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# Synthesis of enkephalins by adrenal medullary chromaffin cells: Reserpine increases incorporation of radiolabeled amino acids

(high-performance liquid chromatography/cell culture/opiate-like peptides/induction/endorphins)

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ABSTRACT **Adrenal medullary chromaffin cells maintained in a chemically defined, serum-free medium retain high levels of both catecholamines and opiate-like peptides. At least 25% of the opioid activity as measured by radioreceptor assay is present as [Met]- and [Leu]enkephalin. Incubation of chromaffin cells with [$^3$H]tyrosine and [$^{35}$S]methionine for 3 days results in incorporation of $^3$H and $^{35}$S into [Met]enkephalin and $^3$H incorporation into [Leu]enkephalin, demonstrating *de novo* enkephalin synthesis. Addition of 100 nM reserpine to the culture medium causes an 80% depletion of catecholamines and a 100% increase in opioid peptide content 3–4 days later. [Met]Enkephalin content is preferentially induced (290% above control) compared with [Leu]enkephalin (70%). Reserpine increases the fractional radiolabeling of [Met]- and [Leu]enkephalin in excess of control cultures, suggesting that the increase in opiate-like peptides results from an increased rate of synthesis rather than decreased degradation, decreased secretion, or increased processing of preformed precursor(s).**

Several laboratories have recently established the presence of large amounts of opiate-like peptides (OLP) in the adrenal medulla of several mammalian species, including man (1–5). These OLP are stored with catecholamines in the storage vesicles of chromaffin cells and are secreted with catecholamines from the adrenal in response to splanchnic activation, acetylcholine, or other stimuli (3, 5). Although the physiological function of the adrenomedullary enkephalins is unclear, stimuli that elicit catecholamine secretion from the gland also release large quantities of enkephalins into the circulation (5).

Viveros *et al.* (5, 6) have demonstrated that the amounts of adrenomedullary enkephalins can be modified via splanchnic-adrenomedullary activation or after catecholamine depletion induced by reserpine. Insulin-induced hypoglycemia or reserpine treatment increases enkephalin levels within a few days. The reserpine-induced increase in OLP content is blocked by administration of cycloheximide (5, 7) or actinomycin D (7), suggesting that this increase requires new peptide synthesis. In spite of these observations, there is no direct evidence for *de novo* synthesis of enkephalins by chromaffin cells nor is it known how reserpine and nerve stimulation increase the content of OLP. This could result from: (*i*) decreased rate of OLP degradation or secretion or both, (*ii*) increased uptake of circulating enkephalins or precursor(s), (*iii*) increased processing of a previously stored precursor, or (*iv*) increased translation and amino acid incorporation.

Synthesis of enkephalins from radiolabeled amino acids *in vivo* by rat brain (8–10) and *in vitro* by myenteric plexus (9) and striatal slices (11) has been observed. However, studies of enkephalin synthesis in intact animals are difficult to interpret, and experiments in isolated tissues are limited by a short time of survival and cellular heterogeneity. Adrenal medullary chromaffin cells maintained in primary culture provide a convenient model system for study of the cell biology and biochemistry of these adrenergic/peptidergic neuron-like cells. This study reports the *de novo* synthesis of [Met]- and [Leu]-enkephalin from radiolabeled amino acids in cultured chromaffin cells and an increase in the synthesis of enkephalins by these cells after treatment with reserpine.

## MATERIALS AND METHODS

**Materials.** Reserpine was generously provided by Ciba Pharmaceutical as Serpasil phosphate. [*ring*-2,3,5,6-$^3$H]Tyrosine (91.6 Ci/mmol; 1 Ci = $3.7 \times 10^{10}$ becquerels) and [$^{35}$S]methionine (1081 Ci/mmol) were obtained from New England Nuclear. Dulbecco's modified Eagle's medium (cat. no. 320-1885), nutrient mixture F-12 (Ham's) (cat. no. 320-1767), fetal bovine serum, and penicillin G were obtained from GIBCO. Collagenase (cat. no. C-2139), DNase I (cat. no. D-4763), nystatin, 5-fluorodeoxyuridine, gentamicin sulfate, and Amberlite XAD-2 were obtained from Sigma. Percoll was obtained from Pharmacia.

