $df=21$, $p<0.001$; for plasma from 24–96 hr, $r=-0.713$, $r^2=0.508$, $df=7$, $p<0.05$; for cortex from 0.5–7 hr, $r=-0.938$, $r^2=0.966$, $df=17$, $p<0.001$; for cortex from 10–96 hr; $r=-0.910$, $r^2=0.828$, $df=11$, $p<0.001$). Half-lives of peptide were estimated from the regression lines of Fig. 1. Those for plasma up to 7 hr and for cortex up to 10 hr after injection were, respectively, 0.8 and 1.0 hr. During the second, slower phase of disappearance the half-life in plasma was estimated to be 33 hr and that in cortex, 42 hr. An increase with time in the ratio of labeled cyclo(Leu-Gly) concentration in the ICS to that in the ECS was observed (Table 1). Since cortical peptide concentrations were indistinguishable from those of other brain areas except 10 min after peptide injection, it is apparent that these values for the cortex apply as well to the whole hemisphere.

EXPERIMENT 2: DISTRIBUTION AND HALF-LIFE OF CYCLO(LEU-^{14}C(U)GLY) IN SUBCELLULAR FRACTIONS OF MOUSE BRAIN FROM 4 TO 72 HR AFTER ITS SC INJECTION

Method

Procedures largely followed those of Dratman *et al.* [6]. Each mouse received an injection of 1 μmole containing 7 μCi cyclo(Leu-^{14}C(U)Gly) for the 4 hr time point, 10μCi for the 10 hr, 17 μCi for the 24 hr and 27 μCi for the 72 hr time point. In each experiment the cerebral hemispheres of 3 mice

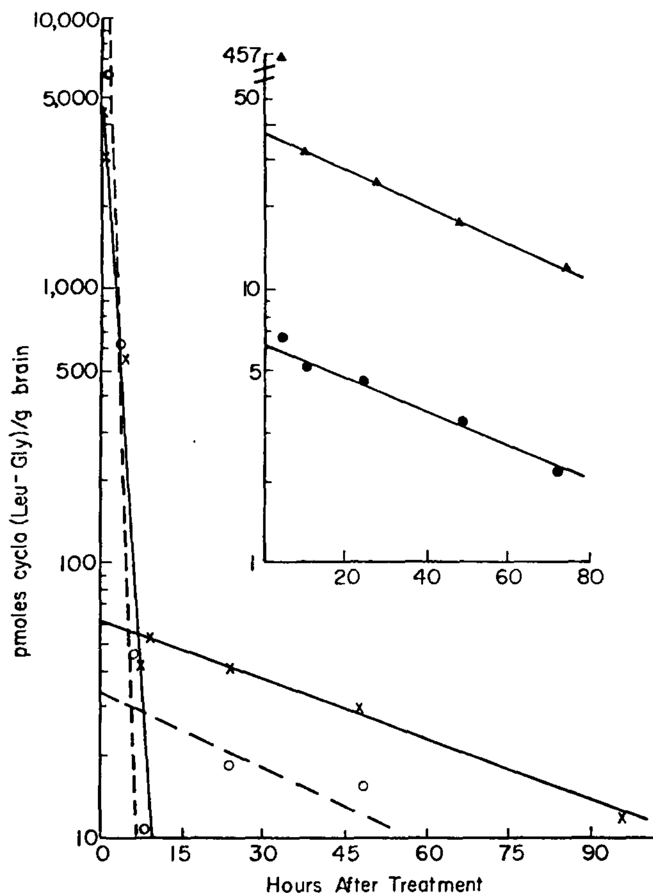

FIG. 1. Disappearance of cyclo(Leu-^{14}C(U)Gly) from plasma (O--O), cerebral cortex (X——X), and cytosol (▲——▲) and synaptosomal (●——●) fractions of cerebral hemispheres. ICR mice were injected SC with 1 μmole of cyclo(Leu-^{14}C(U)Gly). Extrapolated values of the slow exponential phase of disappearance from plasma and cortex were subtracted from observed values of the rapid phase. Not shown in the figure: plasma at 0.17, 0.5 and 96 hr (20124, 13124 and 4.1 pmoles/ml, respectively). Cytosol at 4 hr (457 pmoles/g) is part of the rapid phase. Values are medians (3–4 determinations per time point).

