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The Degradation of Dynorphin A in Brain Tissue *In Vivo* and *In Vitro*

ELIZABETH A. YOUNG, J. MICHAEL WALKER,†
RICHARD HOUGHTEN* AND HUDA AKIL

Mental Health Research Institute, University of Michigan
205 Washtenaw Place, Ann Arbor, MI 48109
**Scripps Clinic and Research Institute, Department of Immunopathology*
10666 N. Torrey Pines Rd., La Jolla, CA 92036
†*Department of Psychology, Brown University, Providence, RI 02912*

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YOUNG, E A., J. M WALKER, R HOUGHTEN AND H AKIL. *The degradation of dynorphin A in brain tissue* in vivo *and* in vitro. PEPTIDES 8(4) 701–707, 1987.—The demonstration of analgesia following *in vivo* administration of dynorphin A (Dyn A) has been difficult In contrast, a number of electrophysiological and behavioral effects reported with *in vivo* injection of Dyn A can be produced by des-tyrosine dynorphin A (Dyn A 2–17). This suggested the extremely rapid amino terminal degradation of dynorphin A To test this hypothesis, we examined the degradation of dynorphin A following *in vivo* injection into the periaqueductal gray (PAG) as well as *in vitro* using rat brain membranes under receptor binding conditions. *In vivo*, we observed the rapid amino terminal cleavage of tyrosine to yield the relatively more stable des-tyrosine dynorphin A This same cleavage after tyrosine was observed *in vitro*. Inhibition of this aminopeptidase activity *in vitro* was observed by the addition of dynorphin A 2–17 or dynorphin A 7–17 but not after the addition of dynorphin A 1–13, dynorphin A 1–8, dynorphin B or α-neo-endorphin suggesting a specific enzyme may be responsible The detection of the behaviorally active des-tyrosine dynorphin A following *in vivo* injection of dynorphin A suggests that this peptide may play an important physiological role.

Dynorphin A *In vivo* *In vitro* Periaqueductal gray

DYNORPHIN A has been shown to be an extremely potent opioid peptide active at several opioid receptors in a number of *in vitro* preparations [4, 11, 16–19, 29, 43]. Despite the demonstration of its high affinity binding to brain membrane receptors, and its substantial potency in guinea pig ileum preparations, demonstration of analgesia following *in vivo* injection has been difficult [13–15, 19, 27, 28, 38, 42]. A number of behaviorial and electrophysiological effects have been reported following *in vivo* injection of pharmacological doses of dynorphin A [1, 2, 10, 19, 25, 27, 28, 35, 36, 38, 42]. With the exception of diuresis, the behavioral and physiological effects often appear to be non-opioid since they cannot be reversed by high doses of naloxone (10 mg/kg) or other opiate antagonists, and, these same effects can be produced by dynorphin A 2–13 or dynorphin A 2–17 [10, 28, 40, 42]. One explanation for the lack of potency of dynorphin A in tests of thermal pain stems from findings that kappa agonists are weak in tests of thermal pain [23]. However, the kappa opioid U-50488H was found to be active in the tail flick test after either systemic or intracerebroventricular injections in rats [37]. Early studies with another opioid peptide, enkephalin, showed similar difficulties demonstrating analgesia after *in vivo* injection of pharmacological doses [3,9]. In the case of enkephalin, these difficulties resulted from extremely rapid breakdown of enkephalin following *in vivo* injection [9, 12, 21, 22, 24, 33, 34]. The availability of stable analogues of enkephalin led to the demonstration of a variety of opioid effects with these compounds [6, 7, 26, 39, 41]. However, in the case of dynorphin A, the stable analogues produced thus far have altered the receptor selectivity of dynorphin A, and thus changed the pharmacological profile of dynorphin A [5]. Since suitable stable analogues are not available, it is critical to examine the stability of dynorphin A following *in vivo* injection and see if we could detect any fragments of dynorphin A that are known to be behaviorally active such as dynorphin A 2–17 or dynorphin A 1–8.