**Chromaffin Cell Culture.** Chromaffin cells were isolated from bovine adrenal medullae by the method of Livett and coworkers (12, 13). Briefly, this includes dissociation of single chromaffin cells from the gland by digestion with collagenase and DNase I and purification of cells by density gradient centrifugation in Percoll. Purified cells were resuspended in plating medium (45% Dulbecco's modified Eagle's medium/45% nutrient mixture F-12/10% fetal bovine serum/10 μM 5-fluorodeoxyuridine/9 mM Hepes/3.2 mM glucose/100 μg of penicillin per ml/10 μg of gentamicin per ml/25 USP units of nystatin per ml) and plated on tissue culture plasticware at 100,000–200,000 cells, in 0.2 ml, per cm$^2$ of surface area. Cultures were maintained at 37°C in a humidified atmosphere containing 5% $CO_2$ in air. After 2 days in culture, the plating medium was removed and replaced with a similar volume of serum-free maintenance medium (50% Dulbecco's modified Eagle's medium/50% nutrient mixture F-12/10 μM 5-fluorodeoxyuridine/10 mM Hepes/3.5 mM glucose/100 μg of penicillin per ml/10 μg of gentamicin per ml/25 units of nystatin per ml). In some experiments, 5-fluorodeoxyuridine was omitted from the maintenance medium; this had no effect on the OLP or catecholamine content of the cells or on their response to reserpine treatment. The medium was replaced every 2 days, except in radiolabeling experiments. Reserpine (100 nM) was added to the cultures for either 1 hr or 1–3 days. These two methods of reserpine administration were equivalent in producing increased OLP and decreased catecholamine levels in chromaffin cells. A more detailed description of the methods for isolating and culturing chromaffin cells will be published elsewhere.

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Abbreviations: OLP, opiate-like peptides; HPLC, high-performance liquid chromatography.

Cells were harvested for assay of OLP and catecholamines by removing the culture medium and washing the cell monolayer twice at 4°C with buffer A (145 mM NaCl/5.4 mM KCl/1.0 mM $NaH_2PO_4$/11.2 mM glucose/15 mM Hepes/1.8 mM $CaCl_2$/0.8 mM $MgSO_4$, pH 7.4; 315 mosM) followed by extraction on ice with 0.4 M perchloric acid. After removal of the perchloric acid extract, the protein residue was dissolved in 0.6 M NaOH for protein determination.

**Simultaneous Incorporation of [$^3$H]Tyrosine and [$^{35}$S]-Methionine.** Chromaffin cells were plated at $1.1 \times 10^7$ per 100-mm tissue culture dish. After 4 days in culture, the maintenance medium was removed, cultures were washed once with nutrient mixture F-12 containing 10 $\mu$M 5-fluorodeoxyuridine and antibiotics, and then 10 ml of nutrient mixture F-12 containing 10 $\mu$M fluorodeoxyuridine, antibiotics, and approximately 100 $\mu$Ci each of [$^3$H]tyrosine and [$^{35}$S]methionine was added. The concentration of tyrosine and methionine in nutrient mixture F-12 was 30 $\mu$M. Cells were maintained for 3 days with or without 100 nM reserpine. Cultures were harvested by removing the medium, washing the cells three times with buffer A at 4°C and then extracted on ice with either 0.4 M perchloric acid or 1 M acetic acid (5–6 ml). Extracts were applied to columns of Amberlite XAD-2 (bed volume, 2 ml) in 0.4 M perchloric acid or 0.2 M acetic acid (extract diluted with water). The column effluent was reapplied to the column two to six times. The resin was then washed with 12–22 ml of 0.2 M acetic acid. Enkephalins were eluted from the resin with 5.0 ml of 0.2 M acetic acid in methanol. The eluate was evaporated to dryness under nitrogen and resuspended in water for application to the high-performance liquid chromatography (HPLC) column.