were homogenized in 9 vol of 0.32 M sucrose. Subcellular fractions of the homogenate were prepared on a discontinuous sucrose density gradient according to the method of Whittaker [38]. The various fractions were pipetted off the density gradient and treated with 6% TCA as described above. Previous electron microscopic studies [8] indicated that the crude synaptosomal fraction prepared by this method contained generally well preserved presynaptic terminals, roughly estimated to be 80% of the intact processes. The remaining 20% included rounded processes lacking vesicles, some of which contained ribosomes. These were tentatively identified as postsynaptic components. The fraction also contained a few scattered free mitochondria, membranes, and a variable—but usually small—amount of myelin sheaths. Mitochondria constituted the bulk of the mitochondrial fraction; presynaptic endings were the most common contaminant [8]. Only the synaptosomal fraction exhibited temperature-dependent uptake of ^{14}C-norepinephrine [1]; ^{14}C-norepinephrine uptake/mg protein was 5–6 fold greater in the synaptosomal fraction than in the homogenate freed of nuclei and tissue fragments by low speed centrifugation (1000 G; 10 min; S_1 supernatant) and 85% of the S_1 supernatant's uptake of ^{14}C-norepinephrine was recovered in the synaptosomal fraction. The method of Lowry *et al.* [24] was used for determination of protein.

To correct for contamination of subcellular fractions by the cytosol during homogenization, 4 μm cyclo(Leu-^{14}C(U)Gly) (the concentration found in brain 30 min after SC injection of 1 μmole of peptide) was added *in vitro* at 4°C to the S_1 supernatant of a brain homogenate. Subcellular fractions were then prepared. The proportions of peptide found in these fraction were subtracted from the proportions obtained in the *in vivo* experiments [7]. The amount of peptide/g brain present in a fraction at a given time was obtained from the total peptide/g brain (Table 1) and the corrected proportion found in the fraction at that time (Table 2). Total peptide/g brain at 72 hr was estimated by interpolation from the values at 48 and 96 hr.

TABLE 1

TIME COURSE OF DISTRIBUTION OF CYCLO(LEU-^{14}C(U)GLY) IN PLASMA AND CEREBRAL CORTEX

Hr after injection	N	Plasma (pmole/ml)	Cerebral Cortex Total* (pmole/g cortex)	ECS† (pmole/g cortex)	ICS (pmole/g cortex)	[ICS]/[ECS]‡
0.17	3	20,124 ± 5,324	2,414 ± 235	2,414	0	0.0
0.5	4	13,124 ± 353	4,424 ± 124	1,575	2,849	0.25
1	4	6,024 ± 1,782	3,143 ± 335	723	2,420	0.46
4	4	641 ± 177	622 ± 159	76.9	545	0.97
7	4	76.5 ± 14.1	97.7 ± 23.5	9.2	88.5	1.31
10	4	42.9 ± 4.1	51.6 ± 5.9	5.1	46.5	1.24
24	3	17.6 ± 5.9	40.7 ± 11.8	2.1	38.6	2.51
48	3	15.3 ± 6.5	28.9 ± 5.9	1.8	27.1	2.05
96	3	4.1 ± 2.9	11.8 ± 2.4	0.5	11.3	3.08

*ICR mice were injected SC with 1 μmole of cyclo(Leu-^{14}C(U)Gly) at varying specific activities; see text for detail. Values are medians ± SE corrected for 3% blood contamination based on hemoglobin content [27].

†ECS taken as 12% of tissue volume [4] and assumed to be in equilibrium with plasma.

‡Ratio pmole/g ECS to pmole/g ICS. Values for cerebral cortex, brain stem, hippocampus plus entorhinal cortex diencephalon and corpus striatum were indistinquishable except at 10 min as noted in the text.

TABLE 2

DISTRIBUTION OF CYCLO(LEU-^{14}C(U)GLY) IN SUBCELLULAR FRACTIONS OF CEREBRAL HEMISPHERES*

Fraction	In Vitro	In Vivo (Hr after injection)			
		4	10	24	72
Cytosol	77.3 ± 2.2	71.3 ± 1.2	60.9 ± 3.3	59.0 ± 1.3	59.7 ± 1.7
Myelin	11.0 ± 0.4	0.4 ± 0.5	1.8 ± 0.8	7.6 ± 1.7	6.9 ± 1.9
Post-myelin	2.3 ± 0.8	0.7 ± 1.2	3.8 ± 0.7	3.4 ± 0.4	5.3 ± 0.8
Synaptosomes	0.6 ± 0.1	1.0 ± 0.2	9.4 ± 1.0	11.0 ± 1.3	11.2 ± 1.0
Post-synaptosomes	0.3 ± 0.1	0.0 ± 0.2	2.2 ± 0.6	1.3 ± 0.3	1.8 ± 0.4
Mitochondria	0.2 ± 0.1	0.1 ± 0.1	0.8 ± 0.3	1.9 ± 0.9	2.1 ± 0.1

*ICR mice received SC injections of 1 μmole cyclo(Leu-^{14}C(U)Gly) of varying specific activities as described in the text. Values are medians ± SE and are % of total cyclo(Leu-^{14}C(U)Gly) in S_1 supernatant applied to gradient. Fractionation was carried out according to the method of Whittaker [38]; 3–4 independent experiments were performed per group for a total of 18. All in vivo values are corrected for contamination by peptide of cytosol during homogenization as noted in text. In vitro studies were carried out at 4°C; for details see text.