Aside from concerns about the stability of dynorphin A following *in vivo* injections, studies on the degradation of brain peptides and other neurotransmitters can yield important information. In the enkephalin system, despite the widespread presence of general aminopeptidases that could use enkephalin as a substrate, *in vivo* degradation studies demonstrated that a family of enzymes, the enkephalinases, were responsible for the *in vivo* breakdown [9, 12, 21, 22, 24, 33, 34]. The existence of such specific degrading enzymes provides another regulatory site in neurotransmitter systems. Inhibitors of such specific enzymes can provide other strategies for studying neuronal regulation or for prolonging the actions of endogenous ligands released under physiological conditions ([31] and cf. monoamine oxidase inhibitors).

In addition to concerns about *in vivo* breakdown of dynorphin A, breakdown of dynorphin A in *in vitro* systems can alter its pharmacological profile. During our own recep-

tor binding studies, we became concerned about the rapid breakdown of this endogenous peptide in our membrane preparation. Since opioid binding activity is dependent upon an intact tyrosine at the N-terminal, it is particularly critical to assure that the peptide is stable under these conditions. Similar studies with CCK-33 binding have demonstrated breakdown under binding conditions that can be inhibited by bacitracin, yielding a 2-fold increase in specific binding [32]. Previous studies by Leslie and Goldstein [20] in rat brain membrane preparations using [^{125}I] dynorphin A 1–13 had demonstrated extremely rapid NH_2-terminal cleavage of the tyrosine as well as rapid progressive COOH-terminal shortening. Similarly, *in vitro* rat membrane data by Robson *et al.* [30] using dynorphin A 1–9 demonstrated that nonspecific inhibition of the NH_2-terminal cleavage of tyrosine led to COOH-terminal shortening and formation of numerous other opioid-active dynorphin fragments, making true pharmacologic characterization of dynorphin A impossible under these conditions Interestingly, the data from Leslie and Goldstein [20] suggested the existence of a specific membrane bound exopeptidase in rat brain that could be inhibited by COOH-terminal fragments of dynorphin A 1–13 (dynorphin A 6–13), but only in concentrations that affected opioid binding. To explore these problems, we undertook (a) characterization of the time course of dynorphin breakdown *in vivo* and *in vitro*, (b) identification of products following *in vitro* incubation with washed brain membranes, (c) characterization of the ability of dynorphin fragments and related peptides to inhibit the breakdown of [^{3}H]dynorphin A, and (d) comparison of the *in vitro* date with *in vivo* studies of the breakdown of [^{3}H]dynorphin A injected into the periaquaductal gray (PAG)

METHOD

Tissue Preparation

Rat and guinea pig brains minus the cerebellum were homogenized with a Brinkman polytron in 50 mM Tris buffer (pH 7.4 at 24°C) at a concentration of 50 mg tissue (wet weight) per ml of buffer. After a 40 minute 37°C incubation to dissociate bound ligands, the homogenates were centrifuged at 40,000 × g, then resuspended in 50 mM Tris at a concentration of 37.5 mg tissue/ml.

Product Identification

The [^{3}H]dynorphin A used for all these studies was synthesized by one of us (R.H.). For breakdown studies *in vitro*, 1 nM [^{3}H]dynorphin A in Tris buffer was incubated with 15 mg tissue in microfuge tubes at 0°C for 30–90 minutes in the presence or absence of possible inhibitory peptides. The pH of Tris at this temperature is 7.2 with no change over the time course of the incubation. These peptides were added in a 10 microliter volume dissolved in a 50·50 mixture (v/v) of methanol to 0.1 N HCl (MeOH:HCl). Bound [^{3}H]dynorphin A was separated from free [^{3}H]dynorphin A by centrifugation for 5 minutes at 4°C in a Beckman microfuge.