**Other Methods.** Separation of opioid peptides by HPLC was performed on a Partisil PXS-10, 10/25 ODS column (Whatman) with 15% acetonitrile/10 mM ammonium acetate, pH 4.2, as the solvent (40 min). This was followed by elution with 50% acetonitrile or a gradient of 15–50% acetonitrile. OLP were assayed by displacement of $^{125}$I-labeled [D-Ala$^2$, D-Leu$^5$]enkephalin from rat brain membrane receptors (14, 15). OLP levels are expressed as the molar equivalent of a [Leu]enkephalin standard. [Leu]- and [Met]enkephalin were equally potent in displacing the labeled ligand. Catecholamines (epinephrine and norepinephrine) were assayed by the method of Anton and Sayre (16). Protein was determined by the method of Lowry *et al.* (17) with bovine serum albumin as the standard. Simultaneous determination of $^3$H and $^{35}$S radioactivities was by liquid scintillation spectrometry with external standardization.

## RESULTS

Cultures of bovine adrenal medullary chromaffin cells contained both catecholamines and OLP (Table 1) and maintained constant levels of protein for at least 3 weeks. These values compare favorably with those found for the bovine adrenal medulla—590 nmol of catecholamines per mg of protein (18) and 100–200 pmol of OLP per mg of protein (3)—although there is some loss of catecholamines and an increase of OLP content with time in culture (7). Addition of 100 nM reserpine to the culture medium resulted in an 80% loss of catecholamines and a 100% increase in OLP 3–4 days later. Other experiments showed that, at this time, reserpine-induced OLP levels were at a maximum. The protein content of the cultures was not altered by reserpine treatment.

In order to characterize the opioid peptides that are increasing with reserpine treatment, to establish if chromaffin cells in culture are able to synthesize OLP *de novo*, and to determine if the reserpine-induced increase in OLP results from increased synthesis or decreased secretion/degradation, cells were cultured with [$^3$H]tyrosine and [$^{35}$S]methionine for 1 or 3 days. The acid extracts of these cultures were subjected to chromatography on Amberlite XAD-2 followed by reverse-phase HPLC. Recovery of total OLP in perchloric acid extracts of chromaffin cells was 84% from Amberlite XAD-2 chromatography; this was similar to the 90% recovery of [$^3$H][Leu]-enkephalin external standards (Table 2). Recovery of total OLP from HPLC was 92% of the applied activity (recovery of $^3$H-labeled [Leu]- or [Met]enkephalin added to adrenal medullary extracts was 100%). Treatment of chromaffin cells with reserpine had no effect on the recoveries of total OLP throughout the fractionation procedure.

Table 1. Reserpine alters catecholamine and opiate-like peptide content of chromaffin cells in culture

| Treatment | Catecholamines, nmol/mg protein | OLP, pmol/mg protein |
|---|---|---|
| None | 366 ± 57 (5) | 180 ± 33 (14) |
| Reserpine (100 nM) | 68 ± 19* (5) | 364 ± 63† (15) |

Reserpine was added to the culture medium of chromaffin cells after 4–7 days of culture. Catecholamines and OLP were extracted from treated and untreated cultures 3 or 4 days later in 0.4 M perchloric acid. The data shown represent results from four (catecholamines) or six (OLP) separate cell preparations maintained under similar conditions and are expressed as mean ± SEM (number of cultures is given in parentheses).
* $P < 0.005$.
† $P < 0.02$.