Results

The distribution of cyclo(Leu-Gly) among subcellular fractions from 4 to 72 hr after SC injection of 1 μmole labeled peptide is given in Table 2. From 10–72 hr, the correlation for the relation of concentration of peptide in cytosol to time after injection was significant ($r=-0.990$, $r^2=0.980$, $df=8$, $p<0.001$) as was also the case for the synaptosomal fraction from 4–72 hr ($r=-0.855$, $r^2=0.730$, $df=12$, $p<0.001$). We were consequently able to estimate the half-lives of the peptide in the 2 fractions during the slow phase of disappearance from the regression lines of Fig. 1. As noted above, the half-life for cortex was 42 hr; that for cytosol was approximately 44 hr and for the synaptosomal fraction was 48 hr. It therefore appeared that cyclo(Leu-Gly) was not selectively retained to an important degree by either of these fractions.

Correlation coefficients for the relationship between peptide concentration and time after injection were not significant for the myelin and mitochondrial fractions. This result precluded further analysis of these fractions.

EXPERIMENT 3: EVALUATION OF THE DEGREE OF PROTECTION AGAINST PUROMYCIN-INDUCED AMNESIA AND THE CONCENTRATION OF LABELED CYCLO(LEU-GLY) IN A PARTICULAR SUBCELLULAR FRACTION

Method

The behavioral procedures have been fully described [7]. Mice were trained in a single session in a Y-maze [7] to a criterion of 9 out of 10 correct responses. Intermittent foot shock was given for failure to move from the stem of the Y within 5 sec and for errors of left-right discrimination. The same procedure was used 1 week after treatment with puromycin for testing of performance of the training experience. Performance was evaluated in terms of percentage savings of errors [7]. Negative savings were scored as zero.

The injection technique for puromycin has also been fully described [10]. Combined bitemporal plus biventricular [10] injections of puromycin 2 HCl (neutralized with NaOH) consistently produced amnesia in the ICR mice used in the present experiments. Each injection site received 60 μg of puromycin in 12 μl of water. All injections of puromycin were made under light Evipal (150 mg/kg) anesthesia 24 hr after training.

The concentration of peptide in subcellular fractions at various times after its injection was estimated from the data given in Tables 1 and 2. Preliminary experiments had shown that the cerebral concentration of peptide was proportional to the amount injected.

Results

The behavioral results are given in Table 3. The dose-response relationship found for cyclo(Leu-Gly) when ICR mice were injected 4 hr before training was indistinguishable from that in Swiss Webster mice treated with the peptide immediately after training [9], as was the absence of protection against puromycin-induced amnesia following injection of 1 μmole of labeled cyclo(Leu-^{14}C(U)Gly) 72 hr before training [11].

The concentrations of cyclo(Leu-Gly) in the cytosol and in the synaptosomal fraction are also shown in Table 3. The correlation coefficient for the relationship between peptide concentration in the synaptosomal fraction and % savings of errors was highly significant ($r=0.921$, $r^2=0.848$, $df=5$, $p<0.005$). The coefficient for the cytosol fraction was not significant, nor was that for myelin, post-myelin, post-synaptosomal and mitochondrial fractions.

DISCUSSION

Cyclo(Leu-Gly) protects mice against puromycin-induced amnesia: the degree of protection provided depends upon the dose of the peptide and the time of injection before training [9,11]. In addition to having these protective effects, cyclo(Leu-Gly) weakly increases resistance to extinction of a pole jumping avoidance response [37]. It has been reported to facilitate development of physical dependence on and tolerance to morphine in rats [28], but to inhibit the same phenomena in mice [35]. An understanding of distribution

TABLE 3

CORRELATION BETWEEN CYCLO(LEU-^{14}C(U)GLY) CONCENTRATIONS AND PROTECTION AGAINST PUROMYCIN-INDUCED AMNESIA*

Injection Time		Dose of Peptide	Cytosol	Synaptosomes	% Saving Errors
Hr before training	N	(μmole)	(pmoles/g Brain)		
4	4	1.0	457	6.4	85.5 ± 4.9
4	4	0.6	274	3.8	58.5 ± 20.1
4	4	0.3	137	1.9	0.0 ± 0.0
24	5	1.0	25	4.5	75.0 ± 5.9
48	5	1.0	17	3.2	40.0 ± 19.2
72	5	1.0	12	2.1	0.0 ± 0.0
72	5	2.0	24	4.2	37.0 ± 19.1

*ICR mice received SC injection of cyclo(Leu-^{14}C(U)Gly) at the times indicated, and puromycin was injected as described in the text at 24 hr after training. Controls injected intracerebrally with puromycin had savings of 0.0 ± 0.0 (N=5), and animals injected with water, 88.0 ± 5.5 (N=5). Values are medians ± SEM.

and survival of this peptide in the brain is consequently of interest from several biological points of view.