Two methods of chromatography were employed, a rapid molecular seiving with a short Sephadex column, followed by more complete identification with reverse phase HPLC. The supernatant was applied to a 12 centimeter G-10 column equilibrated in 2 N acetic acid with 0.10% BSA for molecular sieving. Using MeOH:HCl as an eluant, fractions (0.5 ml) were collected directly into scintillation vials. [^{3}H]dynorphin A, [^{3}H]Leu-enkephalin and [^{3}H]tyrosine were used as standards This column reliably separates dynorphin A from dynorphin A 1–8 from Leu-enkephalin. However, it does not separate Leu-enkephalin from tyrosine. Dynorphin A 1–13 co-migrates with dynorphin A on this column. The fractions were suspended in 10 ml of scintillation cocktail for aqueous samples and counted in a Beckman LS 9000 scintillation counter. The bound (tissue pellet) was also counted to calculate recovery of [^{3}H]dynorphin A as well as to determine both specific and nonspecific tissue binding by centrifugation. Dynorphin A (Dyn A), dynorphin A 2–17 (dT dynorphin) and dynorphin A 7–17 were a generous gift of Dr. David Coy. Dynorphin A 1–8 and dynorphin A 1–13 were purchased from Peninsula (Belmont, CA) and α-neo-endorphin was purchased from Bachem (Torrance, CA).

For final identification of products, separation by reverse phase HPLC was undertaken. Buffer A consisted of 0.1% trifluoroacetic acid (TFA) in water with 0.05% triethylamine (TEA). Buffer B consisted of 0.1% TFA, 80% acetonitrile, 19.9% water with 0 05% TEA. Beginning at 4% Buffer A, a 10-minute gradient rising to 12% B produced separation of Tyr, Tyr-Gly and Tyr-Gly-Gly fragments A 5-minute gradient from 12% to 27% B was followed by a 45-minute gradient between 27–38% buffer B which produced good separation of Leu-enkephalin, dynorphin A 1–8 and dynorphin A For use in these breakdown experiments, the [^{3}H]dynorphin A was repurified on the HPLC so all radioactivity co-migrated with the dynorphin A standard This repurified [^{3}H]dynorphin A was tested for opiate binding activity to further insure that it was [^{3}H]dynorphin A After 0, 15, 30, 60 and 90 minutes incubation of 1 nM [^{3}H]dynorphin A with brain homogenates, the supernatant was lyophilized, resuspended in MeOH HCl and then applied to the HPLC for separation. To insure proper identification of dynorphin and dynorphin fragments, unlabelled peptide fragments and tyrosine were added as standards in each HPLC sample run, and the fractions were counted with a Beckman LS 9000 Scintillation Counter to determine whether radioactivity co-migrated with dynorphin A or other dynorphin fragments as monitored by UV (220 nm). Recovery was monitored by quantitifying the radioactivity applied to column and the counts recovered For those studies in which dynorphin fragments were measured by radioimmunoassay, dynorphin A, dT dynorphin and dynorphin A 1–8 were not added. To assess the elution profile of those standards, a calibration HPLC run followed the sample runs on each day

In Vivo *Studies*

For *in vivo* studies, 25 ga stainless steel guide cannulae were implanted into the periaqueductal gray (PAG) using the coordinates 5 1 mm posterior to bregma, 0.5 mm lateral to midline, with the guide cannulae inserted to a depth of 4.5 mm (Nose + 0.5 mm tilt) (Pellegrino, Pellegrino and Cushman, 1971). Seven to 10 days following surgery, the animals received an injection of 0.1 nanomole of [^{3}H]dynorphin A plus 0.9 nanomole of unlabelled dynorphin A through a 30 ga stainless steel needle extending 2 mm beyond the guide into the PAG. The injection volume was 1 microliter administered over 1 minute. To minimize backflow, the needle remained in place for 30 seconds after the end of the injection. After varying time periods (0, 2.5, 5 and 10 minutes), the rats were decapitated, their brains rapidly removed, and using a stereotactic block, a 2 mm slice of the PAG and the area 2 mm immediately rostral to the PAG were dissected The cortex and colliculi were trimmed