Reverse-phase HPLC revealed that cultured chromaffin cells contain OLP with retention times identical to those of [Met]- and [Leu]enkephalin standards (Fig. 1 *A*, *B*, and *D*). On the basis of more detailed analytical studies carried out on the OLP extracted from homogenates of bovine adrenal medulla (3, 19), the identities of these OLP as [Met]- and [Leu]enkephalin have been firmly established. However, the opioid activity under the [Met]- and [Leu]enkephalin peaks accounted for only 45% of the perchloric acid-extractable OLP in chromaffin cells (Table

Table 2. Fractionation of chromaffin cell OLP

| Fraction | OLP, pmol: Perchloric acid, Control | Perchloric acid, Reserpine | Acetic acid, Reserpine | Cumulative recovery, %* |
|---|---|---|---|---|
| Acid extract | 123 | 273 | 473 | 100 |
| Amberlite eluate | 103 | 230 | 209 | 90 |
| HPLC, total | 95 | 214 | 159 | 90 |
| HPLC, [Met]enkephalin + [Leu]enkephalin | 50 | 125 | 119 | 90 |

Cultures containing $1.1 \times 10^7$ cells were treated, extracted with perchloric or acetic acid, and fractionated. HPLC was performed for 40 min (flow rate, 1 ml/min) with 15% acetonitrile/10 mM ammonium acetate, pH 4.2, as the buffer, followed by elution with 50% acetonitrile or a gradient of 15–50% acetonitrile. The fractions collected were assayed for opioid activity by radioreceptor assay. The amounts of [Met]- and [Leu]enkephalin were calculated from the radioreceptor activity with retention times identical to [Met]- and [Leu]enkephalin standards. Enkephalin recovery was determined by adding 20,000 dpm of [$^3$H][Leu]enkephalin to either perchloric or acetic acid followed by adsorption/desorption on Amberlite XAD-2, drying the eluate under $N_2$, and resuspension in $H_2O$. Other experiments in which acetic acid extraction was compared to perchloric acid extraction of sister cultures also showed 70–100% more total OLP extracted with acetic acid. This was equally true in both control and reserpine-treated cultures.
* Enkephalin recovery on HPLC was determined by addition of [$^3$H][Leu]enkephalin to adrenal medullary extracts.