Because of its diversity of activities, it might be anticipated that cyclo(Leu-Gly) would be widely distributed in the CNS. Consistent with this, we have observed no significant variation, except at 10 min, in its distribution among the 5 brain areas examined for up to 96 hr after injection. These results with cyclo(Leu-^{14}C(U)Gly) are in line with studies of Greenberg *et al.* [13], who 15 sec and 10 min after intra-arterial injection of the behaviorally active peptides, ^{3}H-MIF, ^{3}H-α-MSH and ^{14}C-arginine vasopressin, found label distributed uniformly in the major brain regions; however, these results differ from those found in a study by Kastin *et al.* [15] of the distribution of radioactivity in brain at 15 sec and 30 min after intra-arterial injection of ^{3}H-α-MSH. In that study, radioactivity accumulated in certain areas of brain—i.e., occipital cortex, pons-medulla, and cerebellum—to a greater degree than in other areas at both time points studied. Similarly, 5 min after intraventricular injection of ^{125}I-α-MSH, radioactivity appeared to accumulate in specific areas [25]. However, in all of these latter studies there is always the uncertainty that only accumulation of radioactivity is being measured, rather than accumulation of intact peptide. This problem was avoided in the present study in view of the enzymatic stability of cyclo(Leu-Gly).

The half-lives of cyclo(Leu-Gly) during its initial, rapid phase of disappearance from the plasma of mice (48 min) and during its later slower phase (33 hr) are remarkably longer than those derived from the 2 exponential components of disappearance reported for two other CNS-active peptides, i.e., oxytocin and vasopressin. The half-life of oxytocin during the rapid phase of disappearance from the plasma of rats has been reported to be 2.2 min and during the slow component, 19.2 min. With vasopressin in rabbits the half-life of the peptide during the rapid component was 0.6 min and during the late component, 5.9 min [22]. These differences between cyclo(Leu-Gly) on the one hand and oxytocin and vasopressin on the other can be attributed to the enzymatic stability of the dipeptide and the rapid degradation of the nonapeptides [14].

Considerations such as these regarding the half-lives of peptide hormones, e.g., α-MSH [15] and lysine vasopressin [29], have led to the suggestion that their continued presence are not necessary for expression of behavioral effects. While this concept in a strict sense may well be correct, it may be too restrictive. For example, these hormones may only represent precursor molecules, which are subject to enzymatic fragmentation in limited and well defined areas in the animal. The released fragments, which may possess completely different half-lives and distribution profiles from their precursor molecule(s), may actually be the biologically active peptides. The first case in point to be reported was the enzymatic breakdown of oxytocin to form Pro-Leu-Gly-NH_2 (MIF) [2,32], which not only has been shown to inhibit pituitary release of MSH [2] and ACTH [31], but also to affect a wide spectrum of CNS-activities [16, 28, 33, 35].

Nothing is known of the natural occurrence of cyclo(Leu-Gly). It could be speculated that it might be formed *in vivo* in 2 steps from oxytocin. In the first step enzymatic cleavage of the Pro-Leu peptide bond of oxytocin (e.g., by the serine protease post-proline cleaving enzyme (EC3.4.21—[18]) would yield Leu-Gly-NH_2. When tested *in vitro* in blood, Leu-Gly-NH_2 was subject to rapid attack by aminopeptidases (e.g., [34]), and a minor amount of this dipeptide spontaneously cyclized with elimination of ammonia to yield the diketopiperazine, cyclo(Leu-Gly) (unpublished).

The brain as a whole and, during the period we have studied, its cytosolic and synaptosomal fractions show a similar pattern of disappearance of cyclo(Leu-Gly) as that found in plasma, but possibly at a slightly slower rate. With time one observes an increase in the ratio of cyclo(Leu-^{14}C(U)Gly) concentration in the ICS to that in the ECS (Table 1), suggesting that the passage of the dipeptide from brain back to the plasma is restricted. Of the 6 subcellular fractions examined, the concentration of cyclo(Leu-Gly) only in the synaptosomal fraction was significantly correlated with the degree of protection against puromycin amnesia. This finding suggests that cyclo(Leu-Gly) interacts with membranes of nerve endings or even penetrates these cells, and the possibility exists that cyclo(Leu-Gly) acts there as an

enzyme inhibitor. While such a possibility fits the current idea that synaptic modifications are essential for the establishment and maintenance of memory and that active peptides combine with receptors on synaptic membranes, leading in some way to a strengthening of consolidation, we consider our finding only suggestive. We are ignorant, for example, of the effects of cyclo(Leu-Gly) on peripheral organs that might result in secondary actions on the CNS.

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