TABLE 1

BREAKDOWN OF [^{3}H] DYNORPHIN A AFTER 90 MINUTE INCUBATION AT 0°C (SEPHADEX G-10 SEPARATION)

	Control	1 μM Dyn A 1–17	1 μM dTDyn	[^{3}H]Dyn A Standard
Rat Brain				
[^{3}H]Dynorphin A 1–17 size	23%	62%	62%	90%
[^{3}H]Tyrosine size	67%	32%	28%	
Guinea Pig Brain				
[^{3}H]Dynorphin A 1–17 size	40%	70%	55%	88%
[^{3}H]Tyrosine size	50%	22%	36%	

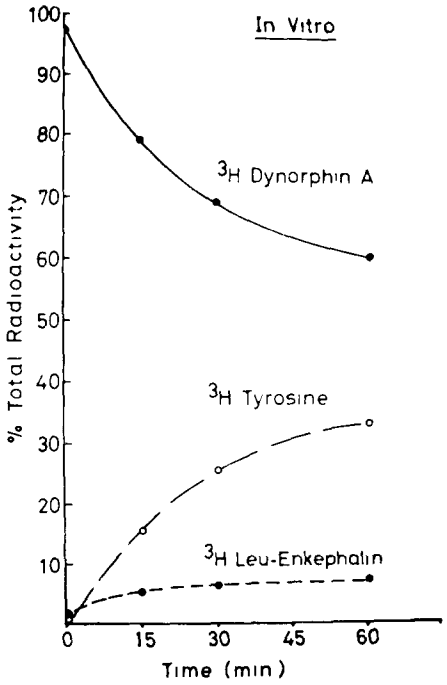

FIG 1. *In vitro* time course for the breakdown of [^{3}H]dynorphin A in double washed rat brain membranes After incubation, supernatant was analyzed by HPLC for co-migration of the radioactivity with dynorphin A, tyrosine or other dynorphin fragments as standards. Data are expressed as percent of total radioactivity recovered

from these regions and then these regions were immediately homogenized in extraction buffer (3:1 acetone:0.2 N HCl). The time from decapitation to homogenization averaged 1.5 minutes. After centrifugation, the supernatant was lyophilized, then resuspended in MeOH:HCl for the HPLC separation and identification. The radioactivity recovered in the tissue averaged 10% of the infused counts. It is unclear if the low recovery in tissue occurred because of nonspecific loss to the cannula or guide or because of diffusion of radioactivity from the site of injection. However, when we extracted the areas surrounding the dissected regions little radioactivity was found. More than 95% of the radioactivity in the tissue was present in the supernatant after extraction. The extracted material was applied to HPLC in MeOH:HCl and separated using the gradient described above.

After HPLC, half of each fraction was counted for radioactivity. The total radioactivity recovered from the HPLC runs averaged 90% of the material applied to the column. To characterize the forms of dynorphin immunoreactivity present after *in vivo* injection, the other half of each fraction was radioimmunoassayed with a dynorphin A antibody raised in our laboratory to dynorphin A 1–17 [8]. This antibody is fully cross reactive with dynorphin A 2–17, but does not recognize dynorphin A 1–8 and is only 50% cross reactive with dynorphin A 1–13.

RESULTS

In Vitro *Results*

Time course studies using single washed membranes with HPLC identification and quantitation demonstrated rapid degradation of dynorphin A following *in vitro* incubation with brain homogenates. At the end of 60 minutes incubation only 23% of the dynorphin A is intact. In contrast, double washed membranes showed slower breakdown (Fig. 1). The HPLC separation identified the major radioactive breakdown fragment as [^{3}H]tyrosine. The rest of radioactivity co-migrated with dynorphin A or Leu-enkephalin.