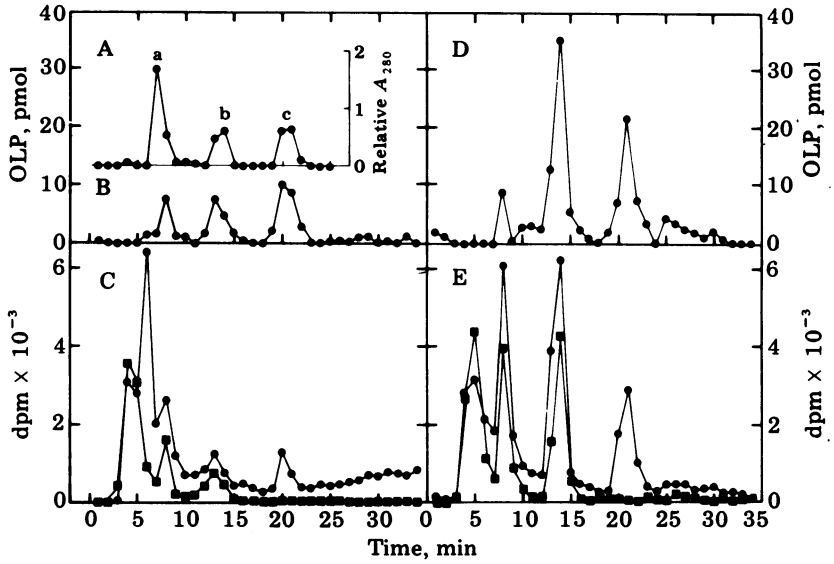


FIG. 1. HPLC of newly synthesized enkephalins from chromaffin cell cultures. Perchloric acid (0.4 M) extracts of $7.7 \times 10^6$ chromaffin cells incubated with [$^3$H]tyrosine and [$^{35}$S]methionine for 3 days and purified by chromatography on Amberlite XAD-2 were subjected to isocratic chromatography on a Partisil PXS-10, 10/25 ODS column in 15% acetonitrile/10 mM ammonium acetate, pH 4.2. The flow rate was 1 ml/min. Fractions were subsequently assayed for OLP and radioactivity. Incorporation of [$^3$H]tyrosine into perchloric acid-precipitable protein was $2.01 \times 10^7$ dpm in control cultures and $2.02 \times 10^7$ dpm in reserpine-treated cultures; [$^{35}$S]methionine incorporation was $1.35 \times 10^7$ and $1.30 \times 10^7$ dpm, respectively ($7.7 \times 10^6$ cells). (*A*) Separation of a mixture of standards containing 20 nmol each of Tyr-Gly-Gly-Phe (peak a), [Met]-enkephalin (peak b), and [Leu]enkephalin (peak c). (*B*) Separation of OLP in untreated cells. (*C*) Separation of $^3$H-labeled (●) and $^{35}$S-labeled (■) material from untreated chromaffin cells. (*D*) Separation of OLP obtained from cells treated with 100 nM reserpine for 3 days. (*E*) Separation of $^3$H-labeled (●) and $^{35}$S-labeled (■) material from reserpine-treated cells.

2, corrected for [$^3$H][Leu]enkephalin recovery). Treatment of chromaffin cell cultures with reserpine produced a 290% increase in [Met]enkephalin and a 70% increase in [Leu]enkephalin (Fig. 1 *B* and *D*; see Table 3). Because reserpine also increases the total OLP, [Leu]- plus [Met]enkephalin still account for only 51% of the corrected opioid activity in the perchloric acid-extracted cells (Table 2). Cultures extracted with perchloric acid, but not those extracted with acetic acid, contained another opiate-receptor binding component with a retention time of 8 min, slightly longer than that of Tyr-Gly-Gly-Phe. Oxidized [Met]enkephalin has a similar retention time and it is likely that some oxidized [Met]enkephalin would result from extraction with perchloric acid, an oxidant. Gradient elution of the HPLC column with 15–50% acetonitrile revealed several small peaks of opioid activity representing, in total, 9% of the applied OLP.

Extraction of the cells with acetic acid precipitated only 60% of the protein precipitated with perchloric acid. Acetic acid extracts had 70% more total OLP than did 0.4 M perchloric acid extracts of sister cultures (Table 2); however, recovery of OLP from Amberlite XAD-2 chromatography was only 44% of the acetic acid extract, so that similar amounts of OLP were recovered from Amberlite XAD-2 and from the HPLC column regardless of the acid used to extract the cultures. In conclusion, only about one-fourth of the total radioreceptor-assayable opioid peptides in the chromaffin cells are present as [Met]- and [Leu]enkephalin. These results are similar to those previously reported (3, 19).

Analysis of the HPLC fractions also showed well-defined peaks of $^3$H eluting with [Met]- and [Leu]enkephalin and an $^{35}$S-labeled peak with a retention time identical to that of [Met]enkephalin but not that of [Leu]enkephalin (Fig. 1 *C* and *E*). The high proportion of essential to nonessential amino acids in [Met]- and [Leu]enkephalin, the nature of the radioactive amino acids used in these studies, and the constancy of the almost 1:1 ratio of $^{35}$S and $^3$H incorporated into the [Met]enkephalin peak with the different treatments (Table 3) make it highly likely that the radioactive label is contained in the original amino acids. In addition, other $^3$H- and $^{35}$S-labeled peaks with shorter retention times were present. The first eluted peak may correspond to contamination by the free radioactive amino acids since they eluted at the front and that peak showed a marked decrease when the cultures were pulsed with tracer for 24 hr followed by a 48-hr chase. In all probability, the second $^3$H peak in control cultures, which did not contain $^{35}$S, corresponds to radioactive catecholamines synthesized by control, but not reserpine-treated, chromaffin cells. Cathecholamine standards had similar retention times. The $^3$H and $^{35}$S radioactivities at retention time 8 min corresponded to a peak of