Initial inhibitor studies were done following 90 minutes incubation at 0°C, the optimal time for [^{3}H]dynorphin A binding in brain homogenates. Table 1 shows the data from one experiment. Molecular sieving with G-10 columns was used to quantitate breakdown and inhibition by other peptides. Thus, after 90 minutes incubation at 0°C with rat membranes, only 23% of the radioactivity co-migrates with [^{3}H]dynorphin A size material, while 67% of the radioactivity is [^{3}H]tyrosine size. In contrast, in guinea pig brain homogenates at 0°C, 40% of the radioactivity elutes with the [^{3}H]dynorphin A standard, while only 50% of the radioactivity is [^{3}H]tyrosine size material. In both species, the addition of 1 micromolar unlabelled dynorphin A substantially inhibits the breakdown of the [^{3}H]dynorphin A. It should be noted, however, that even this high concentration of unla-

TABLE 2

SIXTY MINUTES INCUBATION SEPHADEX G-10 SEPARATION

Inhibitor	Number of Replications	% [^{3}H]Dyn A Size	%[^{3}H]Tyr Size	% Inhibition
None	17	23 ± 0 5	69 ± 0 9	0
1 μM Dyn A	15	50.6 ± 1 6	40 8 ± 2	36 ± 2
10 μM Dyn A	3	63 ± 6 7	26 ± 5 3	52 ± 9
1 μM dT-Dyn A	1	62	28	51
1 μM Dyn A 7–17	3	39 ± 3 2	54 ± 1 7	20 ± 4
10 μM Dyn A 7–17	2	65 ± 4 2	27 ± 5 6	55 ± 5
1 μM NAc Dyn A	2	43 ± 4 2	49 5 ± 2 1	26 ± 5
10 μM Dyn A 1–13	4	35.8 ± 7 6	56 ± 13 6	17 ± 10
10 μM Dyn B	6	32 6 ± 4 7	61 ± 4 1	13 ± 6
10 μM α-neo-end	3	23 7 ± 4	66 ± 6 5	0 ± 5
10 μM Dyn 1–8	3	24 3 ± 3 6	66 ± 4	2 ± 4

To calculate % inhibition the following formula was used

$$100 - \frac{100 - \%\text{ Dyn A intact with inhibitor}}{100 - \%\text{ Dyn A intact without inhibitor}}$$

Overall % inhibition is expressed as mean ± standard error of mean

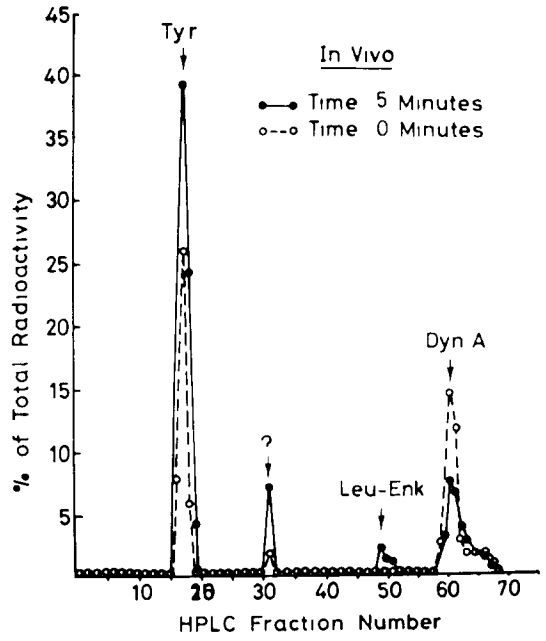

FIG. 2. HPLC profile from time course in PAG, showing the dissappearance of [^{3}H]dynorphin A and the appearance of [^{3}H]tyrosine Standards are marked with arrows A very small proportion of the radioactivity co-migrates with Leu-enkephalin. Data are expressed as percent of total radioactivity recovered.

belled dynorphin A does not totally prevent the breakdown of the [^{3}H]dynorphin A in either species.

Using the same molecular sieving quantitation, destyrosine dynorphin A (dT-Dyn or dynorphin A 2–17) appears to inhibit the breakdown of [^{3}H]dynorphin A to the same extent as dynorphin A in rat brain. This is not the case in guinea pig brain, where dynorphin A is more effective at preventing breakdown of [^{3}H]dynorphin A than is dT dynorphin Thus, there appear to be species differences in the aminopeptidases that cleave the NH_2-terminal tyrosine from dynorphin A. The ability of dynorphin 2–17 to block the breakdown may represent classic end-product inhibition through an allosteric site, or, alternatively, it may be that the recognition site for this dynorphin aminopeptidase is dependent upon the non-opiate core of dynorphin. Consequently we explored the effects of other dynorphin related peptides on the breakdown of [^{3}H]dynorphin A in rat brain (Table 2). Since we wanted to minimize the breakdown of the competing peptides, we used a shorter incubation time of 60 minutes at 0°C.

Again using molecular sieving quantitation, it can be seen in Table 2 that the non-opiate containing sequence of dynorphin A, dynorphin A 7–17, is able to partially inhibit the breakdown of [^{3}H]dynorphin A. It is not as effective an inhibitor as dynorphin A of dT dynorphin, but it posesses approximately 50% of the activity of dynorphin A itself. When the concentration is increased to 10 μM, dynorphin A 7–17 shows similar inhibition as 1 μM dynorphin A. Dynorphin A 1–13, dynorphin B and α-neo-endorphin show some small inhibitory actions at 10 μM concentration. Met-enkephalin 2–5, a classic enkephalinase inhibitor, does not affect the breakdown (data not shown), nor does dynorphin A 1–8, another prodynorphin derived peptide. If a specific enzyme exists, the failure to demonstrate inhibition by dynorphin A 1–8 may indicate either a lack of recognition of this peptide or that dynorphin A 1–8 is broken down so rapidly that by 60 minutes there is no longer any "inhibitor" left. Thus, similar studies were undertaken at 30 minutes. Even after 30 minutes incubation at 0°C, dynorphin A 1–8 showed no inhibition of the amino terminal cleavage of tyrosine from dynorphin A, when compared to the [^{3}H]dynorphin A breakdown standard for that experiment.

In Vivo *Results*

Using HPLC, *in vivo* breakdown was quantitated and

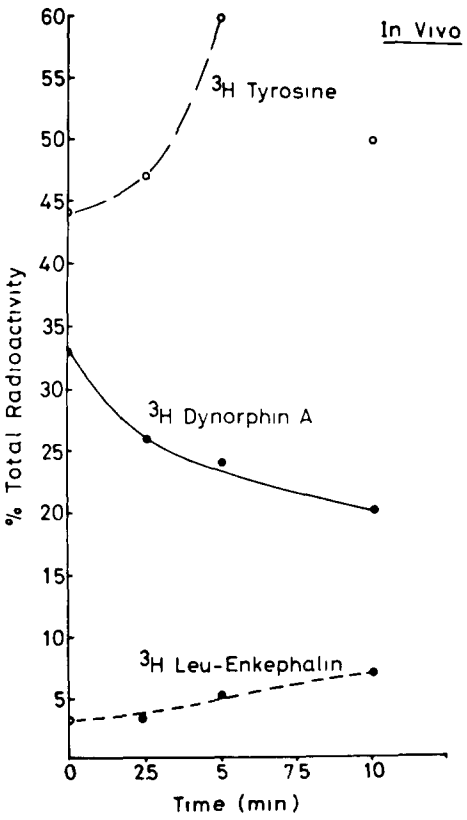

FIG. 3. Summary graph of *in vivo* time course As [^{3}H]dynorphin disappears, [^{3}H]tyrosine appears There is a slight accumulation of [^{3}H]material that co-migrates with Leu-enkephalin.