Table 3. Incorporation of [$^3$H]tyrosine and [$^{35}$S]methionine into enkephalins of chromaffin cells in culture

| Treatment | Enkephalin | Opioid activity, pmol | [$^3$H]Tyr, pmol | [$^{35}$S]Met, pmol | Fraction of OLP labeled |
|---|---|---|---|---|---|
| None | Met | 13.8 | 1.7 | 2.1 | 0.14 |
| | Leu | 21.4 | 2.1 | 0 | 0.10 |
| Reserpine | Met | 53.4 | 12.8 | 10.5 | 0.22 |
| (100 nM) | Leu | 35.9 | 6.5 | 0 | 0.18 |
| Reserpine | Met | 57.2 | 17.2 | 15.6 | 0.29 |
| (100 nM)* | Leu | 26.1 | 6.1 | 0 | 0.23 |

Data taken from Fig. 1 and from a similar HPLC separation of an acetic acid extract of a sister reserpine-treated culture. Values represent extracts of $7.7 \times 10^6$ cells for each treatment. The amount of radioactivity above baseline in the OLP peaks that eluted in the positions of [Met]- and [Leu]enkephalin is expressed as pmol based on the initial specific activities of these amino acids in the medium (715 and 572 dpm/pmol, respectively). The fraction of [Met]enkephalin labeled was calculated as the average of both radiolabels incorporated by the formula [(pmol [$^3$H]tyrosine + pmol [$^{35}$S]methionine)/2]/pmol OLP; the fraction of [Leu]enkephalin labeled pmol [$^3$H]tyrosine/pmol OLP. Radioactivity in the 8-min retention time peak was not considered for these calculations because the high levels of background radioactivity precluded accurate quantitation.

* Culture extracted with 1 M acetic acid.

opioid receptor activity, had the same retention time as oxidized [Met]enkephalin, and was markedly decreased when cultures were extracted with acetic acid rather than perchloric acid (see above).

If the assumption is made that the intracellular pools of tyrosine and methionine rapidly equilibrate with the extracellular amino acids, we can use the initial specific activities of the radiolabeled amino acids in the labeling culture medium to calculate the incorporation of [$^3$H]tyrosine and [$^{35}$S]methionine into chromaffin cell [Met]- and [Leu]enkephalin (Table 3). These values probably represent minima because dilution of the radiolabeled amino acids with cellular pools is likely to occur. Reserpine does not effect the specific activity of the radiolabeled amino acid pool available for protein synthesis as judged by the identical incorporation of [$^3$H]tyrosine and [$^{35}$S]methionine into perchloric acid-precipitable material in both control and reserpine-treated cultures (see legend to Fig. 1). Reserpine increased the incorporation of radiolabeled amino acids in excess of the increased OLP levels as measured by radioreceptor assay. In both control and reserpine-treated cultures, incorporation of [$^3$H]tyrosine into [Met]enkephalin was approximately one-third greater than into [Leu]enkephalin (compare fraction of OLP labeled). In control cultures, 10–14% of the enkephalins found had been synthesized from radiolabeled amino acids during the 72-hr labeling period. With reserpine treatment, this value increased to 18–29%.

## DISCUSSION

Prior to this report there was no direct evidence that adrenal chromaffin cells could synthesize OLP from component amino acids. Because the cultured cells do not have other sources of enkephalins or precursors (cultures are in serum-free media), the reserpine effect cannot result from an increased uptake. Although accumulation of OLP by decreased secretion or a decreased rate of degradation should keep the fraction of OLP labeled with radioactive amino acids the same as control, an increased rate of conversion from a stored precursor should decrease the fraction of OLP labeled. The larger fraction of [Met]- and [Leu]enkephalin labeled during reserpine treatment clearly indicates that the increase in opioid peptides induced by this drug corresponds to an acceleration of synthesis from precursor amino acids. The larger fraction of enkephalins labeled during treatment with reserpine also argues against the possibility that reserpine acts through increasing the extractability of chromaffin cell OLP. Other studies have shown that the reserpine-induced increase in OLP is blocked by actinomycin D and cycloheximide (ref. 7; unpublished data), further strengthening the conclusion that this increase represents increased synthesis.