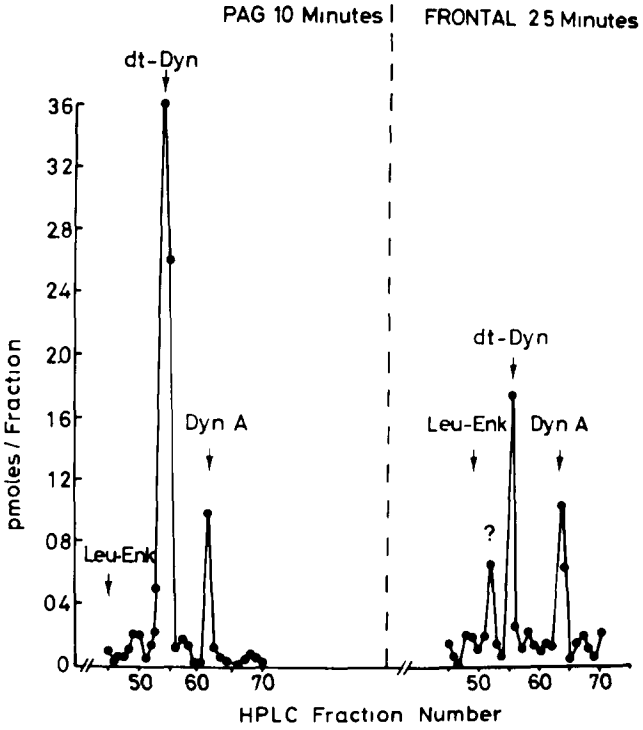

FIG 4 Dynorphin radioimmunoassay of HPLC separation demonstrates the formation of des-tyrosine dynorphin A (dT-Dyn) from dynorphin A (Dyn A). In both the area of injection (PAG) and the region rostral to the PAG (frontal) dTdynorphin can be detected Standards are marked with arrows

products were identified. *In vivo*, the breakdown of [^{3}H]dynorphin A proceeds extremely rapidly (Figs. 2 and 3). Thus, even time 0 samples show less than 50% of the radioactivity co-migrating with dynorphin A on HPLC. The rest of the radioactivity co-migrates with tyrosine. This aminoterminal cleavage of tyrosine appears to be common to both *in vitro* and *in vivo* preparations. Since dT dynorphin has been shown to be biologically active, it was of interest to see if dT dynorphin was formed and stable following the loss of tyrosine from dynorphin. The results are in Fig. 4. Substantial dT dynorphin can be demonstrated by radioimmunoassay. In the area of injection (PAG) at the end of 10 minutes, our longest time point, 84% of the dynorphin A-IR co-migrates with dT dynorphin. The total of dT dynorphin-IR plus dynorphin A-IR equaled the amount injected, suggesting that there is little loss of dynorphin A by conversion of dT dynorphin to other fragments that were not recognized by our antibody. The ratio of [^{3}H]dynorphin A to [^{3}H]tyrosine (80%:20%) is the same as the ratio of dynorphin A to dT dynorphin (84%:16%) in these same fractions. This suggests that injection of dynorphin A is followed by rapid amino terminal cleavage with the formation of a relatively more stable compound, dT dynorphin. However, without a time course of dT dynorphin breakdown it is not possible to conclusively demonstrate the stability of dT dynorphin *in vivo*. But, at the 10 minute time point 5–6-fold more dT dynorphin then dynorphin A is present.

DISCUSSION

The difficulty in demonstrating [^{3}H]dynorphin A binding to rat brain membranes suggested that the breakdown of [^{3}H]dynorphin A was extremely rapid. Leslie and Goldstein had published similar findings with [^{125}I]dynorphin A 1–13 [20]. Our time course studies *in vitro* confirm this notion. In single washed membranes, the breakdown proceeded very rapidly even at 0°C, resulting in less than 25% of the material intact at 30 minutes. In contrast, using double washed membranes substantially slowed the breakdown of [^{3}H]dynorphin A *in vitro* Therefore, the proteolytic enzymes may be loosely associated with the membranes, suggesting nonspecific soluble enzymes may be involved in breakdown *in vitro*. In all cases, the radiolabelled breakdown product appeared to be [^{3}H]tyrosine, without evidence of cleavage at the other sites such as between Gly-Gly or Gly-Phe to yield Tyr-Gly or Tyr-Gly-Gly. This suggests that enkephalinases are not the primary enzymes involved in the breakdown under these conditions. In the double washed membrane preparation, the continued co-migration on HPLC of [^{3}H]dynorphin A with the unlabelled standard suggests that extensive carboxy terminal cleavage is not occurring under these conditions. This is in contrast to the data of Leslie and Goldstein for dynorphin A 1–13 [20] and Robson *et al.* [31] for dynorphin A 1–9, who found that carboxy terminal cleavage proceeds at a rate similar to amino terminal cleavage. It

may be that the native full structure (Dyn A) is more resistant to carboxy-peptidase activity. Likewise, we were unable to demonstrate by HPLC any [^{3}H]dynorphin A 1–8 size peaks following *in vitro* incubation or *in vivo* injection, suggesting conversion of [^{3}H]dynorphin A to smaller opiate active forms is not a major pathway of degradation In contrast, we did observe a small but consistent peak of radioactivity that co-migrates on HPLC with Leu-enkephalin and co-elutes with the tyrosine on G-10 sieving column. Without another system for identification such as thin layer chromatography for amino acid sequence we are unable to conclude that this radioactive material is Leu-enkephalin, however, authentic Leu-enkephalin would be so rapidly degraded under these circumstances that we would not expect to see Leu-enkephalin [9, 33, 34]. Since we are unable to demonstrate [^{3}H]Tyr-Gly-Gly formation that would be generated by the breakdown of [^{3}H]Leu-enkephalin, the generation of Leu-enkephalin would appear to be a relatively minor pathway if at occurs at all.

Although the aminopeptidase cleavage of tyrosine could be accomplished by a general aminopeptidase, the inhibition of this action by dT dynorphin *and* dynorphin A 7–17, suggests there may be a specific enzyme that cleaves dynorphin A with a recognition site in the COOH-terminal domain of dynorphin A. The inhibition of this amino peptidase activity by a compound which cannot function as a substrate (dynorphin A 7–17) supports a specific enzyme hypothesis Similar findings have been reported for dynorphin A 6–13, i.e., that dynorphin A 6–13 blocks the breakdown of [^{125}I]dynorphin A 1–13. Since we were able to demonstrate only a slight inhibition of the breakdown of [^{3}H]dynorphin A by dynorphin A 1–13, it is unclear if this enzyme is the same enzyme described by Leslie and Goldstein [20]. However, this may be due to rapid breakdown including carboxy terminal cleavage of dynorphin A 1–13. Obviously, further studies are necessary to confirm the existence of such an enzyme, but possible inhibitors of this enzyme could provide other avenues of approach to studying the *in vivo* pharmacological effects of dynorphin A.

We have included data from guinea pig brain, even though preliminary, to point out there may be species differences in the stability of dynorphin A under binding conditions across species. In view of the slower breakdown of dynorphin A *in vitro*, it may be of interest to pursue a comparison of dynorphin A and dynorphin A 2–17 effects *in vivo* in guinea pigs.

In conclusion, in rat, it appears that dynorphin A is rapidly broken down to dynorphin A 2–17, a non-opioid but behaviorally active fragment of dynorphin. The rapidity of this breakdown *in vivo* may explain the difficulty in demonstrating opioid effects of dynorphin A *in vivo* in this species. The large number of non-opioid effects observed following *in vivo* injection of dynorphin A, which can be mimicked by dynorphin A 2–17, may occur through the conversion of dynorphin A to the behaviorally active dynorphin A 2–17 fragment

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