On the other hand, the increase in the amounts of [Leu]- and [Met]enkephalin as calculated from the initial specific activities in the culture medium accounts for only approximately 30% of the total increase in [Leu]- and [Met]enkephalin as measured by the radioreceptor assay (Table 3). This difference may be due to a high degree of mixing of the radiolabeled amino acid with the total cell amino acids during 3 days in culture or it may indicate that reserpine induction has more than one primary mode of action—i.e., increasing synthesis plus increasing precursor conversion or decreasing enkephalin degradation.

The pathway for extrahypophyseal biosynthesis of enkephalins is as yet unknown. Although [Met]enkephalin can be formed from $\beta$-endorphin by an extracellular enzyme (20), its biosynthesis in the adrenal medulla as well as in other tissues such as the striatum apparently is different because neither $\beta$-endorphin nor its precursors have been found in these two tissues (2, 3, 21). At present, we can only speculate on the presence and nature of enkephalin precursors in the adrenal medulla. Further work is necessary to establish which of the many putative precursors described (2, 4, 5, 19, 22) truly belong to the enkephalin synthesis chain(s) or are final opioid products in their own right or only accidentally contain part or all of the enkephalin sequences.

Although similar incorporations of [$^3$H]tyrosine and [$^{35}$S]-methionine into [Met]enkephalin was observed, a lower rate of incorporation of amino acids into [Leu]enkephalin was seen on comparison of the fraction of OLP labeled (Table 3). A lower rate of incorporation of [$^3$H]tyrosine into [Leu]enkephalin has also been reported for slices of guinea pig ileum and striatum (9, 11). Furthermore, reserpine not only preferentially increases [Met]enkephalin labeling but it also preferentially increases the accumulation of total [Met]enkephalin over total [Leu]enkephalin. A similar preferential increase of [Met]enkephalin by reserpine has been found in guinea pig adrenals (unpublished data). These results may indicate independent regulation of synthesis, processing, degradation, storage, or secretion of these two enkephalins in adrenomedullary cells.

In intact animals, neurogenic stimulation of the adrenal medulla or catecholamine depletion by reserpine results in induced synthesis of tyrosine hydroxylase and catecholamine storage vesicles, as monitored by an increase in dopamine-$\beta$-hydroxylase and vesicular catecholamine uptake (23, 24). This effect of reserpine is commonly thought to result from the decreased secretion of catecholamines leading to hypotension and reflex stimulation through the splanchnic nerve, and it has been called "trans-synaptic induction" (25). We have recently shown that splanchnic stimulation (5, 26) or reserpine treatment (5, 6) produces large increases in the total OLP and [Met]- and [Leu]enkephalin contents of guinea pig and cat adrenals. Here we have shown that reserpine administration produces similar increases in chromaffin cell OLP, including [Met]- and [Leu]-enkephalin, in the absence of innervation. We have also found an increase in tyrosine hydroxylase in reserpine-treated cultures (ref. 7; unpublished data). Our results suggest that synaptic activation is not the only long-term regulator of enkephalin (and catecholamine) biosynthesis. This is in agreement with *in vivo* studies indicating that reserpine treatment leads to induction of dopamine-$\beta$-hydroxylase at doses that do not produce synaptic activation or in the presence of ganglionic blocking agents (24). A critical pool of catecholamines may be involved in the regulation of OLP levels in cultured chromaffin cells. Enkephalin levels in the striatum are also subject to regulation because various treatments produce increases in striatal enkephalin content (22, 27–29). Although induction of adrenomedullary OLP by reserpine can occur without synaptic activation, increases in striatal enkephalins by decreased dopaminergic activation may require a multineuronal pathway (28).

In conclusion, the data presented here demonstrate that adrenal medullary chromaffin cells maintained in culture can synthesize [Met]- and [Leu]enkephalin *de novo* and that OLP synthesis in these cells is subject to regulation. Elucidation of the reason for a physiological mechanism to control OLP synthesis in the adrenal medulla awaits an understanding of the neuroendocrine role of these peripherally released peptides.

**Note Added in Proof.** Using cultured chromaffin cells, Rossier *et al.* (30) recently incorporated [$^{35}$S]methionine into a putative [Met]enkephalin precursor of 26,000 daltons.

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